Investigation of biologically active peptaibol compounds produced by members of the filamentous fungal genus *Trichoderma*

Ph.D. dissertation

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2. ABBREVIATIONS

act1: actin 1

Aib: α -aminoisobutyric acid

AI: aggressivity index

ALM: alamethicin

cal1: calmodulin

EIC: extracted ion chromatogram

ESI: electrospray ionization

hfb4: hydrophobin 4

HPLC: high-performance liquid chromatography

IT: ion trap

ITS: internal transcribed spacer

LB: Luria–Bertani

Lxx: leucine/isoleucine

MEA: malt extract agar

MIC: minimum inhibitory concentration

MS: mass spectrometry

NRPS: non-ribosomal peptide synthetase

PK-15: porcine kidney tubular epithelial cell line

PI: propidium iodide

rpb2: RNA polymerase B subunit II

SF: subfamily

SZMC: Szeged Microbiological Collection

tef1: translation elongation factor 1 α

TIC: total ion chromatogram

TUCIM: TU Collection of Industrially Important Microorganisms

Vxx: valine/isovaline

3. INTRODUCTION

3.1 Genus *Trichoderma*

Trichoderma species are cosmopolitan, free-living organisms, which play role mostly in decomposing organic matter in soil (Harman et al. 2004). Certain species have great economic importance as sources of antibiotics, enzymes, as plant growth promoters, decomposers of xenobiotics, and as commercial biofungicides (Chet and Inbar 1994, Harman et al. 2004, Hermosa et al. 2012). Probably the most known species with the ability to produce hydrolytic enzymes degrading cellulose or hemicellulose is *T. reesei*, belonging to the clade Longibrachiatum of the genus (Harman and Kubicek 1998, Kubicek et al. 2009). Species like *T. viride*, *T. virens*, *T. atroviride*, *T. asperellum* and *T. harzianum* are also frequently studied due to their ability to produce enzymes and antibiotics valuable in agriculture (Schuster and Schmoll 2010, Contreras-Cornejo et al. 2016), as well as their antagonistic effect against phytopathogenic fungi such as *Botrytis cinerea*, *Alternaria solani* and *Rhizoctonia solani* (Harman et al. 2004). Furthermore, incubation of a “*T. harzianum*” strain, which was later re-identified as *T. atroviride* (Röhrich et al. 2014), with the cell wall of *B. cinerea* led to the secretion of cell wall hydrolyzing enzymes and antibiotic fractions of peptaibols, which inhibited *B. cinerea* spore germination, resulting in a fungicidal effect. Also, peptaibols and hydrolytic enzymes were found to act synergistically in this antagonistic interaction (Schirmböck et al. 1994).

3.1.1 Taxonomy of *Trichoderma*

More than 300 species of the genus *Trichoderma* (Ascomycota, Hypocreales, Hypocreaceae) have been reported so far (Bissett et al. 2015, Zhang and Zhuang 2018). The majority of *Trichoderma* species was described after the year 2000, only a few species were initially included in the genus (Bisby 1939, Rifai 1969). The last 15 years refined the phylogeny of the genus at the species level. Especially, the sequence analysis of introns 4 and 5 of translation elongation factor 1 α (*tef1*) gene provided the highest resolution to distinguish the species from each other, which resulted in the resolution of this hyperdiverse genus (Jaklitsch 2009, Jaklitsch 2011). *Trichoderma* species are categorised into different clades (Jaklitsch and Voglmayr 2015). Recently, 12 *Trichoderma* species that are most frequently observed in nature and belonging to three different *Trichoderma* sections/clades were also selected to study the evolution of their genome sequences (Kubicek et al. 2019).

Clade Viride contains some of the most complicated groups of species considering species recognition and distinction (Jaklitsch and Voglmayr 2015). It forms one of the largest and most diverse groups of genus *Trichoderma*. Bissett (1991a) proposed to include *Hypocrea rufa*/*T. viride* and its relatives in section Trichoderma along with *T. koningii* (Oudem.) and *T. atroviride* (P. Karst.). The monophyly of this group, earlier referred to as section Trichoderma (Kullnig-Gradinger et al. 2002) and recently as clade Viride, was confirmed after DNA sequence analysis of the internal transcribed spacer 1 and 2 (ITS1 and 2) regions, as well as fragments of the actin 1 (*act1*), calmodulin (*cal1*) and *tefl* genes (Samuels et al. 2002). Since the work of Lieckfeldt et al. (1999) many additional species and cultures referable to clade Viride were obtained and the taxonomy of this clade has also been revised (Jaklitsch et al. 2006). The two large subclades of this clade, Koningii and Viride are treated separately and their composition has changed over time (Jaklitsch et al. 2006, Samuels et al. 2006). Among the species of Viride subclade, warted conidia can be found, while species with verruculose conidia occur in the Koningii subclade. Overall, clade Viride is complex and it contains further subclades, such as the Hamatum/Asperellum subclade, the Rogersonii subclade, the Neorufum subclade and several smaller subclades. Hence, Jaklitsch and Voglmayr (2015) integrated these subclades into clade Viride.

The group of *Trichoderma* species with green ascospores contain two major clades, the clade Harzianum/Virens and a paraphyletic group containing clades Ceramica, Chlorospora, Spinulosa and several smaller clades, such as Strictipilosa, Gelatinosa (Jaklitsch and Voglmayr 2015, Kubicek et al. 2019). The species of these clades all have green ascospores. Clade Helicum is closely related to this group. Clades Stromaticum (Samuels et al. 2012a) and Semiorbis are small clades and the latter one interestingly contains both species with hyaline and green ascospores.

Polysporum group is paraphyletic and does not form a monophyletic clade therefore it is referred as a group rather than a clade. It is correlated to the Pachybasium core group (Jaklitsch and Voglmayr 2015). Species were added to the former section Hypocreanum and revised several times, which resulted in clade Hypocreanum (Overton et al. 2006a, Overton et al. 2006b, Jaklitsch et al. 2008, Jaklitsch 2011, Kim et al. 2012, Kim et al. 2013, Jaklitsch and Voglmayr 2015). Clade Megalocitrina (Chaverri and Samuels 2003) was revised and named as clade Psychrophila (Jaklitsch and Voglmayr 2015, Kubicek et al. 2019), which contains species with characteristic light- or bright-coloured stromata and white-conidial gliocladium-like *Trichoderma* asexual morphs. Clade Brevicompactum was defined by Degenkolb et al.

(2008a) and augmented by Jaklitsch (2011). The formerly called clade Lutea was renamed as clade Deliquescens due to *T. deliquescens*, the asexual form of *H. lutea* (Jaklitsch 2011).

Section Longibrachiatum 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 has been designated recently as clade Longibrachiatum (Druzhinina et al. 2012). It is one of the evolutionarily 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 opportunistic members including both clinically relevant and ecologically restricted species. *Trichoderma longibrachiatum*, *T. bissettii*, *T. orientale* and *T. citrinoviride* are opportunistic human pathogens causing infections, mainly in immunocompromised patients (Kuhls et al. 1999, Kredics et al. 2003, Sandoval-Denis et al. 2014, Hatvani et al. 2013). *T. longibrachiatum* or its transformants have also been suggested for use as biocontrol agents against phytopathogens like *Pythium ultimum* or members of the *Fusarium solani* species complex (Migheli 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 (Druzhinina et al. 2008). In 2014, Sandoval-Denis et al. introduced *T. bissettii* as a new species, which was separated from *T. longibrachiatum*. Later, on the basis of ITS1 and 2, *act1*, *cal1*, *tef1*, hydrophobin 4 (*hfb4*), and RNA polymerase B subunit II (*rpb2*) sequence analysis, *T. bissettii* was proposed to be a phylotype of *T. longibrachiatum* – *T. longibrachiatum* f. *bissetti* – instead of a distinct species (Hatvani et al. 2019). The cellulase producer *T. reesei* is also capable of sexual reproduction (Seidl et al. 2009), whereas its sympatric species *T. parareesei* is genetically isolated and has a clonal lifestyle (Atanasova et al. 2010, Druzhinina et al. 2010). While *T. longibrachiatum* and *T. orientale* are cosmopolitan, the related *T. pinnaum* and *T. aethiopicum* are rare and restricted species (Druzhinina et al. 2010). Numerous other species, including *T. reesei*, *T. parareesei*, *T. pseudokoningii*, *T. sinense*, *T. effusum*, *T. konilangbra*, *T. andinense* or *T. novae-zelandiae* are also geographically restricted (Druzhinina et al. 2012). This clade contains species with ornamented conidia, which only occur here apart from clade Viride (Samuels et al. 1998, Jaklitsch 2011, Samuels et al. 2012b).

Several species form lone lineages or miscellaneous species. Few of them have stable phylogenetic sister group relationship with other large clades, e.g. *T. voglmayrii* is closely related to clade Viride, while *T. ghanense*, *T. capillare*, *T. andinense*, *T. effusum* and *T.*

britdaniae to clade Longibrachiatum (Druzhinina et al. 2012). These species usually do not form green conidia, their conidia are either hyaline or there are no asexual morphs at all (*T. peltatum*) (Samuels et al. 2011, Jaklitsch et al. 2013).

3.1.2 *Trichoderma* species as biocontrol agents

Trichoderma species can directly attack phytopathogenic fungi through their mycoparasitism, meanwhile promoting plant growth. Their fast growth and quick nutrient uptake also contributes to their aggressive competition (Verma et al. 2007, Chaverri et al. 2015, Degenkolb et al. 2015, Waghunde et al. 2016), therefore they can rapidly colonise soil, which rises them into the group of good biocontrol agents against several pathogenic organisms (Olabiya and Ruocco 2013, Kushwaha and Verma 2014, Shahid et al. 2014). The most important biocontrol agents are *T. harzianum*, *T. atroviride*, *T. asperellum*, *T. virens* and *T. viride* (Hermosa et al. 2004). *T. harzianum* is thoroughly studied as biocontrol agent against phytopathogenic fungi, such as *F. oxysporum*, *Sclerotinia sclerotiorum* and *R. solani* (Lopes et al. 2012, Geraldine et al. 2013, Qualhato et al., 2013, Pereira et al. 2014). The species of clade Viride can be isolated from very diverse sources with a wide geographic distribution (Kredics et al. 2014) and apart from their biocontrol ability, they were also reported as beneficial organisms in industrial, agricultural, and medicinal fields (Verma et al. 2007, Schuster and Schmoll 2010).

3.1.3 *Trichoderma* species as the causal agents of green mould disease in mushroom cultivation

Certain *Trichoderma* species can be harmful to cultivated mushrooms such as *Agaricus bisporus* and *Pleurotus ostreatus*, commonly known as the white button mushroom and oyster mushroom, respectively. The production of both crops can be seriously affected by the so-called green mould disease caused by certain members of the mycoparasitic fungal genus *Trichoderma* such as *T. aggressivum*, *T. pleuroti* and *T. pleuroticola*. *T. aggressivum* from clade Harzianum/Virens of the genus can be most frequently identified as the causal agent of green mould in the cultivation of *A. bisporus* (Samuels et al. 2002, Hatvani et al. 2007, Kredics et al. 2010). The dominant green mould agents in the cultivation of *P. ostreatus* proved to be different from *T. aggressivum* (Woo et al. 2004, Hatvani et al. 2007, Hatvani et al. 2008), and were described as the new clade Harzianum species *T. pleuroticola* and *T. pleuroti* (Park et al. 2006, Komoń-Zelazowska et al. 2007). *T. pleuroti* – originally incorrectly named as *T. pleurotum* (Park et al. 2006, Komoń-Zelazowska et al. 2007) – possesses *Gliocladium*-like conidiophore morphology, which is different from that of the genetically

closely related other oyster mushroom green mould agent *T. pleurotica*, showing a *Pachybasium*-like phenotype (Komoń-Zelazowska et al. 2007). Most interestingly, to the best of our knowledge, neither *T. aggressivum* nor *T. pleuroti* are known from nature so far, but have only been isolated from mushroom farms, which suggests their specialisation to a yet unknown habitat most likely associated with mushrooms of the order *Agaricales*.

3.1.4 *Trichoderma* species as opportunistic human pathogens

Trichoderma strains – mostly from the clade Longibrachiatum – have also been reported in an increasing number of cases as etiologic agents in human infections (Hatvani et al. 2013). The patients at risk of *Trichoderma* infections are usually the immunocompromised transplant recipients, patients undergoing continuous ambulatory peritoneal dialysis, those with haematological malignancies or human immunodeficiency virus (HIV) infection (Hatvani et al. 2013). Out of the strains collected from cases of *Trichoderma* infections, most of them proved to be *T. longibrachiatum*, while a few of them were *T. orientale* or *T. citrinoviride*. Later, Hatvani et al. (2019) reidentified several clinical samples of *T. longibrachiatum* and certain strains proved to be *T. bissettii*. All these four species are widely distributed, though *T. orientale* is more often found in warmer, tropical areas, while *T. citrinoviride* has never been found in Africa or the Indian subcontinent (Kubicek et al. 2008). The number of infections caused by *Trichoderma* species is relatively low, only few hundred cases have been reported (Walsh and Groll 1999, Kredics et al. 2003, Hatvani et al. 2013). However, this can be an underestimated number due to the lack of recognition, as *Trichoderma* strains detected in clinical specimens are usually considered as mere environmental contaminants. These *Trichoderma* strains are also present in environmental habitats including agricultural systems therefore they can be potential sources of human infections (Hatvani et al. 2019). The idea to use *T. longibrachiatum* as a biocontrol agent appeared in tropical countries, as these fungi can grow at higher temperature, unlike other *Trichoderma* species (Zhang et al. 2014, Zhang et al. 2015). However, this raises a high risk of human infections as these strains show intensive growth at 36 °C, which is the human body temperature (Antal et al. 2005). Therefore, instead of using the whole organism, an alternative option could be to use only their metabolites with biocontrol activities.

3.2 Peptaibols

A number of economically important secondary metabolites and extracellular enzymes are produced by species of the genus *Trichoderma* (Sivasithamparam and Ghisalberti 1998, Vinale et al. 2014). *Trichoderma* species also produce a wide range of bioactive secondary

metabolites with antibacterial, antifungal, and antiviral properties, including a unique peptide group of antibiotics, the peptaibols (Reino et al. 2008). Although these peptide-type molecules are known from other organisms as well, most of them are produced by this genus or by species from closely related genera, such as *Stilbella*, *Gliocladium* or *Acremonium* (Jaworski and Brückner 2001, Degenkolb et al. 2003).

3.2.1 Structure

Peptaibols are short peptides, usually 5–20 amino acid residues long, with characteristic molecular weight falling into the range 500–2200 Da, linear sequences and various types of bioactivity (Toniolo and Brückner 2009, Neumann et al. 2015). Characteristic residues of peptaibols include α -aminoisobutyric acid (Aib) and isovaline, as well as acetylated N-terminus and 1,2-amino alcohols such as Leuol, Valol, Pheol, Tyrol, Ileol, Alaol, and Prool at the C-terminus (Degenkolb et al. 2008a, Stoppacher et al. 2013, Neumann et al. 2015). Many peptaibol sequences contain an Aib–Pro bond in the middle of the peptide chain, which appears to be the most often fragmented bond in investigations using high-performance liquid chromatography (HPLC) mass spectrometry (MS) instruments. The name ‘peptaibol’ is formed from the word **peptide** and the two most characteristic residues, **Aib** and the C-terminal amino alcohol (Benedetti et al. 1982, Brückner and Graf 1983).

3.2.2 Biosynthesis

The sequences of peptaibols usually contain non-proteinogenic amino acids, and large modular enzymes known as non-ribosomal peptide synthetases (NRPSs) assemble them from a remarkable range of precursors by the multiple carrier thiotemplate mechanism (Marahiel 1997, Marahiel et al. 1997, May et al. 2002, Degenkolb et al. 2003, Degenkolb et al. 2007, Bushley and Turgeon 2010). In the case of NRPSs, a single large protein is responsible for the activation, incorporation and elongation of the peptides. Each NRPS module is a semiautonomous unit capable of recognizing, activating and modifying a single amino acid residue. The individual modules consist of distinct domains: (1) adenylation domain for selection and activation of amino acid monomers, (2) thiolation domain for transferring the monomers/growing chain to various catalytic sites and (3) condensation domains for catalysing the formation of peptide bonds, which together represent a minimal repeating unit of NRPSs (Marahiel 1997, Marahiel et al. 1997). 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 enable the acceptance of closely related residues (such as valine/isovaline: Vxx vs.

leucine/isoleucine: 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 herewith further increasing the variability of the products (Marahiel 1997, Marahiel et al. 1997). The first *Trichoderma* NRPS was *tex1* described from *T. virens* (Wiest et al. 2002).

3.2.3 Classification

Peptaibols can be classified based on their length and chemical characteristics into the following three subclasses (Rebuffat et al. 2000). Long-sequence peptaibols consist of 18–20 residues with high Aib content, short peptaibols are 11–16 residues long with several Aib–Pro–Xaa–Xaa motives and lipopeptaibols contain 7–11 residues with high Gly content and an N-terminus acylated by a short lipid chain instead of the acetyl group (Rebuffat et al. 2000). In 2001, Chugh and Wallace also categorised all the peptaibols known that time (more than 200) into subfamilies (SFs) based on the alignments of the sequences. In total, 9 SFs were introduced. SF1 is the largest SF, which contains long peptaibols between the length of 17 and 20 residues. The characteristics of the sequences belonging here are the presence of Gln mostly at the 6th or 7th positions, Aib-Pro bonds near the middle of the sequences and Gln or Glu at the 18th or 19th positions. All sequences belonging to this group can be characterised with the frequent occurrence of Aib, indicating that these molecules often form α -helical structures. SF2 contains around 30 compounds, which are the most similar to the peptaibols belonging to SF1. They are shorter peptaibols, consisting of 14–16 residues, still classified to the group of long peptaibols. They have aromatic residues (Phe) at both termini. They all have high Aib content, and Gly and Gln residues conserved in positions 6 and 11, respectively. A crucial difference of this group from SF1 is that the sequences contain Pro or Hyp in both the 10th and 13th positions. The closely related SF3 contains sequences with 2 characteristic differences from the members of SF2: (1) the 6th position is conserved and always contains Thr, (2) and Gly residues are absent from all the sequences. SF4 is completely different from the other SFs and contains either 11- or 14-residue-long peptaibols. The 2nd position always contains Gln or Asn. The 11-residue-long members contain Pro at positions 9 and 13, while the 14-residue-long peptaibols contain one more Pro at the 5th position. Furthermore, no aromatic amino acids or charged residues are present in the sequences of this group. SF5 contains short peptaibols with either 7 or 11 residues. Their sequences never contain Pro, Gln or any charged residues, but many Gly. SFs 6, 7, 8 and 9 are extremely small with only few members. SF6 peptaibols were considered as long peptaibols because the sequences are 15-

residue-long, while SF7 peptaibols are only 11-residue-long, belonging to the short peptaibols. SF 8 and 9 could not fit into any other group. SF 8 contains Clonostachin, which is 14-residue-long (Chikanishi et al. 1997), while SF9 contains the smallest peptaibol with 5 residues, peptaibolin (Hülsmann et al. 1998).

3.2.4 Biological activity

Peptaibols are membrane-active compounds forming several hydrophobic transmembrane helices surrounding a central pore with the ability to aggregate and establish ion channels in lipid bilayer membranes (Duclohier 2004). They usually show bioactivity against bacteria and fungi. Several studies have reported that Gram-positive bacteria are more sensitive to membrane active compounds than Gram-negative bacteria (Brückner and Graf 1983, Lorito et al. 1996a, Benítez et al. 2004). The key variances between the two types of bioactivity rely on the difference of their cell walls. Yeasts are less sensitive against peptaibols than Gram-positive bacteria or filamentous fungal cultures (Song et al. 2006). In the antagonistic property of *Trichoderma* species against phytopathogenic fungi, the production of secondary metabolites such as peptaibols plays a substantial role (Schirmböck et al. 1994, Howell 2003, Benítez et al. 2004, Küçük and Kivanç 2004). Also, synergism between peptaibols and the inhibition of the β -1,3-glucan synthase could inhibit the re-synthesis of β -glucans of the host cell wall and sustain the disruptive action of β -glucanases, which resulted in an enhanced fungicidal activity (Lorito et al. 1996b). Antiviral activity of peptaivirins A and B has also been reported against tobacco mosaic virus infection of tobacco plants (Yun et al. 2000). Nagaraj et al. (2001) reported also antimalarial properties of peptaibols.

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 of their secondary metabolites have also been described (Rippa et al. 2010, Shi et al. 2016). Peptaibols can also induce plant defence reactions through salicylate signal pathway, which may lead to systemic acquired resistance (Engelberth et al. 2001, Howell 2003, Harman et al. 2004, Brunner et al. 2005). 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 of *Arabidopsis* (Shi et al. 2016). Alamethicin (ALM) was shown to induce resistance in plants (Engelberth et al. 2000, Engelberth et al. 2001, Maischak et al. 2010, Li et al. 2016), but can also be toxic

causing lesions on *Arabidopsis* leaves (Rippa et al. 2010). However, it should also be considered that the commercially available ALM mixture (Sigma-Aldrich A4665) may also contain the trichothecene-type mycotoxin harzianum A produced by the *T. brevicompactum* strain used for ALM fermentations (Degenkolb et al. 2006a).

Peptaibols were also tested on animals, such as insect larvae, shrimps, mussels and mice (Hou et al. 1972, Ritieni et al. 1995, Sallenave et al. 1999, Landreau et al. 2002). Peptaibols produced by *T. koningii* (isolated from shellfish) injected into the last abdominal segment of blowfly (*Phormia terrae novae*) larvae could immobilize them (Landreau et al. 2002). ALM induced changes in the microenvironment of rat lung particulate guanylate cyclase, which resulted in an increased activity (Lad and White 1979). Trichotoxin A proved to be much less toxic to mice by oral administration, than intraperitoneal injection (Hou et al. 1972). Mice fed with 600 mg kg⁻¹ toxin could be maintained even for 4 months under standard conditions with no discernible effects, which was remarkably higher than the lethal intraperitoneal 4.36 mg kg⁻¹ dose. The lack of oral toxicity can be explained either with detoxification by the digestive system or the failure of the toxin to be transported across the intestinal wall (Hou et al. 1972). The neuroleptic activity of peptaibols was also investigated (Berg et al. 2003). Peptaibols could damage the cell membrane of mammalian cell cultures, such as boar spermatozoa and human lung epithelial carcinoma cells (Peltola et al. 2004). Antitumor activity of peptaibols of *Culcinomyces clavisporus* was also studied by He et al. (2006). Certain peptaibols showed effect on membrane-bound enzymes, activation of guanylate cyclases or inhibition of adenylate cyclases (Lad and White 1979, Sulakhe and Höehn 1984, Szekeres et al. 2005). Altogether, majority of their bioactivity is the consequence of their pore-forming ability in the cell membrane which results in leakage and cell death.

3.2.5 ALM, the most studied peptaibol

The most thoroughly studied peptaibol, ALM (Nagaraj and Balaram 1981, Mathew and Balaram 1983, Mak and Webb 1995, Futaki and Asami 2007, Woolley 2007, Kredics et al. 2013, Forbrig et al. 2018, Tyagi et al. 2019a) was reported first in 1967 and identified from the fermentation broth of '*T. viride*' NRRL 3199 (Meyer and Reusser 1967). This strain was later reidentified as *T. arundinaceum*, a species belonging to clade Brevicompactum (Degenkolb et al. 2008b). *T. brevicompactum*, *T. protrudens* and *T. turrialbense* – all belonging to clade Brevicompactum – are also capable of producing ALM compounds (Degenkolb et al. 2006a, Degenkolb and Brückner 2008). The first attempt to identify the primary structure of ALM resulted in a cyclic compound (Payne et al. 1970). Later, the

structure of a linear peptide was confirmed by using nuclear magnetic resonance (NMR) spectroscopy, which also detected the acetylated N-terminus and the C-terminal Pheol, although incorrectly as a sidechain of Gln18 (Martin and Williams 1976). Based on retention factor values in thin layer chromatography, the group of ALMs is composed of two major subgroups, the acidic and the neutral group distinguished by Glu/Gln18 exchange and named ALM F30 (85%) and ALM F50 (12%), respectively, and an additional minor component (ALM F20) (Kirschbaum et al. 2003, Degenkolb et al. 2007). *T. arundinaceum* produces both ALM F30 and F50 compounds depending on the media used for fermentation. The main difference between them is the amino acid exchange of the acidic Glu18 and the neutral Gln18 residues, respectively. Moreover, some further groups of ALM (ALM-F40, ALM-F60 and ALM-F70) were also recognised by thin layer chromatography in low concentrations (Melling and McMullen 1975). ALMs consist of 20 amino acids with the characteristics of long peptaibols or SF1 peptaibols. ALM compounds are available from several companies. The original material of ALM F30 (U-22,324) was released by Upjohn Company and contains equal proportions of ALM F30/3 and ALM F30/7 (Meyer and Reusser 1967). ALM from Sigma-Aldrich (A4665) contains a mixture of 4 compounds of ALM F50. The product of Fluka (product No. 05125) contains 75% of ALM F50/5 and further minor components of ALM.

4. AIMS

Along with the increasing number of described *Trichoderma* species, the chances to find novel, yet undescribed peptaibol compounds or even new peptaibol families are promising. Furthermore, peptaibols are bioactive compounds, they can play role in interactions with phytopathogens or during the infection of mushroom crops or humans, therefore the changes in the peptaibol profiles and their bioactivities are important to study. During this work we intended to focus on the following topics:

- Optimization of the HPLC-MS to identify peptaibol compounds
- Investigation of the peptaibol production of *Trichoderma* species with potential biocontrol abilities
- Investigation of the peptaibol production of *Trichoderma* species causing green mould disease of cultivated mushrooms
- Comparative investigation of the peptaibol profiles of species from the clinically relevant clade Longibrachiatum
- Examination of the bioactivity of peptaibols on bacteria, fungi, plants and mammalian cells

5. MATERIALS AND METHODS

5.1 Strains and culture conditions

All strains used in this study were obtained from the Szeged Microbiological Collection (SZMC, http://www.wfcc.info/ccinfo/collection/by_id/987, Szeged, Hungary). Those *Trichoderma* species were selected from the literature, which were not investigated for their peptaibol production before (Table 1). Two strains belonging to clade Viride, *T. gamsii* SZMC 1656 and *T. koningiopsis* SZMC 12500, were identified by Nucleotide BLAST analysis (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) of a fragment of the *tef1* gene amplified according to Castagnoli et al. (2018a), and proved to be very closely related to *T. gamsii* S582 (GenBank accession number: KJ665495) and *T. koningiopsis* UNISS 17b-36a (GenBank accession number: EF488124), respectively. Two strains known to cause green mould infections of cultivated mushrooms were also included in our studies, strain *T. aggressivum* f. *europaeum* CBS 433.95 (SZMC 1811) and strain *T. pleuroti* TPhu1 (SZMC 12454). Nevertheless, 22 strains from 17 *Trichoderma* species belonging to clade Longibrachiatum were also obtained from the TU Collection of Industrially Important Microorganisms (TUCIM, www.vt.tuwien.ac.at/tucim/, Vienna, Austria) and from the SZMC for the investigation of their peptaibol production (Table 1). All the selected strains were maintained on yeast extract – glucose agar medium (5 g l⁻¹ glucose, 1 g l⁻¹ yeast extract, 5 g l⁻¹ KH₂PO₄, 20 g l⁻¹ agar in distilled water). For peptaibol production, the strains were inoculated on malt extract agar (MEA) medium (30 g l⁻¹ malt extract, 3 g l⁻¹ soy peptone, 15 g l⁻¹ agar in distilled water) and incubated for 7 days at 25 °C. The strains used for confrontation and toxicity tests, *A. bisporus* SZMC 23395, *P. ostreatus* SZMC 23392, *Candida boidinii* SZMC 0673, *Kluyveromyces lactis* SZMC 0683, *Saccharomyces cerevisiae* SZMC 0425, *Schizosaccharomyces pombe* SZMC 0142, *A. alternata* SZMC 16085, *Aspergillus fumigatus* SZMC 23245, *F. falciforme* SZMC 11407, *F. keratoplasticum* SZMC 11414, *F. solani* species complex SZMC 11467, *R. solani* SZMC 6252J, *Phoma cucurbitacearum* SZMC 16088 were maintained on MEA supplemented with yeast extract (2.5 g l⁻¹ yeast extract, 5 g l⁻¹ malt extract, 10 g l⁻¹ glucose, 20 g l⁻¹ agar in distilled water, pH 6.5). *Escherichia coli* SZMC 0582, *Micrococcus luteus* SZMC 0264, *Pseudomonas aeruginosa* SZMC 0568 and *Staphylococcus aureus* SZMC 0579 were maintained on Luria-Bertani (LB) agar medium (10 g l⁻¹ tryptone, 5 g l⁻¹ yeast extract, 10 g l⁻¹ NaCl and 20 g l⁻¹ agar in distilled water). To increase peptaibol production, *Trichoderma* strains were inoculated on large (40 × 40 cm) MEA plates and incubated for 7 days at 25 °C.

TABLE 1 | *Trichoderma* strains involved in this study

SZMC identifier	Other identifier	Clade / Subclade	Species	Origin	Reference
1811	CBS 433.95	Harzianum/Virens	<i>T. aggressivum</i> f. <i>europaeum</i>	<i>A. bisporus</i> compost, Northern Ireland, UK, (1991)	Samuels et al. 2002
12454	TPhu1	Harzianum/Virens	<i>T. pleuroti</i>	wheat straw of <i>P. ostreatus</i> cultivation, Kecskemét, Hungary	Marik et al. 2017
12500	TWA3a/7	Viride / Koningii	<i>T. koningiopsis</i>	<i>A. bitorquis</i> natural substrate, Szeged, Hungary	Castagnoli et al. 2018a
1656	R19	Viride / Viridescens	<i>T. gamsii</i>	Wheat rhizosphere, Rúzs, Hungary	Castagnoli et al. 2018a
1773	CECT 2412	Longibrachiatum / Orientale*	<i>T. bissettii</i> **	Mushroom compost, Wales	Druzhinina et al. 2008
1775	CECT 2937	Longibrachiatum / Orientale*	<i>T. longibrachiatum</i>	Antarctica	Kuhls et al. 1997
1776	CECT 20105	Longibrachiatum / Orientale*	<i>T. bissettii</i> **	Biocontrol strain, Egypt	Antal et al. 2005
12546	UAMH 7956	Longibrachiatum / Orientale*	<i>T. bissettii</i> **	Bone marrow transplant recipient, lung tissue	Richter et al. 1999, Druzhinina et al. 2008
12556	UAMH 9573	Longibrachiatum / Orientale*	<i>T. orientale</i>	Peritoneal catheter tip, Canada	Kredics et al. 2003
22602	TUCIM 1817	Longibrachiatum / Orientale*	<i>T. aethiopicum</i>	<i>Coffea arabica</i> rhizosphere, Jimma, Ethiopia	Druzhinina et al. 2008
22603	TUCIM 3421	Longibrachiatum / Orientale*	<i>T. pinnatum</i>	Sri Lanka	Samuels et al. 2012b
22614	TUCIM 917, QM6a	Parareesei / Reesei*	<i>T. reesei</i>	canvas of US army, Solomon Islands	Reese et al. 1950
22616	QM9414	Parareesei / Reesei*	<i>T. reesei</i>	Mutant of QM9123 (which is mutant of QM6a)	Kuhls et al. 1996
22617	QM9414 G2 Δ lae1	Parareesei / Reesei*	<i>T. reesei</i>	lae1 null mutant (Δ lae1) of <i>T. reesei</i> QM9414	Seiboth et al. 2012
22615	TUCIM 661	Parareesei / Reesei*	<i>T. parareesei</i>	Subtropical rain forest, Iguazu Falls, Argentina	Atanasova et al. 2010
22606	TUCIM 1267	Saturnisporum*	<i>T. saturnisporum</i>	Italy	Samuels et al. 2012b
22607	TUCIM 132	Konilangbra / Sinensis*	<i>T. konilangbra</i>	Uganda	Samuels et al. 1998
22608	TUCIM 3350	Konilangbra / Sinensis*	<i>T. flagellatum</i>	<i>Coffea arabica</i> rhizosphere, Ethiopia	Belayneh Mulaw et al. 2010
22609	TUCIM 527	Konilangbra / Sinensis*	<i>T. sinensis</i>	Taiwan	Bissett et al. 2003
22618	SJ40	Citrinoviride / Pseudokoningii*	<i>T. citrinoviride</i>	Office bookshelf, settled dust, Espoo, Finland	Castagnoli et al. 2018a
22613	TUCIM 1277	Citrinoviride / Pseudokoningii*	<i>T. pseudokoningii</i>	the bark of <i>Beilschmiedia tawa</i>	Samuels et al. 1998
22612	TUCIM 4158	Novae-zelandiae / Saturnisporopsis*	<i>T. novae-zelandiae</i>	native <i>Notophagus</i> forest, New Zealand	Samuels et al. 1998
22604	TUCIM 2057	lone lineage	<i>T. ghanense</i>	<i>Agaricus</i> compost, Hungary	Hatvani et al. 2007
22605	TUCIM 2883	lone lineage	<i>T. capillare</i>	Wall of a mushroom growing cellar, Hungary	Hatvani et al. 2007
22610	TUCIM 1291	lone lineage	<i>T. andinense</i>	Venezuela, high elevation	Samuels et al. 1998
22611	TUCIM 254	lone lineage	<i>T. effusum</i>	Soil isolation, Himalaya, India	Bissett et al. 2003

* Subclades were defined based on Samuels et al. (2012b). ** Proposed as *T. longibrachiatum* f. *bissettii* (Hatvani et al. 2019)

5.2 Peptaibol extraction

After 7 days of incubation on MEA, peptaibols were extracted from the cultures with chloroform (3×5 ml per Petri dish) for identification of the compounds produced. Crude extracts were evaporated to dryness on a rotary evaporator (IKA RV 10, IKA Works, USA), then dissolved in 1.5 ml methanol (analytical reagent grade, VWR International, Hungary) and centrifuged ($10000 \times g$ for 10 min) in a Biofuge Primo (Heraeus, Germany) to remove the insoluble particles from the solutions. Finally, the crude extracts were evaporated under nitrogen blow down, re-suspended in methanol (200 μ l) and stored at -20 °C. Peptaibols were directly extracted also from the surface of *in vitro* confrontation plates ($n = 3$, see below) with chloroform (3×5 ml). Extracts were evaporated and re-dissolved in methanol (1 ml) in order to pellet the insoluble residues. Finally, the supernatants were collected, evaporated and dissolved in 200 μ l methanol (HPLC grade, VWR International, Hungary).

From the large plates, after 7 days of incubation of the strains on MEA medium, mycelium of the cultures was harvested and collected. Then, 300 ml chloroform/methanol (analytical reagent grade, VWR International, Hungary) 2/1 (v/v) solution was added, and the mixture was shaken for 2 h (100 rpm). The lower phase was collected and evaporated to dryness (IKA RV 10, IKA Works, Wilmington, NC, USA). The extraction steps were repeated three times in total. After the extraction, the dry residue was dissolved in methanol (HPLC grade, VWR International, Hungary), centrifuged ($10000 \times g$ for 10 min) in a Biofuge Primo centrifuge (Heraeus, Hanau, Germany) and stored at -20 °C. The samples were diluted $100 \times$ for HPLC-MS analysis and set to 100 mg ml^{-1} for inhibition tests.

5.3 Peptaibol purification

The extracted crude samples were purified on a Flash chromatograph (CombiFlash EZ Prep UV-VIS Teledyne Isco). The peptaibol compounds could not be separated from each other, but this method resulted in a pure mixture containing only the peptaibol compounds with the same rate of the compounds as in the original crude extracts. The cartridge (CombiFlash EZ Prep) was filled with 60 cm^3 silica (30–40 μm), and 1.5 g of crude peptaibol 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 (HPLC grade, VWR International, Hungary), respectively (gradient solvent B: 0% – 0 min, 0% – 5 min, 100% – 15 min, 100% – 18 min). Fractions were automatically collected into collector tubes (18 $\times$ 180 mm, 30 ml) based on the slope of the UV signal. Fractions were evaporated,

dissolved in methanol (100 mg ml⁻¹, HPLC-MS grade, VWR International, Hungary) 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, a calibration curve was prepared from the integrated peaks area of all y₇-ion fragments of the ALM mixture (Sigma-Aldrich A-4665, Hungary), which was dissolved in methanol (HPLC-MS grade, VWR International, Hungary) in different concentrations. After this, the summarized integrated peaks area of all the appearing y₇-ion fragments of the purified samples were compared to this calibration curve.

5.4 Analytical procedures and data analysis

HPLC parameters after the optimization of the system: Agilent 1100 system (Agilent Technologies, Palo Alto, CA, USA) controlled by a ChemStation software (A09.03, Agilent Technologies, Palo Alto, CA, USA) and equipped with a binary pump, a vacuum degasser and a μ Well-plate autosampler as well as a Jones Model 7990 Space column heater (Jones Chromatography Ltd., Lakewood, CO, USA), Gemini NX-C18 HPLC column (150 mm $\times$ 2.0 mm, 3 μ m, Phenomenex Inc., Torrance, CA, USA), guard column with the same phase (C18), Solvent A: H₂O (Gradient A10) with 0.05% (v/v) trifluoroacetic acid, Solvent B: acetonitrile/methanol (HPLC-MS grade, VWR International, Hungary) 1/1 (v/v) with 0.05% (v/v) trifluoroacetic acid (HPLC-MS grade, VWR International, Hungary), flow rate: 0.2 ml min⁻¹, gradient program for Solvent B: 65% – 0 min, 65% – 5 min, 80% – 45 min, 100% – 70 min, 100% – 75 min, 65% – 76 min, 65% – 81 min, column temperature: 40 °C, injection volume: 5 μ l. Electrospray ionization (ESI) - ion trap (IT) - MS parameters: Varian 500 MS (Agilent Technologies, Palo Alto, CA, USA) with ESI source in positive mode at normal scan speed controlled by the 500-MS Mass Spec module driver of the Varian Workstation software (6.6/SP1, Varian Inc., Palo Alto, CA, USA), ESI parameters: spray chamber temperature 50 °C, drying gas (N₂) pressure 30 psi, drying gas temperature 350 °C, nebulizer gas (N₂) pressure 50 psi, needle voltage 5704 V, spray shield voltage 600 V, General parameters: maximum scan times 2.78, 2 μ Scans averaged, data rate 0.36 Hz, multiplier offset 0. Ionization control parameters: target total ion chromatogram (TIC) 100%, max ion time 250 000 μ s, Scan parameters: capillary voltage 66 V, RF loading 147%, MS scan parameters: Low mass m/z 200, High mass m/z 2000, MS² measurements of selected y₆ and y₇ fragments were carried out with the following excitation storage level (m/z)/excitation amplitude (V) conditions: m/z 612.3 (170.1/2.42), m/z 626.3 (173.4/2.47) m/z 660.4 (181.5/2.60), m/z 674.4 (184.9/2.65), m/z 754.5 (204.5/2.95), m/z 755.5 (204.8/2.96) m/z 768.5 (208.0/3.00), m/z

769.5 (208.3/3.00), m/z of 774.4 (209.4/3.02), m/z of 775.4 (209.7/3.03), m/z of 788.4 (212.9/3.08), and m/z of 789.4 (213.2/3.08).

From the year 2019, the initial Varian 500MS data were further confirmed by a newly acquired HPLC-Orbitrap-MS: Dionex UltiMate 3000 system (Thermo Scientific, CA, USA), which was controlled by the Xcalibur 4.2 software (Thermo Scientific, CA, USA) and equipped with a quaternary pump, a vacuum degasser, an autosampler and a column heater. Gemini NX-C18 HPLC column (50 × 2.0 mm, 3 μm, Phenomenex Inc., Torrance, CA, USA) was used for the separation with a guard column containing the same phase (C18). Solvent A was H₂O/methanol/acetonitrile (HPLC-MS grade, VWR International, Hungary) 8/1/1 with 10 mM ammonium-acetate (HPLC-MS grade, VWR International, Hungary) and 0.1% (v/v) acetic acid (HPLC-MS grade, VWR International, Hungary), while solvent B was acetonitrile/methanol 1/1 (v/v) with 10 mM ammonium-acetate and 0.1% (v/v) acetic acid. The flow rate was set to 0.2 ml min⁻¹ and the gradient program for Solvent B was 10% – 0 min, 10% – 2 min, 78% – 3 min, 89% – 16 min, 95% – 16.5 min, 95% – 19.5 min, 10% – 20 min, 10% – 24 min. The column temperature was kept at 30 °C and the injection volume was 5 μl. An Orbitrap-MS, Thermo Scientific Q Exactive Plus (Thermo Scientific, CA, USA) with heated ESI source in positivemode controlled by Xcalibur 4.2 software (Thermo Scientific, CA, USA) was used for the MS measurements. Heated ESI parameters: spray voltage 3 kV, sheath gas flow rate 30 arbitrary units, aux gas flow rate 15 arbitrary units, capillary temperature 350 °C, aux gas heater 250 °C. The acquisition mode was Full-MSddMS². Full-MS parameters: resolution 70,000 at m/z 200, AGC target 3e6, maximum injection time 100 ms, scan range 350–2200 m/z . The ddMS² parameters: fixed first scan at m/z 80, resolution 17500 at m/z 200, AGC target 1e6, maximum injection time 50 ms, isolation window 1 m/z , collision energy 30 NCE. The minimum AGC target for ddMS² triggering was 1e5.

Based on a calibration with ALM standard (Sigma-Aldrich A-4665, Hungary), the peptaibol contents of the crude extracts were also calculated based on the method explained in paragraph 5.3. To avoid false results due to the eventual harzianum A contamination of the ALM standard solution, its detection was carried out based on a previous article (Nielsen et al. 2005). The flow rate was set to 0.2 ml min⁻¹ on a Phenomenex Gemini 50 × 2 mm, 3 μm HPLC column, which was attached to a guard column containing the same phase (C18). The column heater was set to 30 °C and the injection volume was 5 μl. An Orbitrap-MS detector was attached to the HPLC system and the parameters were set according to the Orbitrap MS

parameters described above. The measurements ran in negative ionization mode, the spray voltage was set to -3 kV.

Peptaibol profiles of individual strains were analyzed using cluster analysis in the ClustVis web tool (Metsalu and Vilo 2015), and a heat map was constructed using the complete linkage and Euclidian distance settings applied to the columns (strains).

5.5 Nomenclature of the identified peptaibols

As no amino acid analysis was carried out for the determination of the Val/Iva and Leu/Ile isomers, the Vxx/Lxx nomenclature was used for the peptaibol sequences.

The novelty of the sequences was validated according to the “Comprehensive Peptaibiotics Database” (Stoppacher et al. 2013). The former online resource (Neumann et al. 2015) is unavailable since the autumn of 2017, therefore the latest offline version of the “Peptaibiotics Database”, which was kindly provided by Prof. Rainer Schuhmacher, and PubMed searches of publications since 2017 were also performed with the keyword “peptaibol.”

The newly identified peptaibol compounds obtained from *T. gamsii* SZMC 1656 were named according to their elution order (I, II, ... n), attached to the prefix ‘Pept’. In the case of compounds eluting close to each other and differing in their characteristic ion fragments (b_{12} and y_7), Latin letters (a and b) are following the Roman numerals. The sequences obtained from *T. koningiopsis* SZMC 12500 were named koningiopsins and numbered with Roman numerals (I, II, ... n) based on the elution order, and the different variants are distinguished by Latin letters (a and b) as mentioned before (Marik et al. 2018).

The hypomurocin B-like compounds were named according to the following three descriptive characters of the peptaibols: molar mass, the y-ion part after the fragmentation of the most unstable Aib-Pro bond (a or b) and the elution order of the compounds (1, 2, ... n), attached to ‘Pept’. Tripleurins were named with Roman numerals according to their elution order. Where the difference between compounds was detected only in the retention time, suggesting that the compounds differ in the isoforms of amino acids at certain positions with Vxx or Lxx, Latin letters (a, b, c) were added after the Roman numerals (Marik et al. 2017).

All the peptaibol compounds produced by the species of clade Longibrachiatum were named according to the group to which they belong to (A or B) and the elution order of the compounds on the HPLC-Varian MS system (I, II, ..., n), appended to “Pept.” Compounds with the same retention time but different sequences were considered as variants and named

with small latin letters (a, b, ..., n, in decreasing order of amount the variants were produced). Group C peptaibols were named as brevicelsins and numbered according to their elution order (Marik et al. 2019).

5.6 Testing the effect of peptaibol extracts on bacteria and yeasts

For activity tests with bacteria, LB agar medium was prepared. The plates contained 20 ml media LB agar medium as base. Bacteria (10^5 cells per plates) from fresh cultures were mixed into 5 ml covering agar (LB with 10 g l^{-1} agar) at 40°C and poured onto the surface of the basal medium. Fifty μl amounts of the methanolic extracts of the peptaibol extracts were filled into holes (5 mm in diameter) bored into the agar plates. The diameters of the inhibition zones were recorded after 2 days of incubation at 37°C . The same protocol was used for the inhibition assays with yeasts but using MEA medium supplemented with yeast extract. A 10-step 2-fold dilution series of the 100 mg ml^{-1} crude peptaibol extracts - which were also examined for their peptaibol composition - were tested, with methanol as control. The yeast cultures were incubated at 25°C . Photographs were taken with a Nikon Coolpix S2600 camera after 2 days of incubation. Three parallel experiments were set up to measure the inhibition zones.

5.7 Testing the effect of peptaibol extracts on filamentous fungi

MEA medium supplemented with yeast extract was used for the inhibition tests with filamentous fungi. Agar plugs cut from the colonies of the fungal strains were placed in the centre of the plates containing MEA medium supplemented with yeast extract and holes (5 mm in diameter) were bored around in 3 cm distance from the centre of the plate. A 10-step 2-fold dilution series of the 100 mg ml^{-1} crude peptaibol extracts – which were also examined for their peptaibol composition – were tested, using methanol as control. The cultures were incubated at 25°C . Photographs were taken by a Nikon Coolpix S2600 camera at two stages, (1) when the edge of the culture reached the control hole and (2) when it reached the edge of the Petri-dish. Three parallel experiments were set up to measure the inhibition zones.

5.8 *In vitro* confrontation assays

Agar plugs 5 mm in diameter, cut from the edge of *A. bisporus* and *P. ostreatus* colonies freshly growing on MEA were inoculated 1.5 cm apart from the centre of MEA plates (90 mm in diameter). When the colony reached the central point of the plate (after 3 and 8 days for *P. ostreatus* and *A. bisporus*, respectively), the *Trichoderma* strains were inoculated to the opposite side at 1.5 cm distance from the center of the plates. Seven days later, photographs

were taken from the plates by a Nikon Coolpix S2600 camera to calculate aggressivity index (AI) values. Calculation of AI is principally based on the image analysis-based biocontrol index (BCI) determination method of Szekeres et al. (2006) optimized to mushroom green mould strains of *Trichoderma* by Körmöczy et al. (2009). The AI value is given in percentage and calculated from the area covered by the *Trichoderma* strain, which is divided by the total area covered by *Trichoderma* and the confronted mushroom. Each experiment was set in three parallel tests. Self-confrontations of the examined *Trichoderma* strains were used as controls.

5.9 Bioassays for peptaibol toxicity on mushroom mycelia

Agar plugs (5 mm in diameter), cut from the edge of *A. bisporus* or *P. ostreatus* colonies freshly grown on MEA were inoculated to the center of MEA plates and incubated at 25 °C. After the cultures reached the colony diameter of 30 mm, holes 5 mm in diameter were bored into the media at 1 cm distance from the edge of the mushroom colony and immediately filled with the crude extracts (40 µl). Three parallel tests were set of each experiment and *A. bisporus* or *P. ostreatus* colonies without treatment were inoculated as controls. The inhibitory effects were observed and recorded after 3 days of incubation at 25 °C.

5.10 Bioassays for peptaibol toxicity on filamentous fungi

For inhibition tests with filamentous fungi, MEA medium completed with yeast extract was used at 25 °C, following the method described above. 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 ALM (Sigma-Aldrich A-4665, Hungary) and nystatin (Nystatin 2-hydrate BioChemica, AppliChem A3811,0025, Germany) as reference compounds. All solutions were prepared in two-fold 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 using 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.

5.11 Bioassays for peptaibol effects on plants

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 1 M NaOH) in Petri

dishes (90 × 17 mm) five seeds per Petri dish in a single row. 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* seedlings were placed in a greenhouse with a photoperiod of 12 h of light and 12 h of darkness, a light intensity of 300 $\mu\text{mol m}^{-2} \text{s}^{-2}$ and a temperature of 25 ± 1 °C. On 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 and methanol solvent as the control. Plants without any treatment were also used as absolute control. The length 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 extracted by acetone (analytical reagent grade, VWR International, Hungary) and quantified based on their OD (at 470 nm, 534 nm, 643 nm and 661 nm wavelengths) as described by Lichtenthaler (1987).

5.12 Peptaibol bioactivity assays against mammalian cells

5.12.1 Mammalian somatic cell toxicity assays

The porcine kidney tubular epithelial cell line (PK-15) was pre-cultivated in BioWhittaker RPMI 1640 medium supplemented with L-glutamine at 37 °C in an atmosphere of 95% air and 5% CO₂ in a cell culture cabinet (Heracell 150i, Thermo Fisher Scientific, Vantaa, Finland). For the bioassays, separated and detached cells were used in 96-well microtiter plates based on the method of Castagnoli et al. (2018a) in three parallel experiments. Initially, the wells of the first columns were filled with 180 μl , while the rest with 100 μl medium. Into the wells in the first column, 20 μl of the samples were added, mixed and 100 μl amounts were transferred to the next columns. Next, 100 μl of the media containing mammalian cells were added to each well except for the control ones, which contained only the medium without cells. After 2 days of incubation at 37 °C, 10 μl resazurin (Sigma Chemical Co., St. Louis, MO, USA) (400 $\mu\text{g ml}^{-1}$ in physiological saline) was added to each well and incubated for 2 h under the same conditions. After incubation, the plates were investigated by a microtiter plate reader (Fluoroskan Ascent, Thermo Scientific, Vantaa, Finland) at the excitation wavelength of 544 nm and the emission wavelength of 590 nm. The endpoints of toxicity were specified by the differences from the OD of the control holes that means the living cells were less than 50%. Typically, peptaibols have very sharp endpoints between two

dilution steps. After 2 more days of incubation, the NADH^+ production of the living cells causes a colour changing to red for visible examination. Purified ophiobolins were used for references for our peptaibol inhibitions in the bioassays (Bencsik et al. 2014).

5.12.2 Sperm motility inhibition assay

The sperm motility bioassays were performed by using the method of Castagnoli et al. (2018b) in three parallel tests of each experiment. The boar semen was obtained from Figen Oy (Finland) and extended in MR-A (Kubus, S.A., Madrid, Spain) containing 27×10^6 spermatozoa ml^{-1} . Four different amounts, 10 μl , 5 μl , 2 μl and 1 μl of the samples were added to 2 ml of $100 \times$ diluted MR-A boar semen stock solution, while 20 μl methanol (analytical reagent grade, VWR International, Hungary) was added to the blank assay. Two different incubation times, 30 min and 24 h were used for the short- and long-term bioassays, respectively, at room temperature. After the incubation, optimal conditions (shaken by hand for few seconds at 37°C to provide them oxygen) were ensured for 5 min to the sperm cells as a pre-incubation step before the examination of their motility. For the detection of the living, motile sperm cells, a phase contrast microscope (Olympus CKX31 and Software CellSens standard version 11.0.06, Olympus Soft Imaging Solution GmbH, Münster, Germany, 2009–2012) was used to check the tail beating of the sperm cells proving that they are alive. A normal living cell seems as if it had two tails by looking at the pictures of the microscope and the ratio of these “two-tailed” cells were compared to the blank samples in order to define the effect of the peptaibol samples.

5.12.3 Membrane integrity disruption assay

Disruption of the cell membrane integrity of motile sperm cells was assayed by staining with propidium iodide (PI) (Molecular Probes, Eugene, OR, USA) in sperm cells induced by shaking to swim at 37°C as described by Bencsik et al. (2014) with small modifications. The test was performed as follows: 50 μl aliquots of Dulbecco’s phosphate buffered saline were pipetted into 96 wells of a microtiter plate. Fifty μl of the test suspension (methanol extracts of peptaibols) was added to the first column of the wells and a 2-fold serial dilution was prepared until the 11th column. The wells of the 12th column representing reagent blank was filled with 200 μl Dulbecco’s phosphate buffered saline and with methanol (analytical reagent grade, VWR International, Hungary) as solution control. One hundred and fifty μl aliquots of commercially extended boar semen (27×10^6 sperm cells ml^{-1}) were pipetted into the microtiter plate wells and pre-incubated for 2 h on an orbital shaker (Innova 5000, New Brunswick Scientific, Enfield, CT, USA) at 160 rpm and 37°C . Three parallel dilutions were

performed for each sample. After incubation, PI solution (stock solution stored as frozen 1 mg ml⁻¹ in Dulbecco's phosphate buffered saline) was diluted to a concentration of 10 µg ml⁻¹. One hundred µl of this stain mixture was added to the exposed boar semen. This suspension was incubated at 37 °C in darkness for 15 min and examined by a Fluoroskan Ascent microplate reader (Thermo Scientific, Vantaa, Finland) at excitation and emission wavelengths of 544 nm and 600 nm, respectively. During the observation of plasma membrane damage by staining with PI, the positive control was frozen-thawed semen (for mortality of 100%) representing the maximal fluorescence emitted by the cells permeable to PI. Dulbecco's phosphate buffered saline was used as the background control (blank). The frozen semen was represented by the wells in the last horizontal row. Loss of viability in the sample was calculated as described by Alm et al. (2001). The frozen sample represented 100% loss of viability. The assay was calibrated with triclosan (Sigma Chemical Co., St. Louis, MO, USA), in 5 parallel tests, the EC₅₀ was 2 µg ml⁻¹ (SD ± 0.6). The cell membrane permeability assay on static sperm cells at room temperature was performed by viability staining with PI and calcein-acetoxymethyl (calcein-AM, Molecular Probes, Eugene, OR, USA) as described by Hoornstra et al. (2003).

5.13 Statistical analysis

All the statistical analyses were performed using GraphPad Prism Version 6.00 for Windows (GraphPad Software, San Diego, CA, USA, 2007, www.graphpad.com). The significant differences between the peptaibol production of the strains in *in vitro* confrontation assays and their respective controls were determined by unpaired t-analysis using parametric test. Statistical analyses of the peptaibols' effect on *Arabidopsis* plants 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.

6. RESULTS AND DISCUSSION

6.1 Optimization of ESI-IT-MS system for the identification of peptaibol compounds

At first, the ESI-IT-MS system was set for peptaibol identification using ALM standard (Sigma-Aldrich A-4665, Hungary) to examine whether the compounds produced by *Trichoderma* strains can be identified using our HPLC-ESI-IT-MS system. In order to optimize the system, the MS parameters including RF loading, capillary- and needle voltages were tuned in both positive and negative modes by continuous infusion ($5 \mu\text{l min}^{-1}$) of an ALM standard solution ($100 \text{ ng } \mu\text{l}^{-1}$) with the built-in syringe pump of the instrument. Based on the reported data, the purchased standard mainly represents the neutral ALM F50 peptides (Kirschbaum et al. 2003), thus during the optimization, the following protonated/deprotonated molecular ions ($[\text{M}+\text{H}]^+ / [\text{M}-\text{H}]^-$) of microheterogeneity mixture of ALM components were monitored in positive/negative mode: m/z 1963/1961 for ALM F50-5, m/z 1977/1975 for ALM F50-6a/6b/7 and m/z 1991/1989 for ALM F50-8b/8c. After the setup of ion optic parameters, the optimal ion source conditions such as nebulizer gas- and drying gas pressures and source temperatures were estimated via flow injection analysis in the mobile phase constitution 1/1 (v/v) of eluent A/B.

For further investigations, ALM compounds were separated by HPLC on an analytical octadecyl silica (ODS) column equivalent to the stationary phase used by Krause et al. (2006a) as the general method for the peptaibol analysis, but other than applied by Kirschbaum et al. (2003) specifically for the ALM separation (Figure 1).

Figure 1 | HPLC-UV (A) and HPLC-ESI-MS (B) chromatogram of ALM components

MS measurements of peptaibols usually generate molecular ions and more-or-less complete series of characteristic fragment ions (Kirschbaum et al. 2003, Krause et al. 2006a). However, in our case, the molecular ions showed much lower abundance due to their disintegration than the sodium adduct form in positive mode with exception of ALM-F50-8b falling its correspondent m/z value out of the examined mass range (Figure 2 and 3), which could make the identification of the unknown peptaibols difficult in our further screening studies. With the ESI negative mode ionization the $[M-H]^-$ masses were visible only with moderate intensity.

Figure 2 | HPLC-ESI-MS spectra of ALM-F50-5 (A), ALM-F50-6a (B), ALM-F50-7 (C) and ALM-F50-8b (D)

Figure 3 | Abundances of the characteristic fragments of ALM components appearing on the HPLC-ESI-MS spectra

The most abundant ions among the fragment ions were the y_7 and b_{13} fragments resulting from cleavage of the extremely unstable Aib–Pro bond of the 20 residue peptides at position 6–7 from the carboxyl terminus in positive mode. Thus, the summation of the proper y and b ions could serve the mass of hydrogen adduct of the molecular ion. It is interesting that the double-charged adduct ions showed high intensity in the used MS instrument such as $(M+2Na)^{2+}$ and $(M+Na+H)^{2+}$ (Figure 2 and 3), which was not observed earlier. The signals of the b_{10} fragments due to the fragmentation of the Aib–Gly bond were relatively high, although their y_{10} counter-fragments were not detected. At the lower m/z values with lower intensity, but detectable level, the series of shorter b fragments were recorded including b_{10} , b_8 , b_4 and b_3 ions (Figure 2 and 3). From the mass differences (Δm) of fragment ions, the presence of the marker amino acid Aib, characterised by m/z 85.1 Da (Krause et al. 2006a) could not be deduced. The fragment ions and the acquired corresponding masses are shown in Table 2.

TABLE 2 | Identifications of characteristic ions (m/z) of ALM components acquired in positive mode

	ALM components			
	5	6a	7	8b
Rt (min)	43.7	46.2	48.5	51.1
[M+H]⁺	1963	1977	1977	1991
[M+Na]⁺	1985	1999	1999	Out of range
[M+2Na]²⁺	1004	1011	1011	1018
[M+Na+H]²⁺	993	1000	1000	1007
[M+2H]²⁺	982	989	989	996
[M-H₂O]⁺	1945	1959	1959	1973
b₁₃	1189	1189	1203	1203
b₁₀	934	934	948	948
b₈	750	750	764	764
b₄	381	381	381	381
b₃	310	310	310	310
y₇	774	788	774	788

The resulting peaks were in accordance with the ALM components reported previously (Kirschbaum et al. 2003), eluted with proper resolutions (Figure 1). Furthermore, based on the mass spectra acquired from certain peaks, the different components could be identified, some of which were denoted earlier only as mixture of isomers including ALM-F50-4b/5, ALM-F50-6a/6b and ALM-F50-8b/8c (Kirschbaum et al. 2003). In the case of ALM-F50-4b/5 eluted at 43.7 min, the characteristic ions of the 4b component were not detectable due to the single charged and double charged sodium adducts at the m/z 1971 and 998, respectively (Figure 2A). The peak of ALM-F50-6a/6b at 46.2 min contains also only the 6a component, because of the absence of m/z 930 (b_{10} fragment) of ALM-F50-6b (Figure 2B). Similarly, in the case of ALM-F50-8b/8c at 51.1 min, only the ALM-F50-8b was involved due to the lack of y_7 (m/z 744) and b_{10} (m/z 962) fragments originated from the fragmentation of ALM-F50-

8c (Figure 2D), while the ALM-F50-8c, eluted at 48.5 min showing the highest peak area (Figure 2C). There was a peak with a remarkable area on the UV chromatogram at approximately 35 min, but it was not active mass spectrometrically.

The results achieved here could contribute to the identification of other unknown peptaibol molecules isolated from different *Trichoderma* species based on the gathered data about the MS fragmentation properties.

6.2 Identification of the peptaibols produced by *Trichoderma* species

The method applied for peptaibol identification described in the Materials and Methods section 5.4 was based on the strategy developed during the investigation of ALM, which was described above. The sequences were determined based on the observation of the characteristic ions of the compounds ($[M+Na]^+$, $[M+2Na]^{2+}$, b_{12} and y_7 ion) and the retention time. Throughout the investigation of the species causing green mould disease and the species from clade Viride, the $[M+Na]^+$ ions were clearly detected. All through the investigation of the species belonging to clade Longibraciatum, the single-charged pseudomolecular ions, such as $[M+Na]^+$ or $[M+H]^+$, were scarcely detectable in the spectra, whereas double-charged ($[M+2Na]^{2+}$) ions were present and could be used for identification. All full scan MS spectra contained the series of the fragment ions from the N-terminal part (shown in Supplementary Figures 1–5), except for the ion fragment where the Gln–Aib bond is present in the compounds (Krause et al. 2006a). This is shown on the spectra as the characteristic mass difference $\Delta m = 213$ Da, due to the stability of this bond under the fragmentation conditions of ESI-MS (Jaworski et al. 1999, Kirschbaum et al. 2003, Krause et al. 2006a). The C-terminal y_7 fragment was consistently observed and could also provide a good reference for the quantification of the peptides, where $[M+Na]^+$ ions could not be observed on the MS spectra. The first part of the sequences till the Aib–Pro bond could be identified from the full scan MS spectra, but MS² experiments were performed for the identification of residues at the C-terminus. Usually, the last four residues could be identified directly from the MS² spectra (Supplementary Figures 6–8) measured on the Varian MS system. The y_7 –AA(19–15) could not be detected with MS² during the investigations of the species from the clade Viride, therefore the amino acids in positions 14 and 15 (Vxx14–Aib15) were predicted based on the Comprehensive Peptaibotics Database showing the frequent presence of the Aib–Pro–Vxx–Aib motif in this region (Stoppacher et al. 2013, Neumann et al. 2015). The y_7 –AA(19–15) ions (Pro–AA) were also not shown on the spectra during the investigation of the species belonging to clade Longibraciatum, therefore another MS² fragmentation was performed on

an Orbitrap-MS system from these y_7 ions (Supplementary Figure 9). Thus, all the detected peaks could also be reidentified at high resolution on the HPLC-Orbitrap-MS system, except for y_7-H_2O (Supplementary Tables 1–6). Instead of $[M+Na]^+$ and $[M+2Na]^{2+}$ ions, $[M+H]^+$ could be observed on the spectra of the reidentification. As no amino acid analysis has been performed, discrimination between isobaric amino acids was not possible.

6.3 Peptaibols from *T. gamsii* and *T. koningiopsis* (clade Viride)

6.3.1 Peptaibol production of *T. gamsii* and *T. koningiopsis*

The sequences of the compounds identified from the two examined strains and listed in Table 3 and Table 4 are derived from the *de novo* MS-based sequencing. The diagnostic fragment ions of the peptaibols found in this study are shown in Supplementary Tables 7–10, presented according to Röhrich et al. (2013). The peptaibols produced by *T. gamsii* SZMC 1656 strain (Table 3, Supplementary Table 7, 9, Supplementary Figures 1, 6 and 10) proved to be completely different from the ones detected in the extract of *T. koningiopsis* SZMC 12500 (Table 4, Supplementary Table 8, 10, Supplementary Figures 2, 6 and 11). The main differences between the peptaibols of the two species could be identified in 4 positions of their sequences. In the 2nd position, peptaibols produced by *T. gamsii* SZMC 1656 contain Gly or Ala in Pept-X, -XI, and -XII, while only Ala was observed in this position in the peptaibol sequences identified from *T. koningiopsis* SZMC 12500. Another difference is at the 5th residue of the sequences, where mostly Lxx, in some cases Vxx was identified in the sequences of *T. gamsii* SZMC 1656, while the compounds of *T. koningiopsis* SZMC 12500 exhibited mostly Aib at this position. The third main difference between the sequences of the two examined species was observed at the 9th position, where mostly Aib was identified in the peptaibols produced by *T. gamsii* SZMC 1656, while those from *T. koningiopsis* SZMC 12500 mostly showed Lxx and in some cases Aib. The 18th position contains Gln in the sequences of *T. gamsii* SZMC 1656, but *T. koningiopsis* SZMC 12500 produces compounds with Glu in this position causing 2 more variants of the y_7 ion. The sequences Pept-Vb, -VIb, and -VII were matching with trikoningin KA V, though the isomeric positions of Vxx and Lxx were not identified. All other sequences proved to be new and showed similarities to the peptaibol groups of trikoningins, tricholongins, trichostrigocins, and trichorzianins (see references in Tables 3 and 4).

TABLE 3 | Sequences of the newly identified peptaibol compounds produced by *Trichoderma gamsii* SZMC 1656 and their similarity to known peptaibols available in the “Comprehensive Peptaibiotics Database”

Peptide	M	[M+Na] ⁺	[M+2Na] ²⁺	b ₁₂	y ₇	rt. (min)	R	R1	<u>R2</u>	R3	R4	<u>R5</u>	R6	R7	R8	R9	<u>R10</u>	R11	R12	R13	R14	<u>R15</u>	<u>R16</u>	R17	R18	R19	Compound identical or positionally isomeric with	Reference
Pept-Ia	1861.1	1884.1	953.55	1108.5	754.4	50.504	Ac	Aib	<i>Gly</i>	<i>Ala</i>	Aib	<u>V_{xx}</u>	Gln	Aib	Aib	Aib	Ser	Lxx	Aib	Pro	Vxx	Aib	<u>V_{xx}</u>	Gln	Gln	Lxxol	New: Trikoningin KA V: [Ile] ⁵ →[V _{xx}] ⁵ and [Ile] ¹⁶ →[V _{xx}] ¹⁶ New: Tricholongin LBII, LBIV: [Phe] ³ →[Ala] ³ and [Aib] ⁵ →[V _{xx}] ⁵ New: Tricholongin BII (LBII): [Phe] ³ →[Ala] ³ and [Aib] ⁵ →[V _{xx}] ⁵	Goulard et al. 1995 Degenkolb et al. 2006b Rebuffat et al. 1991
Pept-Ib	1876.2	1899.2	961.1	1108.5	768.4	51.073	Ac	Aib	<i>Gly</i>	<i>Ala</i>	Aib	<u>V_{xx}</u>	Gln	Aib	Aib	Aib	Ser	Lxx	Aib	Pro	Vxx	Aib	Lxx	Gln	Gln	Lxxol	New: Trikoningin KA V: [Ile] ⁵ →[V _{xx}] ⁵ (Positional isomer of Pept-Ilb; Pept-IIlb; Pept-IVb)	Goulard et al. 1995
Pept-IIa	1876.1	1899.1	961.05	1122.5	754.4	51.175	Ac	Aib	<i>Gly</i>	<i>Ala</i>	Aib	<i>Lxx</i>	Gln	Aib	Aib	Aib	Ser	Lxx	Aib	Pro	Vxx	Aib	<u>V_{xx}</u>	Gln	Gln	Lxxol	New: Trikoningin KA V: [Ile] ¹⁶ →[V _{xx}] ¹⁶ (Positional isomer of Pept-IIIa; Pept-IVa; Pept-Va; Pept-VIa; Pept-VIIIa) New: Tricholongin LBII, LBIV: [Phe] ³ →[Ala] ³ and [Aib] ⁵ →[Lxx] ⁵ New: Tricholongin BII: [Phe] ³ →[Ala] ³ and [Aib] ⁵ →[Lxx] ⁵ New: Trichostrigocin TSG-A, TSG-B: [Ala] ² →[Gly] ² and [Aib] ³ →[Ala] ³	Goulard et al. 1995 Degenkolb et al. 2006b Rebuffat et al. 1991 Degenkolb et al. 2006b
Pept-IIb	1875.2	1898.2	960.6	1108.5	768.4	52.316	Ac	Aib	<i>Gly</i>	<i>Ala</i>	Aib	<u>V_{xx}</u>	Gln	Aib	Aib	Aib	Ser	Lxx	Aib	Pro	Vxx	Aib	Lxx	Gln	Gln	Lxxol	New: (Positional isomer of Pept-Ib; Pept-IIlb; Pept-IVb)	→ Pept-Ib
Pept-IIIa	1876.2	1899.2	961.1	1122.5	754.4	53.005	Ac	Aib	<i>Gly</i>	<i>Ala</i>	Aib	<i>Lxx</i>	Gln	Aib	Aib	Aib	Ser	Lxx	Aib	Pro	Vxx	Aib	<u>V_{xx}</u>	Gln	Gln	Lxxol	New: (Positional isomer of Pept-IIa; Pept-IVa; Pept-Va; Pept-VIa; Pept-VIIIa)	→ Pept-IIa
Pept-IIIb	1875.3	1898.3	960.65	1108.5	768.4	53.292	Ac	Aib	<i>Gly</i>	<i>Ala</i>	Aib	<u>V_{xx}</u>	Gln	Aib	Aib	Aib	Ser	Lxx	Aib	Pro	Vxx	Aib	Lxx	Gln	Gln	Lxxol	New: (Positional isomer of Pept-Ib; Pept-IIb; Pept-IVb)	→ Pept-Ib
Pept-IVa	1875.2	1898.2	960.6	1122.5	754.4	53.555	Ac	Aib	<i>Gly</i>	<i>Ala</i>	Aib	<u>V_{xx}</u>	Gln	Aib	Aib	Aib	Ser	Lxx	Aib	Pro	Vxx	Aib	<u>V_{xx}</u>	Gln	Gln	Lxxol	New: (Positional isomer of Pept-IIa; Pept-IIIa; Pept-Va; Pept-VIa; Pept-VIIIa)	→ Pept-IIa
Pept-IVb	1875.2	1898.2	960.6	1108.5	768.4	53.996	Ac	Aib	<i>Gly</i>	<i>Ala</i>	Aib	<u>V_{xx}</u>	Gln	Aib	Aib	Aib	Ser	Lxx	Aib	Pro	Vxx	Aib	Lxx	Gln	Gln	Lxxol	New: (Positional isomer of Pept-Ib; Pept-IIb; Pept-IIIb)	→ Pept-Ib
Pept-Va	1876.2	1899.2	961.1	1122.5	754.4	54.776	Ac	Aib	<i>Gly</i>	<i>Ala</i>	Aib	<i>Lxx</i>	Gln	Aib	Aib	Aib	Ser	Lxx	Aib	Pro	Vxx	Aib	<u>V_{xx}</u>	Gln	Gln	Lxxol	New: (Positional isomer of Pept-IIa; Pept-IIIa; Pept-IVa; Pept-VIa; Pept-VIIIa)	→ Pept-IIa
Pept-Vb	1890.2	1913.2	968.1	1122.5	768.4	55.313	Ac	Aib	<i>Gly</i>	<i>Ala</i>	Aib	<i>Lxx</i>	Gln	Aib	Aib	Aib	Ser	Lxx	Aib	Pro	Vxx	Aib	Lxx	Gln	Gln	Lxxol	Trikoningin KA V (Positional isomer of Pept-VIb; Pept-VII; Pept-VIIIb)	Goulard et al. 1995
Pept-VIa	1876.2	1899.2	961.1	1122.5	754.4	55.617	Ac	Aib	<i>Gly</i>	<i>Ala</i>	Aib	<i>Lxx</i>	Gln	Aib	Aib	Aib	Ser	Lxx	Aib	Pro	Vxx	Aib	<u>V_{xx}</u>	Gln	Gln	Lxxol	New: (Positional isomer of Pept-IIa; Pept-IIIa; Pept-IVa; Pept-Va; Pept-VIIIa)	→ Pept-IIa
Pept-VIb	1890.2	1913.2	968.1	1122.5	768.4	55.798	Ac	Aib	<i>Gly</i>	<i>Ala</i>	Aib	<i>Lxx</i>	Gln	Aib	Aib	Aib	Ser	Lxx	Aib	Pro	Vxx	Aib	Lxx	Gln	Gln	Lxxol	(Positional isomer of Pept-Vb; Pept-VII; Pept-VIIIb)	→ Pept-Vb

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 | Continued

Peptide	M	[M+Na] ⁺	[M+2Na] ²⁺	b ₁₂	y ₇	rt. (min)	R	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15	R16	R17	R18	R19	Compound identical or positionally isomeric with	Reference
Pept-VII	1889.3	1912.3	967.65	1122.5	768.4	56.403	Ac	Aib	Gly	Ala	Aib	Lxx	Gln	Aib	Aib	Aib	Ser	Lxx	Aib	Pro	Vxx	Aib	Lxx	Gln	Gln	Lxxol	(Positional isomer of Pept-Vb; Pept-VIb; Pept-VIIIb) → Pept-Vb	
Pept-VIIIa	1874.2	1897.2	960.1	1122.5	754.4	56.806	Ac	Aib	Gly	Ala	Aib	Lxx	Gln	Aib	Aib	Aib	Ser	Lxx	Aib	Pro	Vxx	Aib	Lxx	Gln	Gln	Lxxol	New: (Positional isomer of Pept-IIa; Pept-IIIa; Pept-IVa; Pept-Va; Pept-VIa) → Pept-IIa	
Pept-VIIIb	1890.2	1913.2	968.1	1122.5	768.4	56.826	Ac	Aib	Gly	Ala	Aib	Lxx	Gln	Aib	Aib	Aib	Ser	Lxx	Aib	Pro	Vxx	Aib	Lxx	Gln	Gln	Lxxol	(Positional isomer of Pept-Vb; Pept-VIb; Pept-VII) → Pept-Vb	
Pept-IX	1874.5	1897.5	960.25	1106.5			Ac	Aib	Gly	Ala	Aib	Lxx	Gln	Aib	Aib	Aib	Ala	Lxx	Aib	Pro	Vxx	Aib	Lxx	Gln	Gln	Lxxol	New: Tricholongin LBIII: [Phe] ³ →[Ala] ³ , [Aib] ⁵ →[Lxx] ⁵ and [Lxx] ¹⁶ →[Lxx] ¹⁶	Degenkolb et al. 2006b
Pept-X	1904.2	1927.2	975.1	1136.4	768.4*	57.55*	Ac	Aib	Ala	Ala	Aib	Lxx	Gln	Aib	Aib	Aib	Ser	Lxx	Aib	Pro	Vxx	Aib	Lxx	Gln	Gln	Lxxol	New: Trikoningin KA V: Gly] ² →[Ala] ² (Positional isomer of Pept-XI; Pept-XII)	Gouldard et al. 1995
																											New: Trichorizianin TSG-A, TSG-B: [Aib] ³ →[Ala] ³ and [Vxx] ¹⁶ →[Lxx] ¹⁶	Degenkolb et al. 2006b
																											New: Trichorizianin TA IIIb: [Aib] ⁵ →[Lxx] ⁵ and [Trpol] ¹⁹ →[Lxxol] ¹⁹	Hajji et al. 1987
																											New: Trichorizianin TA VIb: [Aib] ⁵ →[Lxx] ⁵ and [Pheol] ¹⁹ →[Lxxol] ¹⁹	Hajji et al. 1987
																											New: Trichorizianin TA IVb: [Iva] ⁵ →[Lxx] ⁵ and [Trpol] ¹⁹ →[Lxxol] ¹⁹	Hajji et al. 1987
																											New: Trichorizianin TA VII: [Iva] ⁵ →[Lxx] ⁵ and [Pheol] ¹⁹ →[Lxxol] ¹⁹	Hajji et al. 1987
																											New: Trichorizianin TAP 14a: [Aib] ⁵ →[Lxx] ⁵ and [Trpol] ¹⁹ →[Lxxol] ¹⁹	Pócsfalvi et al. 1998
																											New: Trichorizianin TB IIIc: [Aib] ⁵ →[Lxx] ⁵ and [Trpol] ¹⁹ →[Lxxol] ¹⁹	Rebuffat et al. 1989
																											New: Trichorizianin TB IVb: [Iva] ⁵ →[Lxx] ⁵ and [Trpol] ¹⁹ →[Lxxol] ¹⁹	Rebuffat et al. 1989
																											New: Trichorizianin TB VIb: [Aib] ⁵ →[Lxx] ⁵ and [Pheol] ¹⁹ →[Lxxol] ¹⁹	Rebuffat et al. 1989
																											New: Trichorizianin TB VII: [Iva] ⁵ →[Lxx] ⁵ and [Pheol] ¹⁹ →[Lxxol] ¹⁹	Rebuffat et al. 1989
Pept-XI	1903.3	1926.3	974.65	1136.5	768.4	58.602	Ac	Aib	Ala	Ala	Aib	Lxx	Gln	Aib	Aib	Aib	Ser	Lxx	Aib	Pro	Vxx	Aib	Lxx	Gln	Gln	Lxxol	New: (Positional isomer of Pept-X; Pept-XII) → Pept-X	
Pept-XII	1904.2	1927.2	975.1	1136.6	768.4	59.264	Ac	Aib	Ala	Ala	Aib	Lxx	Gln	Aib	Aib	Aib	Ser	Lxx	Aib	Pro	Vxx	Aib	Lxx	Gln	Gln	Lxxol	New: (Positional isomer of Pept-X; Pept-X) → Pept-X	

*value belonging to both peptides (result of the same peak of y₇ ion)

TABLE 4 | Sequences of the newly identified peptaibol compounds produced by *Trichoderma koningiopsis* SZMC 12500 and their similarity to known peptaibols available in the “Comprehensive Peptaibiotics Database”

Peptide	M	[M+Na] ⁺	[M+2Na] ²⁺	b ₁₂	y ₇	rt. (min)	R	R1	R2	R3	R4	<u>R5</u>	R6	R7	R8	<u>R9</u>	<u>R10</u>	R11	R12	R13	R14	R15	<u>R16</u>	<u>R17</u>	<u>R18</u>	R19	Compound identical or positionally isomeric with	Reference
Koningiopsin Ia	1875.5	1898.5	960.75	1121.9	754.5	53.121	Ac	Aib	Ala	Ala	Aib	<u>Vxx</u>	Gln	Aib	Aib	<u>Aib</u>	Ser	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	<i>Lxxol</i>	New: Trichorzianin TAP-14b: [Pheol] ¹⁹ →[<i>Lxxol</i>] ¹⁹	Pócsfalvi et al. 1998
Koningiopsin Ib	1889.5	1912.5	967.75	1121.5	768.5	54.267	Ac	Aib	Ala	Ala	Aib	<u>Vxx</u>	Gln	Aib	Aib	<u>Aib</u>	Ser	Lxx	Aib	Pro	Vxx	Aib	Lxx	Gln	Gln	<i>Lxxol</i>	New: Trichorzianin TA IVb: [Trpol] ¹⁹ →[<i>Lxxol</i>] ¹⁹ New: Trichorzianin TA VII: [Pheol] ¹⁹ →[<i>Lxxol</i>] ¹⁹	Hajji et al. 1987 Hajji et al. 1987
Koningiopsin IIa	1890.5	1913.5	968.25	1136.7	754.5	54.69	Ac	Aib	Ala	<i>Ala</i>	Aib	Aib	Gln	Aib	Aib	<i>Lxx</i>	Ser	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	<i>Lxxol</i>	New: Trichorzianin TA IVb: [Aib] ⁹ →[<i>Lxx</i>] ⁹ and [Trpol] ¹⁹ →[<i>Lxxol</i>] ¹⁹ New: Trichostrogocin TSG-A, TSG-B: [Aib] ³ →[<i>Ala</i>] ³ and [Aib] ⁹ →[<i>Lxx</i>] ⁹	Hajji et al. 1987 Degenkolb et al. 2006b
Koningiopsin IIb	1889.6	1912.6	967.8	1120.8	768.6	55.513	Ac	Aib	Ala	Ala	Aib	Aib	Gln	Aib	Aib	<u>Aib</u>	<u>Vxx</u>	Lxx	Aib	Pro	Vxx	Aib	Lxx	Gln	Gln	<i>Lxxol</i>	New: Trichorzianin TA IIIb, TA IIIc: [Ser] ¹⁰ →[<i>Vxx</i>] ¹⁰ and [Trpol] ¹⁹ →[<i>Lxxol</i>] ¹⁹ New: Trichorzianin TA VIb: [Ser] ¹⁰ →[<i>Vxx</i>] ¹⁰ and [Pheol] ¹⁹ →[<i>Lxxol</i>] ¹⁹ New: Trichorzianin TAP-14a: [Ser] ¹⁰ →[<i>Vxx</i>] ¹⁰ and [Pheol] ¹⁹ →[<i>Lxxol</i>] ¹⁹	Hajji et al. 1987 Hajji et al. 1987 Pócsfalvi et al. 1998
Koningiopsin IIIa	1891.6	1914.6	968.8	1122.7	755.6	55.593	Ac	Aib	Ala	<i>Ala</i>	Aib	<u>Ala</u>	Gln	Aib	Aib	<i>Lxx</i>	Ser	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	<u>Glu</u>	<i>Lxxol</i>	New: Trichorzianin TAP-14b: [Aib] ⁵ →[<i>Ala</i>] ⁵ , [Aib] ⁹ →[<i>Lxx</i>] ⁹ , [Gln] ¹⁸ →[<i>Glu</i>] ¹⁸ and [Pheol] ¹⁹ →[<i>Lxxol</i>] ¹⁹ New: Trichorzianin TB IIIc, TB IVb: [Aib] ⁵ →[<i>Ala</i>] ⁵ , [Aib] ⁹ →[<i>Lxx</i>] ⁹ , [Ile] ¹⁶ →[<i>Vxx</i>] ¹⁶ and [Trpol] ¹⁹ →[<i>Lxxol</i>] ¹⁹ New: Trichorzianin TB VIb, TB VII: [Aib] ⁵ →[<i>Ala</i>] ⁵ , [Aib] ⁹ →[<i>Lxx</i>] ⁹ , [Ile] ¹⁶ →[<i>Vxx</i>] ¹⁶ and [Trpol] ¹⁹ →[<i>Lxxol</i>] ¹⁹ New: Trichostrogocin TSG-A, TSG-B: [Aib] ³ →[<i>Ala</i>] ³ , [Ala] ⁵ →[<i>Lxx</i>] ⁵ , [Aib] ⁹ →[<i>Lxx</i>] ⁹ and [Gln] ¹⁸ →[<i>Glu</i>] ¹⁸	Pócsfalvi et al. 1998 Rebuffat et al. 1989 Rebuffat et al. 1989 Degenkolb et al. 2006b
Koningiopsin IIIb	1873.6	1896.6	959.8	1120.8	754.5	55.936	Ac	Aib	Ala	Ala	Aib	Aib	Gln	Aib	Aib	<u>Aib</u>	<u>Vxx</u>	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	<i>Lxxol</i>	New: Trichorzianin TAP-14b: [Ser] ¹⁰ →[<i>Vxx</i>] ¹⁰ and [Pheol] ¹⁹ →[<i>Lxxol</i>] ¹⁹	Pócsfalvi et al. 1998

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 | Continued

Peptide	M	[M+Na] ⁺	[M+2Na] ²⁺	b ₁₂	y ₇	rt. (min)	R	R1	R2	R3	R4	<u>R5</u>	R6	R7	R8	<u>R9</u>	<u>R10</u>	R11	R12	R13	R14	R15	<u>R16</u>	<u>R17</u>	<u>R18</u>	R19	Compound identical or positionally isomeric with	Reference
Koningiopsin IV	1903.6	1926.6	974.8	1136.7	768.6	56.317	Ac	Aib	Ala	Ala	Aib	Aib	Gln	Aib	Aib	<i>Lxx</i>	Ser	<i>Lxx</i>	Aib	Pro	Vxx	Aib	<i>Lxx</i>	Gln	Gln	<i>Lxxol</i>	New: Trichorzianin TA IIIb, TA IIIc: [Aib] ⁹ →[<i>Lxx</i>] ⁹ and [Trpol] ¹⁹ →[<i>Lxxol</i>] ¹⁹ (Positional isomer of Pept-XVIIa) New: Trichorzianin TA VIb: [Aib] ⁹ →[<i>Lxx</i>] ⁹ and [Pheol] ¹⁹ →[<i>Lxxol</i>] ¹⁹ New: Trichorzianin TAP-14a: [Aib] ⁹ →[<i>Lxx</i>] ⁹ and [Pheol] ¹⁹ →[<i>Lxxol</i>] ¹⁹	Hajji et al. 1987 Hajji et al. 1987 Pócsfalvi et al. 1998
Koningiopsin Va	1903.6	1926.6	974.8	1136.8	768.6	56.739	Ac	Aib	Ala	Ala	Aib	Aib	Gln	Aib	Aib	<i>Lxx</i>	Ser	<i>Lxx</i>	Aib	Pro	Vxx	Aib	<i>Lxx</i>	Gln	Gln	<i>Lxxol</i>	New: (Positional isomer of Koningiopsin IV)	→ Pept-XVI
Koningiopsin Vb	1905.6	1928.6	975.8	1136.8	769.6	57.463	Ac	Aib	Ala	Ala	Aib	Aib	Gln	Aib	Aib	<i>Lxx</i>	Ser	<i>Lxx</i>	Aib	Pro	Vxx	Aib	<i>Lxx</i>	Gln	<u>Glu</u>	<i>Lxxol</i>	New: Trichorzianin TB IIIc: [Aib] ⁹ →[<i>Lxx</i>] ⁹ , and [Trpol] ¹⁹ →[<i>Lxxol</i>] ¹⁹ New: Trichorzianin TB VIb: [Aib] ⁹ →[<i>Lxx</i>] ⁹ , and [Pheol] ¹⁹ →[<i>Lxxol</i>] ¹⁹	Rebuffat et al. 1989 Rebuffat et al. 1989
Koningiopsin VIa	1888.6	1911.6	967.3	1120.7	768.6	57.825	Ac	Aib	Ala	Ala	Aib	Aib	Gln	Aib	Aib	<i>Lxx</i>	<u>Ala</u>	<i>Lxx</i>	Aib	Pro	Vxx	Aib	<i>Lxx</i>	Gln	Gln	<i>Lxxol</i>	New: Trichorzianin TA IIIb, TA IIIc: [Aib] ⁹ →[<i>Lxx</i>] ⁹ , [Ser] ¹⁰ →[<i>Ala</i>] ¹⁰ and [Trpol] ¹⁹ →[<i>Lxxol</i>] ¹⁹ New: Trichorzianin TA VIb: [Aib] ⁹ →[<i>Lxx</i>] ⁹ , [Ser] ¹⁰ →[<i>Ala</i>] ¹⁰ and [Pheol] ¹⁹ →[<i>Lxxol</i>] ¹⁹ New: Trichorzianin TAP-14a: [Aib] ⁹ →[<i>Lxx</i>] ⁹ , [Ser] ¹⁰ →[<i>Ala</i>] ¹⁰ and [Pheol] ¹⁹ →[<i>Lxxol</i>] ¹⁹	Hajji et al. 1987 Hajji et al. 1987 Pócsfalvi et al. 1998
Koningiopsin VIb	1888.7	1911.7	967.35	1120.9	769.5	58.448	Ac	Aib	Ala	Ala	Aib	Aib	Gln	Aib	Aib	<i>Lxx</i>	<u>Ala</u>	<i>Lxx</i>	Aib	Pro	Vxx	Aib	<i>Lxx</i>	Gln	<u>Glu</u>	<i>Lxxol</i>	New: Trichorzianin TB IIIc: [Aib] ⁹ →[<i>Lxx</i>] ⁹ , [Ser] ¹⁰ →[<i>Ala</i>] ¹⁰ and [Trpol] ¹⁹ →[<i>Lxxol</i>] ¹⁹ New: Trichorzianin TB VIb: [Aib] ⁹ →[<i>Lxx</i>] ⁹ , [Ser] ¹⁰ →[<i>Ala</i>] ¹⁰ and [Pheol] ¹⁹ →[<i>Lxxol</i>] ¹⁹	Rebuffat et al. 1989 Rebuffat et al. 1989

The information available in the literature about the peptaibol profiles of *T. koningiopsis* and *T. gamsii* is limited. Peptaibols produced by *T. koningiopsis* were previously identified as trikoningin KA V, together with other 11-residue-long compounds, trikoningin KB I and KB II (McMullin et al. 2017). To the best of our knowledge, other peptaibol compounds produced by *T. koningiopsis* were not reported in the literature previously. Apart from the groups shown in Table 3, the newly identified sequences also showed high similarity to trichorzin HAs, which, however, are only 18-residue-long peptaibols devoid of the Gln/Glu residue (Goulard et al. 1995). Trikoningin KA V, identified firstly from *T. koningii* (Auvin-Guette et al. 1993), is a peptaibol sequence positionally isomeric with sequences Pept-Vb, -VIb, and -VII. This compound was also found to be produced by *T. koningiopsis*, along with the 11-residues lipopeptaibols trikoningin KB I and KB II (McMullin et al. 2017). Other 19-residue-long peptaibols such as trichostrogocins were previously identified from *T. strigosum* (Degenkolb et al. 2006b, Neuhaus et al. 2007, Degenkolb et al. 2008a) and later also from the extracts of *T. paraviridescens* and *T. trixiae* as trichostrogocin-like compounds (Castagnoli et al. 2018a). Tricholongins were detected in *T. longibrachiatum* (Rebuffat et al. 1991) and *T. strigosum* (Degenkolb et al. 2006b), while trichorzianins are known from *T. atroviride* (Hajji et al. 1987, Rebuffat et al. 1989, Pócsfalvi et al. 1998, Röhrich et al. 2014). Further 19-residue-long peptaibols closely related to those of the present study include hypophellins from *T. phellinicola* (syn. *H. phellinicola*), hypopulvins from *T. pulvinatum* (syn. *H. pulvinata*), gelatinosins from *T. gelatinosum* (syn. *H. gelatinosa*), voglmayrins from *T. voglmayrii* (syn. *H. voglmayrii*), minutisporins from *T. minutisporum* (syn. *H. minutispora*) and hypocitrins from *T. citrinum* (syn. *H. citrina*) (Röhrich et al. 2012, Röhrich et al. 2013, Röhrich et al. 2014). This indicates that within the genus the ability to produce 19-residue-long peptaibols is not restricted to clade Viride of section Trichoderma, it also occurs in sections Hypocreanum (*T. phellinicola*, *T. pulvinatum*, *T. citrinum*) and Pachybasium (*T. minutisporum*), as well as in lone lineages (*T. voglmayrii*, *T. gelatinosum*). *T. gamsii* is a widespread species of the genus, also known as an endophyte of the traditional Chinese medicinal plant *Panax notoginseng* (Dang et al. 2010). Although, the investigation of peptaibol production was not yet carried out for this species, *T. gamsii* was shown to produce numerous secondary metabolites including cytochalasans (Ding et al. 2012a, Ding et al. 2012b, Chen et al. 2014, Ding et al. 2014), the spiro-cytochalasan trichodermonone (Ding et al. 2014), trichoderamides A and B originating from the PKS-NRPS hybrid pathway (Ding et al. 2015), trichodenols A and B (Ding et al. 2015), trichoderpyrone (Chen et al. 2017), as well as volatile organic compounds like dimethyl disulfide, dibenzofuran, methanethiol, and ketones (Chen et al. 2016). Among the

detected cytochalasans (trichalasin A, B, C, D, E, F, G, H, aspochalasins D, I, J, K, M, P, and aspergillin PZ), aspochalasins D and I displayed weak inhibitory activity against the HeLa cancer cell line (Ding et al. 2012a, Ding et al. 2012b), trichalasin G proved to be modestly inhibitory to the human cancer cell line MDA-MB-231 (Chen et al. 2016), while trichoderpyrone displayed weak cytotoxic activities against A549, HepG2, and HeLa cancer cell lines (Chen et al 2017).

In certain cases, minor differences were observed between the presently detected and the previously reported peptaibol sequences showing amino acid exchanges only at selected positions of the peptide chain (Table 3 and Table 4). Trichorzianins differ in the position 19 of the peptide chain. This position plays a critical role in the lifetime of the opened ion channel as the substitution of Pheol to Leuol/Ileol has led to increased lifetime of the open channel (Koide et al. 1997). This was also observed previously in the case of peptaibol-formed channels, where the Pheol was substituted with Trpol in both trichorzianin B-IIIc (Trpol) and B-VII (Pheol) (Duclohier et al. 1989). On the other hand, the investigation of synthetic ALM analogues – where all Aib residues were replaced with Leu – revealed that the substitution of the C-terminal residues did not affect the lifetime of the open channel (Molle et al. 1991). A secondary structural study was also carried out in the case of the purified compound trichorzianin TA VII in association with sodium dodecyl sulfate (SDS) micelles, which revealed formation of two right-handed helical segments (1–8 and 11–19) linked by a β -turn (Condamine et al. 1998). The novelty of the peptaibols produced by *T. koningiopsis* SZMC 12500 is in the variation of the C-terminus, which is critical in the lifetime of the ion channels. The name “Koningiopsin” was introduced for these novel compounds.

Based on the calibration made using ALM standard (Sigma-Aldrich Ltd., Budapest, Hungary), the amount of the peptaibol mixture of the crude extracts was determined. This new method for quantification of peptaibols based on the length of different peptides was described by Van Bohemen et al. (2016) and mentioned in the Materials and Methods section. The different length of peptaibols results in different structures, furthermore, shorter peptaibols (11–14 residues) contain more Pro leading to a structural deformation (Rebuffat et al. 1999). Based on the ALM standard, only the longer (17–20-residue-long) peptaibols can be quantified with high accuracy. The calculated concentration of the whole peptaibol molecules were $214.28 \mu\text{g ml}^{-1}$ and $101.26 \mu\text{g ml}^{-1}$ in the crude extracts of *T. gamsii* SZMC 1656 and *T. koningiopsis* SZMC 12500, respectively. In the case of *T. gamsii* SZMC 1656, Pept VIIIb and Pept VIb were the most abundant sequences of peptaibols (Supplementary

Table 7, 9). The sum of the amount of these two molecules was approximately 50%, while the concentration of other peptaibols remained below 10%. In the extract of *T. koningiopsis* SZMC 12500, Pept XVIIa accounted for almost half of the peptaibols produced (Supplementary Table 8, 10).

6.3.2 Effect of peptaibol extracts on bacteria, yeasts and filamentous fungi

M. luteus and *S. aureus* proved to be highly sensitive to both peptaibol extracts (Table 5), *E. coli* was more resistant, while *P. aeruginosa* showed higher sensitivity to the extracts from *T. koningiopsis* than to those from *T. gamsii*. *M. luteus* and *S. aureus* are Gram-positive bacteria, while *E. coli* and *P. aeruginosa* are Gram-negative ones, thus their sensitivity showed correlation with the type of their cell wall. Studies on the bioactivity of paracelsins (Brückner and Graf 1983) or ALM (Jen et al. 1987) showed similar results, i.e., that Gram-positive bacteria proved to be more sensitive to peptaibols. Testing the peptaibols trichorzianine A1 and B1 on Gram-positive and Gram-negative bacteria also revealed similar results, furthermore, synergistic effect could also be detected between peptaibols and different membrane-affecting compounds (MACs) and cell wall-degrading enzymes (CWDEs) (Lorito et al. 1996b, Benítez et al. 2004). In a study of Lorito et al. (1996a), the inhibition of β -D-glucan synthase was reported as a specific effect of peptaibol antibiotics. On the other hand, in the study of Cutler et al. (1991), the purified peptaibol identified as trikoningin KA V (also known as koningin A) seemed to be inactive against both Gram-positive and Gram-negative bacteria. No inhibition zones could be observed in the case of the tested yeasts (Table 5), though in another study, minimum inhibition could be observed after the treatment of trichokonins produced by *T. koningii* on *S. cerevisiae* CGMCC2.395 and *C. albicans* CGMCC2.538 (Song et al. 2006). Complete inhibition could not be observed among the fast-growing fungi (*Trichoderma* and *Fusarium* species), only the speed of their mycelial growth was limited (Table 6). Interestingly, the producer *T. koningiopsis* and *T. gamsii* strains seemed to be more sensitive to their own peptaibol extracts than *T. aggressivum* f. *europaeum* and *T. pleuroti*, known as the causal agents of green mould disease occurring in mushroom cultivation (Table 6).

TABLE 5 | Bioactivity of crude peptaibol extracts (100 mg ml⁻¹) from *Trichoderma gamsii* SZMC 1656 and *T. koningiopsis* SZMC 12500 towards bacteria and yeasts

Tested microbial strains	Sensitivity to <i>T. gamsii</i> SZMC 1656 extract	Sensitivity to <i>T. koningiopsis</i> SZMC 12500 extract
<i>Micrococcus luteus</i> SZMC 0264	+++++	++++
<i>Staphylococcus aureus</i> SZMC 0579	+++++	++++
<i>Escherichia coli</i> SZMC 0582	+	–
<i>Pseudomonas aeruginosa</i> SZMC 0568	+	++
<i>Candida boidinii</i> SZMC 0673	–	–
<i>Kluyveromyces lactis</i> SZMC 0683	–	–
<i>Saccharomyces cerevisiae</i> SZMC 0425	–	–
<i>Schizosaccharomyces pombe</i> SZMC 0142	–	–

–, absence of inhibition, diameter of inhibition zone: +, 5–7 mm, ++, 7–9 mm, +++, 9–11 mm, +++++, 11–13 mm, +++++, 13–15 mm diameter.

TABLE 6 | Bioactivity of crude peptaibol extracts (100 mg ml⁻¹) and their 2-fold serial dilutions from *Trichoderma gamsii* SZMC 1656 and *T. koningiopsis* SZMC 12500 towards cultures of filamentous fungi

Tested filamentous fungal strains	Sensitivity to <i>T. gamsii</i> SZMC 1656 extract (MIC, mg ml ⁻¹)	Sensitivity to <i>T. koningiopsis</i> SZMC 12500 extract (MIC, mg ml ⁻¹)
<i>Alternaria alternata</i> SZMC 16085	1.563 *	3.125 *
<i>Fusarium solani</i> SC SZMC 11467	6.25	12.5
<i>Rhizoctonia solani</i> SZMC 6252J	12.5 *	6.25 *
<i>Phoma cucurbitacearum</i> SZMC 16088	12.5*	3.125 *
<i>Trichoderma aggressivum</i> f. <i>europaeum</i> SZMC 1811	12.5	25
<i>Trichoderma pleuroti</i> SZMC 12454	12.5	25
<i>Trichoderma gamsii</i> SZMC 1656	6.25	6.25
<i>Trichoderma koningiopsis</i> SZMC 12500	6.25	6.25

SC: species complex

*, mycelial growth was completely stopped.

The *F. solani* species complex member appeared to be more sensitive to the peptaibol extract of *T. gamsii* than to the one of *T. koningiopsis*. Inhibition could also be detected in the case of *A. alternata*, *R. solani* and *P. cucurbitacearum*, all growing very slowly (8 days, 12 days, and 11 days, respectively, till they reach the hole containing methanol on the plates), and interestingly, the mycelial growth of these filamentous fungi stopped where the peptaibols were added into the holes and could not reach the edge of the plates (Table 6).

6.4 Peptaibol production of *Trichoderma* species causing mushroom green mould disease

6.4.1 Peptaibols produced by *T. aggressivum* f. *europaeum* and *T. pleuroti*

From the crude extracts of *T. aggressivum* f. *europaeum* CBS 433.95, peptaibols positionally isomeric with hypomurocins B1, B3a, B3b and B4, (Becker et al. 1997) as well as several new, yet unreported compounds with sequences very similar to hypomurocin B were identified based on the MS and MS² spectra (Table 7). The detection of hypomurocin B-like peptaibols in *T. aggressivum* f. *europaeum* CBS 433.95 is also in agreement with the partial sequence data published by Krause et al. (2006b) for isolate CBS 100526, the type strain of *T. aggressivum* f. *europaeum*. The fragment sequence 4 ([286]–Lxx–Aib–213–Vxx–Aib–142–Aib–210–Aib–Aib–Gln–Valol) reported in this previous study corresponds with sequences Pept-1719-a-1 and Pept-1719-a-2 detected in strain CBS 433.95. A further set of our sequences differ from fragment sequence 6 ([286]–Lxx–Vxx–Gln–Aib–170–Ala–Vxx–Aib–210–Aib–Aib–Gln–Valol) of Krause et al. (2006b) by containing Gly instead of Ala in the R10 position. Peptaibols corresponding with fragment sequence 5 or with the 11-residue-long hypomurocin A-like compounds were not detected in strain CBS 433.95. Differences in peptaibol profiles between different strains of the same species were reported in the literature also for *T. virens* (Wei et al. 2005). Another possible explanation for the lack of detection of hypomurocin A-like compounds may be the application of chloroform as an extraction solvent, which is less polar than dichloromethane/methanol (1/1) used in the study of Krause et al. (2006b).

TABLE 7 | Sequences of peptaibols produced by strain *Trichoderma aggressivum* f. *europaeum* CBS 433.95 and their similarity to known peptaibols available in the “Comprehensive Peptaibiotics Database”

Peptide	M	[M+Na] ⁺	[M+2Na] ²⁺	b ₁₂	y ₇	rt. (min)	R	R1	R2	R3	R4	<u>R5</u>	R6	R7	R8	<u>R9</u>	<u>R10</u>	R11	R12	R13	R14	R15	<u>R16</u>	R17	<u>R18</u>	Compound identical or positionally isomeric with	Reference	
Pept-1719-a-1	1719	1742	882.5	1108	612	43.13	AcAib	Ser	Ala	Lxx	<u>Aib</u>	Gln	Aib	Vxx	Aib	Gly	<u>Aib</u>	Aib	Pro	Lxx	Aib	Aib	Gln	Vxxol	Hypomurocin B1 (Positional isomer of Pept-1719-a-2)			Becker et. al. 1997
Pept-1719-a-2	1719	1742	882.5	1107	612	43.67	AcAib	Ser	Ala	Lxx	<u>Aib</u>	Gln	Aib	Vxx	Aib	Gly	<u>Aib</u>	Aib	Pro	Lxx	Aib	Aib	Gln	Vxxol	(Positional isomer of Pept-1719-a-1)			→ Pept-1719-a-1
Pept-1733-a-1	1733	1756	889.5	1122	612	45.34	AcAib	Ser	Ala	Lxx	<i>Vxx</i>	Gln	Aib	Vxx	Aib	Gly	<u>Aib</u>	Aib	Pro	Lxx	Aib	Aib	Gln	Vxxol	New: Hypomurocin B1: [Aib] ⁵ →[<i>Vxx</i>] ⁵			Becker et. al. 1997
Pept-1703-a-1	1703	1726	874.5	1092	612	45.91	AcAib	<u>Ala</u>	Ala	Lxx	<u>Aib</u>	Gln	Aib	Vxx	Aib	Gly	<u>Aib</u>	Aib	Pro	Lxx	Aib	Aib	Gln	Vxxol	Hypomurocin B3a			Becker et. al. 1997
Pept-1733-a-2	1733	1756	889.5	1121	612	46.11	AcAib	Ser	Ala	Lxx	<u>Aib</u>	Gln	<u>Vxx</u>	Vxx	Aib	Gly	<u>Aib</u>	Aib	Pro	Lxx	Aib	Aib	Gln	Vxxol	Hypomurocin B3b			Becker et. al. 1997
Pept-1717-a-1	1717	1740	881.5	1105	612	48.33	AcAib	<u>Ala</u>	Ala	Lxx	<i>Vxx</i>	Gln	Aib	Vxx	Aib	Gly	<u>Aib</u>	Aib	Pro	Lxx	Aib	Aib	Gln	Vxxol	New: Hypomurocin B3a: [Aib] ⁵ →[<i>Vxx</i>] ⁵ (Positional isomer of Pept-1717-a-2)			Becker et. al. 1997
Pept-1733-a-3	1733	1756	889.5	1122	612	48.65	AcAib	Ser	Ala	Lxx	<u>Aib</u>	Gln	Aib	Vxx	Aib	Gly	Vxx	Aib	Pro	Lxx	Aib	Aib	Gln	Vxxol	Hypomurocin B4			Becker et. al. 1997
Pept-1717-a-2	1718	1740	881.5	1106	612	48.92	AcAib	<u>Ala</u>	Ala	Lxx	<i>Vxx</i>	Gln	Aib	Vxx	Aib	Gly	<u>Aib</u>	Aib	Pro	Lxx	Aib	Aib	Gln	Vxxol	New: (Positional isomer of Pept-1717-a-1)			→ Pept-1717-a-1
Pept-1747-a-1	1747	1770	896.5	1135	612	49.56	AcAib	Ser	Ala	Lxx	<i>Vxx</i>	Gln	Aib	Vxx	Aib	Gly	Vxx	Aib	Pro	Lxx	Aib	Aib	Gln	Vxxol	New: Hypomurocin B4: [Aib] ⁵ →[<i>Vxx</i>] ⁵ (Positional isomer of Pept-1747-a-2 and Pept-1747-a-3)			Becker et. al. 1997
Pept-1747-a-2	1747	1770	896.5	1135	612	50.20	AcAib	Ser	Ala	Lxx	<i>Vxx</i>	Gln	Aib	Vxx	Aib	Gly	Vxx	Aib	Pro	Lxx	Aib	Aib	Gln	Vxxol	New: (Positional isomer of Pept-1747-a-1 and Pept-1747-a-3)			→ Pept-1747-a-1
Pept-1747-a-3	1747	1770	896.5	1136	612	51.31	AcAib	Ser	Ala	Lxx	<i>Vxx</i>	Gln	Aib	Vxx	Aib	Gly	Vxx	Aib	Pro	Lxx	Aib	Aib	Gln	Vxxol	New: (Positional isomer of Pept-1747-a-1 and Pept-1747-a-2)			→ Pept-1747-a-1
Pept-1761-a-1	1761	1785	904.5	1150	612	51.58	AcAib	Ser	Ala	Lxx	<i>Vxx</i>	Gln	<u>Vxx</u>	Vxx	Aib	Gly	<i>Vxx</i>	Aib	Pro	Lxx	Aib	Aib	Gln	Vxxol	New: Hypomurocin B3b: [Aib] ⁵ →[<i>Vxx</i>] ⁵ and [Aib] ¹¹ →[<i>Vxx</i>] ¹¹ (Positional isomer of Pept-1761-a-2 and Pept-1761-a-3)			Becker et. al. 1997
																										New: Hypomurocin B4: [Aib] ⁵ →[<i>Vxx</i>] ⁵ and [Aib] ⁷ →[<i>Vxx</i>] ⁷	Becker et. al. 1997	
Pept-1717-a-3	1717	1740	881.5	1105	612	51.61	AcAib	<u>Ala</u>	Ala	Lxx	<u>Aib</u>	Gln	Aib	Vxx	Aib	Gly	<i>Vxx</i>	Aib	Pro	Lxx	Aib	Aib	Gln	Vxxol	New: Hypomurocin B3a: [Aib] ¹¹ →[<i>Vxx</i>] ¹¹			Becker et. al. 1997
Pept-1731-a-1	1731	1754	888.5	1119	612	51.90	AcAib	<u>Ala</u>	Ala	Lxx	<u>Aib</u>	Gln	<u>Vxx</u>	Vxx	Aib	Gly	<i>Vxx</i>	Aib	Pro	Lxx	Aib	Aib	Gln	Vxxol	New: Hypomurocin B3a: [Aib] ⁷ →[<i>Vxx</i>] ⁷ and [Aib] ¹¹ →[<i>Vxx</i>] ¹¹			Becker et. al. 1997
																										New: (Positional isomer of Pept-1731-a-4)		
Pept-1747-a-4	1747	1770	896.5	1136	612	52.22	AcAib	Ser	Ala	Lxx	<u>Aib</u>	Gln	<u>Vxx</u>	Vxx	Aib	Gly	<i>Vxx</i>	Aib	Pro	Lxx	Aib	Aib	Gln	Vxxol	New: Hypomurocin B3b: [Aib] ¹¹ →[<i>Vxx</i>] ¹¹			Becker et. al. 1997
																										New: Hypomurocin B4: [Aib] ⁷ →[<i>Vxx</i>] ⁷	Becker et. al. 1997	
Pept-1731-a-2	1731	1754	888.5	1120	612	52.99	AcAib	<u>Ala</u>	Ala	Lxx	<i>Vxx</i>	Gln	Aib	Vxx	Aib	Gly	<i>Vxx</i>	Aib	Pro	Lxx	Aib	Aib	Gln	Vxxol	New: Hypomurocin B3a: [Aib] ⁵ →[<i>Vxx</i>] ⁵ and [Aib] ¹¹ →[<i>Vxx</i>] ¹¹ (Positional isomer of Pept-1731-a-3)			Becker et. al. 1997
Pept-1761-b-	1761	1784	903.5	1136	626	53.09	AcAib	Ser	Ala	Lxx	<i>Vxx</i>	Gln	Aib	Vxx	Aib	Gly	Vxx	Aib	Pro	Lxx	Aib	Aib	Gln	<u>Lxxol</u>	New: Hypomurocin B5: [Aib] ⁵ →[<i>Vxx</i>] ⁵			Becker et. al. 1997
Pept-1761-a-2	1761	1784	903.5	1149	612	53.69	AcAib	Ser	Ala	Lxx	<i>Vxx</i>	Gln	<u>Vxx</u>	Vxx	Aib	Gly	<i>Vxx</i>	Aib	Pro	Lxx	Aib	Aib	Gln	Vxxol	New: (Positional isomer of Pept-1761-a-1 and Pept-1761-a-3)			→ Pept-1761-a-1
Pept-1731-a-3	1731	1754	888.5	1120	612	53.79	AcAib	<u>Ala</u>	Ala	Lxx	Vxx	Gln	Aib	Vxx	Aib	Gly	Vxx	Aib	Pro	Lxx	Aib	Aib	Gln	Vxxol	New: (Positional isomer of Pept-1731-a-2)			→ Pept-1731-a-2
Pept-1761-a-3	1761	1784	903.5	1150	612	54.18	AcAib	Ser	Ala	Lxx	<i>Vxx</i>	Gln	<u>Vxx</u>	Vxx	Aib	Gly	<i>Vxx</i>	Aib	Pro	Lxx	Aib	Aib	Gln	Vxxol	New: (Positional isomer of Pept-1761-a-1 and Pept-1761-a-2)			→ Pept-1761-a-1
Pept-1731-a-4	1731	1754	888.5	1119	612	54.45	AcAib	<u>Ala</u>	Ala	Lxx	<u>Aib</u>	Gln	<u>Vxx</u>	Vxx	Aib	Gly	Vxx	Aib	Pro	Lxx	Aib	Aib	Gln	Vxxol	New: (Positional isomer of Pept-1731-a-1)			→ Pept-1731-a-1
Pept-1745-a-1	1745	1768	895.5	1134	612	55.66	AcAib	<u>Ala</u>	Ala	Lxx	<i>Vxx</i>	Gln	<u>Vxx</u>	Vxx	Aib	Gly	<i>Vxx</i>	Aib	Pro	Lxx	Aib	Aib	Gln	Vxxol	New: Hypomurocin B3a: [Aib] ⁵ →[<i>Vxx</i>] ⁵ , [Aib] ⁷ →[<i>Vxx</i>] ⁷ and [Aib] ¹¹ →[<i>Vxx</i>] ¹¹			Becker et. al. 1997
Pept-1775-b-1	1775	1798	910.5	1149	626	56.16	AcAib	Ser	Ala	Lxx	<i>Vxx</i>	Gln	<u>Vxx</u>	Vxx	Aib	Gly	Vxx	Aib	Pro	Lxx	Aib	Aib	Gln	<u>Lxxol</u>	New: Hypomurocin B5: [Aib] ⁵ →[<i>Vxx</i>] ⁵ and [Aib] ⁷ →[<i>Vxx</i>] ⁷ (Positional isomer of Pept-1775-b-2)			Becker et. al. 1997
Pept-1775-b-	1775	1798	910.5	1149	626	56.46	AcAib	Ser	Ala	Lxx	<i>Vxx</i>	Gln	<u>Vxx</u>	Vxx	Aib	Gly	Vxx	Aib	Pro	Lxx	Aib	Aib	Gln	<u>Lxxol</u>	New: (Positional isomer of Pept-1775-b-1)			→ Pept-1775-b-1
Pept-1745-a-2	1745	1768	895.5	1133	612	56.62	AcAib	<u>Ala</u>	Ala	Lxx	<i>Vxx</i>	Gln	<u>Vxx</u>	Vxx	Aib	Gly	<i>Vxx</i>	Aib	Pro	Lxx	Aib	Aib	Gln	Vxxol	New: (Positional isomer of Pept-1745-a-1)			→ Pept-1745-a-1

Minor sequence variants in amino acid exchange positions are underlined. Amino acid exchanges in new compounds are set in italic. Diagnostic fragment ions and extracted ion chromatograms (EICs) are shown in Supplementary Tables 11 and 12, and Supplementary Figure 12.

Hypomurocin B-like compounds were first identified from an antagonist of shiitake mushroom (*Lentinula edodes*) in Japan, originally misidentified as *H. muroiana* (Becker et al. 1997) and later reidentified as *T. atroviride* (Samuels et al. 2006). These compounds have a characteristic amino acid sequence with four variable positions (R2, R7, R11 and R18). The sequences reported in our study contain one more variable position, R5 with a Vxx/Aib exchange. 18-residue-long peptaibols relatively close to hypomurocins were also found in a biocontrol strain of *T. simmonsii*, where the sequences had an R3 position different in an Ala/Aib exchange from hypomurocin B1 or B4, while a further 18-residue-long peptaibol similar to hypomurocin B5 with the same difference in the R3 position was identified from *T. harzianum* (Degenkolb et al. 2015). The 3D-structural and folding properties of hypomurocins were recently characterised by Horváth et al (2016).

Six compounds with the highest concentrations (Pept-1733-a-1, Pept-1733-a-3, Pept-1747-a-3, Pept-1747-a-4, Pept-1731-a-3 and Pept-1761-a-3, Supplementary Table 11) account for 57.3% of the microheterogeneous peptaibol mixture, which consists of 25 compounds, all of them identified as 18-residue-long peptaibols. Compounds Pept-1719-a-1, Pept-1703-a-1, Pept-1733-a-2 and Pept-1733-a-3 show sequence similarity to hypomurocins B1, B3a, B3b and B4, respectively, while the other 21 compounds are new (see references in Table 7). During the full scan MS measurements, b_{12} and y_6 ions along the fragment ions resulted from the unstable Aib–Pro bond were the most intensively observed fragments. Based on the MS² experiments, the y_6 ions had the same sequence in 22 compounds with the mass of m/z 612, while the remaining three compounds contained a y_6 ion of m/z 626 (Supplementary Table 12). According to the MS² spectra, all y_6 sequences with m/z 612 have an Lxx residue in the R14 position and a Vxxol residue at the end of the sequence in the R18 position, while the m/z 626 y_6 ions have Lxxol instead of Vxxol at the terminus.

In *T. pleuroti* strain TPhu1, a new group of compounds was detected, which does not match any of the known groups of peptaibols (Table 8). A relatively close group according to the b_{12} ion is the group of trichokindins (Iida et al. 1994, Peltola et al. 2004), which, however, have a leucine in the R8 position and a completely different y_6 ion part with m/z 625. According to the MS² investigations of the y_6 ion part with m/z 660 and m/z 674, the closest group is the trichorzin PA group described from *T. harzianum* (Leclerc et al. 1988, Duval et al. 1997), however, trichorzins PA have Gly–Leu in the R10–R11 positions of the b_{12} ion part and not Ala–Vxx or Ala–Aib like the peptaibols produced by *T. pleuroti*. Furthermore, although the m/z values of the y_6 ion part are the same in the trichorzin PA group as in our sequences, the

variable residue is in position R18 and not in R16 as in the *T. pleuroti* sequences. Another group, trichorzin PA_U was also reported with a further Iva/Aib exchange in position R5 (Urbán et al. 2016). For this new group of peptaibols detected in our study, we introduced the name ‘tripleurin’. The compounds present in highest concentrations were tripleurins VI, VIIIb and XIIc with 7.9%, 10.0% and 12.0% of the [M+Na]⁺ area, respectively. These three compounds gave almost 1/3 (29.9%) of the total peptaibol fraction consisting of 22 compounds, all of them being new (Supplementary Table 13). The 3D-structural and folding property of tripleurin XIIc was recently characterised by Tyagi et al. (2019b).

TABLE 8 | Sequences of peptaibols produced by strain *Trichoderma pleuroti* TPhu1 and their similarity to known peptaibols available in the “Comprehensive Peptaibiotics Database”

Peptide	M	[M+Na] ⁺	[M+2Na] ²⁺	b ₁₂	y ₇	rt. (min)	R	R1	R2	R3	R4	<u>R5</u>	R6	R7	R8	<u>R9</u>	<u>R10</u>	R11	R12	R13	R14	R15	<u>R16</u>	<u>R17</u>	<u>R18</u>	Compound identical or positionally isomeric with	Reference
Tripleurin Ia	1767	1790	906.5	1107	660	43.13	Ac	Aib	Ser	Ala	Aib	<u>Aib</u>	Gln	Aib	<i>Vxx</i>	Aib	Ala	<i>Vxx</i>	Aib	Pro	Lxx	Aib	<u>Aib</u>	Gln	<i>Pheol</i>	New: Trichokindin IIa: [Leu] ⁸ →[<i>Vxx</i>] ⁸ and [Ileol] ¹⁸ →[<i>Pheol</i>] ¹⁸ (Positional isomer of Tripleurin Ib)	Iida et al. 1994
Tripleurin II	1767	1790	906.5	1094	674	43.67	Ac	Aib	Ser	Ala	Aib	<u>Aib</u>	Gln	<i>Aib</i>	<i>Vxx</i>	Aib	Ala	<u>Aib</u>	Aib	Pro	Lxx	Aib	<i>Vxx</i>	Gln	<i>Pheol</i>	New: Trichokindin Ia: [Iva] ⁷ →[<i>Aib</i>] ⁷ , [Leu] ⁸ →[<i>Vxx</i>] ⁸ , [Aib] ¹⁶ →[<i>Vxx</i>] ¹⁶ and [Ileol] ¹⁸ →[<i>Pheol</i>] ¹⁸ New: Trichokindin Ib: [Iva] ⁵ →[<i>Aib</i>] ⁵ , [Leu] ⁸ →[<i>Vxx</i>] ⁸ , [Aib] ¹⁶ →[<i>Vxx</i>] ¹⁶ and [Ileol] ¹⁸ →[<i>Pheol</i>] ¹⁸ New: Trichokindin IIa: [Leu] ⁸ →[<i>Vxx</i>] ⁸ , [Iva] ¹¹ →[<i>Aib</i>] ¹¹ , [Aib] ¹⁶ →[<i>Vxx</i>] ¹⁶ and [Ileol] ¹⁸ →[<i>Pheol</i>] ¹⁸	Iida et al. 1994 Iida et al. 1994 Iida et al. 1994
Tripleurin Ib	1767	1790	906.5	1107	660	45.34	Ac	Aib	Ser	Ala	Aib	<u>Aib</u>	Gln	Aib	<i>Vxx</i>	Aib	Ala	<i>Vxx</i>	Aib	Pro	Lxx	Aib	<u>Aib</u>	Gln	<i>Pheol</i>	New: (Positional isomer of Tripleurin Ia)	→ Tripleurin Ia
Tripleurin IIIa	1782	1805	914.0	1107	674	45.91	Ac	Aib	Ser	Ala	Aib	<i>Vxx</i>	Gln	Aib	<i>Vxx</i>	Aib	Ala	<u>Aib</u>	Aib	Pro	Lxx	Aib	<i>Vxx</i>	Gln	<i>Pheol</i>	New: Trichokindin Ib: [Leu] ⁸ →[<i>Vxx</i>] ⁸ , [Aib] ¹⁶ →[<i>Vxx</i>] ¹⁶ and [Ileol] ¹⁸ →[<i>Pheol</i>] ¹⁸ (Positional isomer of Tripleurin IIIb)	Iida et al. 1994
Tripleurin IIIb	1782	1805	914.0	1108	674	46.11	Ac	Aib	Ser	Ala	Aib	<i>Vxx</i>	Gln	Aib	<i>Vxx</i>	Aib	Ala	<u>Aib</u>	Aib	Pro	Lxx	Aib	<i>Vxx</i>	Gln	<i>Pheol</i>	New: (Positional isomer of Tripleurin IIIa)	→ Tripleurin IIIa
Tripleurin IV	1752	1775	899.2	1077	674	48.33	Ac	Aib	<u>Ala</u>	Ala	Aib	<u>Aib</u>	Gln	Aib	<i>Vxx</i>	Aib	Ala	<u>Aib</u>	Aib	Pro	Lxx	Aib	<i>Vxx</i>	Gln	<i>Pheol</i>	New: Trichokindin Ib: [Ser] ² →[<i>Ala</i>] ⁷ , [Iva] ⁵ →[<i>Aib</i>] ⁵ , [Leu] ⁸ →[<i>Vxx</i>] ⁸ , [Aib] ¹⁶ →[<i>Vxx</i>] ¹⁶ and [Ileol] ¹⁸ →[<i>Pheol</i>] ¹⁸	Iida et al. 1994
Tripleurin V	1782	1805	914.0	1122	660	48.65	Ac	Aib	Ser	Ala	Aib	<u>Aib</u>	Gln	Aib	<i>Vxx</i>	<u><i>Vxx</i></u>	Ala	<i>Vxx</i>	Aib	Pro	Lxx	Aib	<u>Aib</u>	Gln	<i>Pheol</i>	New: Trichokindin IIa: [Leu] ⁸ →[<i>Vxx</i>] ⁸ , [Aib] ⁹ →[<i>Vxx</i>] ⁹ , and [Ileol] ¹⁸ →[<i>Pheol</i>] ¹⁸	Iida et al. 1994
Tripleurin VI	1782	1805	914.0	1107	674	48.92	Ac	Aib	Ser	Ala	Aib	<i>Vxx</i>	Gln	<u>Ala</u>	<i>Vxx</i>	Aib	Ala	<i>Vxx</i>	Aib	Pro	Lxx	Aib	<i>Vxx</i>	Gln	<i>Pheol</i>	New: Trichokindin IIIb: [Aib] ⁷ →[<i>Ala</i>] ⁷ , [Leu] ⁸ →[<i>Vxx</i>] ⁸ , [Aib] ¹⁶ →[<i>Vxx</i>] ¹⁶ and [Ileol] ¹⁸ →[<i>Pheol</i>] ¹⁸ New: Trichokindin Vb: [Aib] ⁷ →[<i>Ala</i>] ⁷ , [Leu] ⁸ →[<i>Vxx</i>] ⁸ , [Aib] ¹⁶ →[<i>Vxx</i>] ¹⁶ and [Ileol] ¹⁸ →[<i>Pheol</i>] ¹⁸ New: Trichokindin VI: [Iva] ⁷ →[<i>Ala</i>] ⁷ , [Leu] ⁸ →[<i>Vxx</i>] ⁸ , [Aib] ¹⁶ →[<i>Vxx</i>] ¹⁶ and [Ileol] ¹⁸ →[<i>Pheol</i>] ¹⁸ New: Trichokindin VII : [Iva] ⁷ →[<i>Ala</i>] ⁷ , [Leu] ⁸ →[<i>Vxx</i>] ⁸ , [Aib] ¹⁶ →[<i>Vxx</i>] ¹⁶ and [Ileol] ¹⁸ →[<i>Pheol</i>] ¹⁸	Iida et al. 1994 Iida et al. 1994 Iida et al. 1994 Iida et al. 1994
Tripleurin VII	1752	1775	899.2	1092	660	49.56	Ac	Aib	<u>Ala</u>	Ala	Aib	<u>Aib</u>	Gln	Aib	<i>Vxx</i>	Aib	Ala	<i>Vxx</i>	Aib	Pro	Lxx	Aib	<u>Aib</u>	Gln	<i>Pheol</i>	New: Trichokindin IIa: [Ser] ² →[<i>Ala</i>] ⁹ , [Leu] ² →[<i>Vxx</i>] ⁸ , and [Ileol] ¹⁸ →[<i>Pheol</i>] ¹⁸	Iida et al. 1994

Minor sequence variants in amino acid exchange positions are underlined. Amino acid exchanges in new compounds are set in italic. Diagnostic fragment ions and EICs are shown in Supplementary Tables 11 and 12, and Supplementary Figure 12.

TABLE 8 | Continued

Peptide	M	[M+Na] ⁺	[M+2Na] ²⁺	b ₁₂	y ₇	rt. (min)	R	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15	R16	R17	R18	Compound identical or positionally isomeric with	Reference
Tripleurin VIIIa	1796	1819	921.0	1121	674	50.20	AcAib	Ser	Ala	Aib	Vxx	Gln	Vxx	Vxx	Aib	Ala	Aib	Aib	Pro	Lxx	Aib	Vxx	Gln	Pheol	New: Trichokindin IIb: [Leu] ⁸ →[Vxx] ⁸ , [Aib] ¹⁶ →[Vxx] ¹⁶ and [Leuol] ¹⁸ →[Pheol] ¹⁸ New: Trichokindin IV: [Leu] ⁸ →[Vxx] ⁸ , [Aib] ¹⁶ →[Vxx] ¹⁶ and [Ileol] ¹⁸ →[Pheol] ¹⁸	Iida et al. 1994 Iida et al. 1994	
Tripleurin IXa	1765	1788	905.5	1106	660	51.31	AcAib	Ala	Ala	Aib	Vxx	Gln	Aib	Vxx	Vxx	Ala	Aib	Aib	Pro	Lxx	Aib	Aib	Gln	Pheol	New: Trichokindin Ib: [Ser] ² →[Ala] ² , [Leu] ⁸ →[Vxx] ⁸ , [Aib] ⁹ →[Vxx] ⁹ and [Ileol] ¹⁸ →[Pheol] ¹⁸	Iida et al. 1994	
Tripleurin X	1765	1788	905.5	1091	674	51.58	AcAib	Ala	Ala	Aib	Vxx	Gln	Aib	Vxx	Aib	Ala	Aib	Aib	Pro	Lxx	Aib	Vxx	Gln	Pheol	New: Trichokindin Ib: [Ser] ² →[Ala] ² , [Leu] ⁸ →[Vxx] ⁸ , [Aib] ¹⁶ →[Vxx] ¹⁶ and [Ileol] ¹⁸ →[Pheol] ¹⁸	Iida et al. 1994	
Tripleurin XI	1796	1819	921.0	1135	660	51.61	AcAib	Ser	Ala	Aib	Aib	Gln	Vxx	Vxx	Vxx	Ala	Vxx	Aib	Pro	Lxx	Aib	Aib	Gln	Pheol	New: Trichokindin IIIa: [Leu] ⁸ →[Vxx] ⁸ , [Aib] ⁹ →[Vxx] ⁹ and [Leuol] ¹⁸ →[Pheol] ¹⁸ New: Trichokindin Va: [Leu] ⁸ →[Vxx] ⁸ , [Aib] ⁹ →[Vxx] ⁹ and [Ileol] ¹⁸ →[Pheol] ¹⁸	Iida et al. 1994 Iida et al. 1994	
Tripleurin VIIIb	1796	1819	921.0	1122	674	51.90	AcAib	Ser	Ala	Aib	Vxx	Gln	Aib	Vxx	Aib	Ala	Vxx	Aib	Pro	Lxx	Aib	Vxx	Gln	Pheol	New: Trichokindin IIIb: [Leu] ⁸ →[Vxx] ⁸ and [Leuol] ¹⁸ →[Pheol] ¹⁸ New: Trichokindin Vb: [Leu] ⁸ →[Vxx] ⁸ and [Ileol] ¹⁸ →[Pheol] ¹⁸	Iida et al. 1994 Iida et al. 1994	
Tripleurin IXb	1765	1788	905.5	1105	660	52.22	AcAib	Ala	Ala	Aib	Vxx	Gln	Aib	Vxx	Vxx	Ala	Aib	Aib	Pro	Lxx	Aib	Aib	Gln	Pheol	New: Trichokindin Ib: [Ser] ² →[Ala] ² , [Leu] ⁸ →[Vxx] ⁸ , [Aib] ⁹ →[Vxx] ⁹ and [Ileol] ¹⁸ →[Pheol] ¹⁸	Iida et al. 1994	
Tripleurin XIIa	1810	1833	928.0	1135	674	52.99	AcAib	Ser	Ala	Aib	Vxx	Gln	Vxx	Aib	Vxx	Ala	Vxx	Aib	Pro	Lxx	Aib	Vxx	Gln	Pheol	New: Trichokindin VI: [Leu] ⁸ →[Aib] ⁸ , [Aib] ⁹ →[Vxx] ⁹ , [Aib] ¹⁶ →[Vxx] ¹⁶ and [Leuol] ¹⁸ →[Pheol] ¹⁸ (Positional isomer of Tripleurin XIIb and Tripleurin XIIc) New: Trichokindin VII: [Leu] ⁸ →[Aib] ⁸ , [Aib] ⁹ →[Vxx] ⁹ , [Aib] ¹⁶ →[Vxx] ¹⁶ and [Ileol] ¹⁸ →[Pheol] ¹⁸	Iida et al. 1994 Iida et al. 1994	
Tripleurin XIIb	1810	1833	928.0	1135	674	53.09	AcAib	Ser	Ala	Aib	Vxx	Gln	Vxx	Aib	Vxx	Ala	Vxx	Aib	Pro	Lxx	Aib	Vxx	Gln	Pheol	New: (Positional isomer of Tripleurin XIIa and Tripleurin XIIc)	→ Tripleurin XIIa	
Tripleurin XIIc	1810	1833	928.0	1135	674	53.69	AcAib	Ser	Ala	Aib	Vxx	Gln	Vxx	Aib	Vxx	Ala	Vxx	Aib	Pro	Lxx	Aib	Vxx	Gln	Pheol	New: (Positional isomer of Tripleurin XIIa and Tripleurin XIIb)	→ Tripleurin XIIa	
Tripleurin XIIIa	1780	1803	913.0	1106	674	53.79	AcAib	Ala	Ala	Aib	Vxx	Gln	Lxx	Aib	Aib	Ala	Aib	Aib	Pro	Lxx	Aib	Vxx	Gln	Pheol	New: Trichokindin Ib: [Ser] ² →[Ala] ² , [Aib] ⁷ →[Lxx] ⁷ , [Leu] ⁸ →[Aib] ⁸ and [Ileol] ¹⁸ →[Pheol] ¹⁸ New: Trichokindin IIb: [Ser] ² →[Ala] ² , [Iva] ⁷ →[Lxx] ⁷ , [Leu] ⁸ →[Aib] ⁸ and [Leuol] ¹⁸ →[Pheol] ¹⁸ New: Trichokindin IV: [Ser] ² →[Ala] ² , [Iva] ⁷ →[Lxx] ⁷ , [Leu] ⁸ →[Aib] ⁸ and [Ileol] ¹⁸ →[Pheol] ¹⁸	Iida et al. 1994 Iida et al. 1994 Iida et al. 1994	

TABLE 8 | Continued

Peptide	M	[M+Na] ⁺	[M+2Na] ²⁺	b ₁₂	y ₇	rt. (min)	R	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15	R16	R17	R18	Compound identical or positionally isomeric with	Reference
Tripleurin XIIIb	1780	1803	913.0	1106	674	54.18	AcAib	<u>Ala</u>	Ala	Aib	Vxx	Gln	<u>Lxx</u>	<u>Aib</u>	<u>Ala</u>	Ala	Vxx	Aib	Pro	Lxx	Aib	<u>Vxx</u>	Gln	<i>Pheol</i>	New: Trichokindin IIIb: [Ser] ² →[<i>Ala</i>] ² , [Aib] ⁷ →[<i>Lxx</i>] ⁷ , [Leu] ⁸ →[<i>Aib</i>] ⁸ , [Aib] ⁹ →[<i>Ala</i>] ⁹ , [Aib] ¹⁶ →[<i>Vxx</i>] ¹⁶ and [Leuol] ¹⁸ →[<i>Pheol</i>] ¹⁸ (Positional isomer of and Tripleurin XIIIc)	Iida et al. 1994	
																									New: Trichokindin Vb: [Ser] ² →[<i>Ala</i>] ² , [Aib] ⁷ →[<i>Lxx</i>] ⁷ , [Leu] ⁸ →[<i>Aib</i>] ⁸ , [Aib] ⁹ →[<i>Ala</i>] ⁹ , [Aib] ¹⁶ →[<i>Vxx</i>] ¹⁶ and [Ileol] ¹⁸ →[<i>Pheol</i>] ¹⁸	Iida et al. 1994	
																									New: Trichokindin VI: [Ser] ² →[<i>Ala</i>] ² , [Iva] ⁷ →[<i>Lxx</i>] ⁷ , [Leu] ⁸ →[<i>Aib</i>] ⁸ , [Aib] ⁹ →[<i>Ala</i>] ⁹ , [Aib] ¹⁶ →[<i>Vxx</i>] ¹⁶ and [Leuol] ¹⁸ →[<i>Pheol</i>] ¹⁸	Iida et al. 1994	
																									New: Trichokindin VII: [Ser] ² →[<i>Ala</i>] ² , [Iva] ⁷ →[<i>Lxx</i>] ⁷ , [Leu] ⁸ →[<i>Aib</i>] ⁸ , [Aib] ⁹ →[<i>Ala</i>] ⁹ , [Aib] ¹⁶ →[<i>Vxx</i>] ¹⁶ and [Ileol] ¹⁸ →[<i>Pheol</i>] ¹⁸	Iida et al. 1994	
Tripleurin XIIIc	1780	1803	913.0	1105	674	54.45	AcAib	<u>Ala</u>	Ala	Aib	Vxx	Gln	<u>Lxx</u>	<u>Aib</u>	<u>Ala</u>	Ala	Vxx	Aib	Pro	Lxx	Aib	<u>Vxx</u>	Gln	<i>Pheol</i>	New: (Positional isomer of and Tripleurin XIIIb)	→ Tripleurin XIIIa	
Tripleurin XIV	1824	1847	935.0	1149	674	55.66	AcAib	Ser	Ala	Aib	Vxx	Gln	<u>Vxx</u>	<u>Aib</u>	<u>Lxx</u>	Ala	Vxx	Aib	Pro	Lxx	Aib	<u>Vxx</u>	Gln	<i>Pheol</i>	New: Trichokindin VI: [Aib] ⁹ →[<i>Lxx</i>] ⁹ , [Aib] ¹⁶ →[<i>Vxx</i>] ¹⁶ and [Leuol] ¹⁸ →[<i>Pheol</i>] ¹⁸	Iida et al. 1994	
																									New: Trichokindin VII: [Aib] ⁹ →[<i>Ala</i>] ⁹ , [Aib] ¹⁶ →[<i>Vxx</i>] ¹⁶ and [Ileol] ¹⁸ →[<i>Pheol</i>] ¹⁸	Iida et al. 1994	
Tripleurin XV	1794	1817	920.0	1120	674	56.16	AcAib	<u>Ala</u>	Ala	Aib	Vxx	Gln	<u>Vxx</u>	<u>Vxx</u>	Aib	Ala	Vxx	Aib	Pro	Lxx	Aib	<u>Vxx</u>	Gln	<i>Pheol</i>	New: Trichokindin VI: [Ser] ² →[<i>Ala</i>] ² , [Aib] ⁸ →[<i>Vxx</i>] ⁸ , [Aib] ¹⁶ →[<i>Vxx</i>] ¹⁶ and [Leuol] ¹⁸ →[<i>Pheol</i>] ¹⁸	Iida et al. 1994	
																									New: Trichokindin VII: [Ser] ² →[<i>Ala</i>] ² , [Aib] ⁸ →[<i>Vxx</i>] ⁸ , [Aib] ¹⁶ →[<i>Vxx</i>] ¹⁶ and [Ileol] ¹⁸ →[<i>Pheol</i>] ¹⁸	Iida et al. 1994	
Tripleurin XVI	1808	1831	927.0	1133	674	56.46	AcAib	<u>Ala</u>	Ala	Aib	Vxx	Gln	<u>Lxx</u>	<u>Aib</u>	<u>Vxx</u>	Ala	Vxx	Aib	Pro	Lxx	Aib	Vxx	Gln	<i>Pheol</i>	New: Trichokindin III: [Ser] ² →[<i>Ala</i>] ² , [Aib] ⁷ →[<i>Lxx</i>] ⁷ , [Leu] ⁸ →[<i>Aib</i>] ⁸ , [Aib] ⁹ →[<i>Vxx</i>] ⁹ , [Aib] ¹⁶ →[<i>Vxx</i>] ¹⁶ and [Leuol] ¹⁸ →[<i>Pheol</i>] ¹⁸	Iida et al. 1994	
																									New: Trichokindin V: [Ser] ² →[<i>Ala</i>] ² , [Aib] ⁷ →[<i>Lxx</i>] ⁷ , [Leu] ⁸ →[<i>Aib</i>] ⁸ , [Aib] ⁹ →[<i>Vxx</i>] ⁹ , [Aib] ¹⁶ →[<i>Vxx</i>] ¹⁶ and [Ileol] ¹⁸ →[<i>Pheol</i>] ¹⁸	Iida et al. 1994	
																									New: Trichokindin VI: [Ser] ² →[<i>Ala</i>] ² , [Iva] ⁷ →[<i>Lxx</i>] ⁷ , [Leu] ⁸ →[<i>Aib</i>] ⁸ , [Aib] ⁹ →[<i>Vxx</i>] ⁹ , [Aib] ¹⁶ →[<i>Vxx</i>] ¹⁶ and [Leuol] ¹⁸ →[<i>Pheol</i>] ¹⁸	Iida et al. 1994	
																									New: Trichokindin VII: [Ser] ² →[<i>Ala</i>] ² , [Iva] ⁷ →[<i>Lxx</i>] ⁷ , [Leu] ⁸ →[<i>Aib</i>] ⁸ , [Aib] ⁹ →[<i>Vxx</i>] ⁹ , [Aib] ¹⁶ →[<i>Vxx</i>] ¹⁶ and [Ileol] ¹⁸ →[<i>Pheol</i>] ¹⁸	Iida et al. 1994	

6.4.2 Changes in the peptaibol profiles of *T. pleuroti* and *T. aggressivum* f. *europaeum* during in vitro antagonistic interactions with *P. ostreatus* and *A. bisporus*

Peptaibol profiles of the green mould agents *T. pleuroti* and *T. aggressivum* f. *europaeum* during interactions with mushroom mycelia were measured in *in vitro* antagonism experiments by confronting the *Trichoderma* strains with *A. bisporus* SZMC 23395 and *P. ostreatus* SZMC 23392. AI values were calculated according to the method of Körmöczi et al. (2009). Strain *T. aggressivum* f. *europaeum* CBS 433.95 – a green mould isolate from *Agaricus* compost – antagonised *A. bisporus* SZMC 23395 after 1 week of incubation with an AI value of 91% (Figure 4). *T. pleuroti* TPhu1 – isolated from *Pleurotus* cultivation substrate affected by green mould – proved to be more effective against *P. ostreatus* SZMC 23392 (AI: 100%) than the *T. aggressivum* strain (AI: 61%). Furthermore, strain TPhu1 was also more aggressive against *A. bisporus* (AI: 98%) than its infectious agent *T. aggressivum* f. *europaeum* (AI: 91%).

Figure 4 | Interaction of *Trichoderma* strains with *A. bisporus* SZMC 23395 and *P. ostreatus* SZMC 23392. A) *A. bisporus* self confrontation, B) *P. ostreatus* self confrontation, 1) *T. aggressivum* f. *europaeum* CBS 433.95 self confrontation, 2) *T. pleuroti* TPhu1 self

confrontation, A1 – B2): interactions in different combinations (A: *A. bisporus*, B: *P. ostreatus*, 1: *T. aggressivum* f. *europaeum* CBS 433.95, 2: *T. pleuroti* TPhu1).

Although the production of certain peptaibol compounds, e.g., Pept-1733-a-3 and Pept-1747-a-3 by *T. aggressivum* f. *europaeum* CBS 433.95 was higher during confrontation with *Agaricus* or *Pleurotus* than in the case of the control (self confrontation), a significant increase could be detected only in the case of Pept-1717-a-1 ($P = 0.0152$) during the confrontation with *P. ostreatus* (Figure 5). On the other hand, during confrontation with *A. bisporus* and *P. ostreatus*, the amounts of certain compounds including Pept-1733-a-1 ($P = 0.0052$ and 0.0395 , respectively), Pept-1717-a-2 ($P = 0.0120$ and 0.0480 , respectively) and Pept-1745-a-2 ($P = 0.0007$ and 0.0011 , respectively) were significantly lower.

Figure 5 | Effects of the *in vitro* confrontation of *T. aggressivum* f. *europaeum* CBS 433.95 with *P. ostreatus* and *A. bisporus* on the production of peptaibols. Significance levels of differences from the control are marked with asterisks: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ ($n = 3$).

Changes in the peptaibol profiles could also be observed during the confrontation of *T. pleuroti* TPhu1 with the two mushrooms (Figure 6). The amount of tripleurin VIIIb increased significantly during the confrontation with both *A. bisporus* ($P = 0.0336$) and *P. ostreatus* ($P = 0.0484$), while a significantly decreased production in the presence of both mushrooms was measured for tripleurins Ib (P values of 0.0020 and 0.0392 , respectively) and XIIIb (P values of 0.0255 and 0.0180 , respectively). Further significant increase (tripleurins IXb and XIIIc, with P values of 0.0008 and 0.0168 , respectively) or decrease (tripleurin IIIa with a P value of

0.0025) in the amount of compounds could be detected during the confrontation of *T. pleuroti* with *A. bisporus*. The results suggest that the mushroom hosts may have an impact on the peptaibol profiles of *Trichoderma* species causing green mould problems. Differences in the production of 18-residue-long trichotoxins could also be recently detected during the *in vitro* confrontation of a mycoparasitic *T. asperellum* strain with phytopathogenic fungi (*F. moniliforme*, *F. culmorum*, *F. graminearum*, *F. oxysporum* species complex, *A. solani* and *R. solani*) (Tamandegani et al. 2020).

Figure 6 | Effects of the *in vitro* confrontation of *T. pleuroti* TPhu1 with *P. ostreatus* and *A. bisporus* on the production of peptaibols. Significance levels of differences from the control are marked with asterisks: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ ($n = 3$).

6.4.3 Effects of peptaibols on *P. ostreatus* and *A. bisporus*

The effects of the crude peptaibol extracts filled into wells bored into the agar plate were also examined. Inhibition could be observed in the growth of *A. bisporus* and *P. ostreatus* after 3 days of incubation (Figure 7), suggesting that peptaibols may play a role during the antagonistic action of aggressive *Trichoderma* species against cultivated mushrooms. The inhibition zones disappeared after further incubation, as the mushroom mycelia could slowly overgrow the holes that contained the extracts. The antifungal activities of certain *Trichoderma* peptaibols are long known from the literature. The review of Szekeres et al. (2005) summarized the previously published information about the biological activities of *Trichoderma* peptaibols, including antifungal properties. The authors discussed the inhibitory activity of 10-residue-long trichopolyns I and II towards the basidiomycete mushrooms

Lentinula edodes, *Flammulina velutipes* and *P. ostreatus*, the yeasts *C. albicans*, *C. utilis* and *Cryptococcus neoformans*, and the filamentous ascomycetes *Penicillium chrysogenum*, *A. niger*, *A. fumigatus* and *Trichophyton mentagrophytes*, as well as the antifungal activities of trichorzins HA/harzianins HC and trichotoxin A against *Sclerotium cepivorum* and *Mucor ramannianus*, respectively. A synergism between membrane-affecting peptaibols and hydrolytic enzymes was also observed during the antifungal antagonism of *Trichoderma* strain ATCC 36042 (Schirmböck et al. 1994) originally identified as *T. harzianum*, then reidentified as *T. atroviride* (Kuhls et al. 1996). This was later explained by inhibition of the membrane-bound β -1,3-glucan synthase of the host by the peptaibols, which inhibit the resynthesis of cell wall β -glucans, sustain the disruptive action of β -glucanases, and thereby enhance the fungicidal activity (Lorito et al. 1996a). A more recent study revealed the antibiotic mechanism of the 20-residue-long trichokonin VI (produced by *T. pseudokoningii* SMF2) against *F. oxysporum* by showing that this peptaibol induces a metacaspase-independent apoptotic programmed cell death in parallel with the accumulation of cytoplasmic vacuoles (Shi et al. 2012).

Figure 7 | Inhibition of *P. ostreatus* SZMC 23392 – upper plates, and *A. bisporus* SZMC 23395 – lower plates, by crude extracts from A) *T. aggressivum* f. *europaeum* CBS 433.95 and B) *T. pleuroti* TPhu1 after 1) 1 day, 2) 2 days, and 3) 1 week of incubation. The *A. bisporus* could not grow further most probably due to lack of nutrients.

6.5 Peptaibol production of *Trichoderma* species from clade Longibrachiatum

6.5.1 Peptaibols produced by the species selected from clade Longibrachiatum

The peptaibol sequences could be categorized into three groups, designated as A (Table 9, Supplementary Tables 1, 4), B (Table 10, Supplementary Tables 2, 5) and C (Table 11, Supplementary Tables 3, 6). EICs resulting from full scan measurements of crude extracts from the examined *Trichoderma* strains are shown in Supplementary Figures 14–35. The producer species of the compounds are listed in Supplementary Tables 15–16. Groups A and B contain 20-residue-long peptaibols, whereas group C sequences had lost a residue in position R6. Several sequences proved to be homologous or positionally isomeric to the peptaibol subgroups of trichobrachins, paracelsins, suzukacillins, saturnisporins, trichoaureocins, trichocellins, longibrachins, hyporientalins, trichokonins, trilongins, metanicins, trichosporins, gliodeliquescins, ALMs, and hypophellins (see references in Tables 9–11). Some sequences had amino acid exchanges in comparison with previously described compounds from the peptaibol groups listed above.

TABLE 9 | Sequences of the newly identified group A peptaibol compounds from *Trichoderma* species of clade Longibrachiatum and their similarities to known peptaibols available in the “Comprehensive Peptaibiotics Database”

Peptide	M	[M+Na] ⁺	[M+2Na] ²⁺	b ₁₃	y ₇	rt. (min)	R	R1	R2	<u>R3</u>	R4	R5	<u>R6</u>	R7	R8	<u>R9</u>	<u>R10</u>	<u>R11</u>	<u>R12</u>	<u>R13</u>	<u>R14</u>	<u>R15</u>	<u>R16</u>	<u>R17</u>	<u>R18</u>	R19	R20	Compound identical or positionally isomeric with	Reference
Pept-A-Ia	1922	1945	984	1149	774	35.35	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	Vxx	Ala	Gly	Lxx	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	Trichoareocin 1d	Brückner et al. 2002
Pept-A-Ib	1922	1945	984	1149	774	36.88	Ac	Aib	Ala	Aib	Ala	Aib	<i>Aib</i>	Gln	Aib	Vxx	Ala	Gly	<i>Vxx</i>	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	New: Trichoareocin 1d: [Lxx] ¹² →[Vxx] ¹²	Brückner et al. 2002
Pept-A-IIa	1923	1946	984.5	1149	775	38.26	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	Vxx	<i>Aib</i>	Gly	<i>Vxx</i>	Aib	Pro	Vxx	Aib	Aib	<i>Glu</i>	Gln	Pheol	New: Longibrachin B II: [Lxx] ¹² →[Vxx] ¹² New: Trilongin CI: [Lxx] ¹² →[Vxx] ¹² New: Hypophellin 2: [Lxx] ¹² →[Vxx] ¹² New: Longibrachin B II, Trilongin CI: [Lxx] ¹² →[Vxx] ¹²	Leclerc et al. 1998 Mikkola et al. 2012 Röhrich et al. 2013 Tamandegani et al. 2016
Pept-A-IIb	1923	1946	984.5	1149	775	37.46	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	Vxx	Ala	Gly	Lxx	Aib	Pro	Vxx	Aib	Aib	<i>Glu</i>	Gln	Pheol	New: Trichoareocin 1d: [Gln] ¹⁷ →[Glu] ¹⁷	Brückner et al. 2002
Pept-A-IIIa	1936	1959	991	1149	788	39.82	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	Vxx	<i>Aib</i>	Gly	<i>Vxx</i>	Aib	Pro	Vxx	Aib	<i>Vxx</i>	Gln	Gln	Pheol	New: Longibrachin A II: [Leu] ⁹ →[Vxx] ⁹ New: Paracelsin F: [Aib] ¹² →[Vxx] ¹² New: Suzukacillin A 03: [Aib] ¹² →[Vxx] ¹² New: Suzukacillin A 10a: [Lxx] ¹² →[Vxx] ¹² New: Trichoareocin 4: [Lxx] ¹² →[Vxx] ¹² New: Trichobrachin II 07, 08, 09 IIb B: [Lxx] ¹² →[Vxx] ¹² New: Trichokonin VII: [Leu] ¹² →[Vxx] ¹² New: Trilongin BII: [Lxx] ¹² →[Vxx] ¹² New: Metanin B: [Leu] ¹² →[Vxx] ¹² New: Hypophellin 3: [Lxx] ¹² →[Vxx] ¹² New: Pept-1951-c: [Lxx] ¹² →[Vxx] ¹² New: Hyporientalin A: [Aib] ¹² →[Vxx] ¹²	Leclerc et al. 1998 Pöcsfalvi et al. 1997 Krause et al. 2006b Krause et al. 2006b Brückner et al. 2002 Krause et al. 2007 Huang et al. 1996 Mikkola et al. 2012 Kimonyo and Brückner 2013 Röhrich et al. 2013 Tamandegani et al. 2016 Touati et al. 2018
Pept-A-IIIb	1936	1959	991	1149	788	38.17	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	Vxx	Ala	Gly	Lxx	Aib	Pro	Vxx	Aib	<i>Vxx</i>	Gln	Gln	Pheol	New: Longibrachin A II: [Aib] ¹⁰ →[Ala] ¹⁰ New: Suzukacillin A 10a: [Aib] ¹⁰ →[Ala] ¹⁰ New: Trichoareocin 4: [Aib] ¹⁰ →[Ala] ¹⁰ New: Trichoareocin 1d: [Aib] ¹⁷ →[Ala] ¹⁷ New: Trichobrachin II 07, 08, 09, IIb B: [Aib] ¹⁰ →[Ala] ¹⁰ New: Trichokonin VII: [Aib] ¹⁰ →[Ala] ¹⁰ New: Trilongin BII: [Aib] ¹⁰ →[Ala] ¹⁰ New: Metanin B: [Aib] ¹⁰ →[Ala] ¹⁰ New: Hypophellin 3: [Aib] ¹⁰ →[Ala] ¹⁰ New: Pept-1951-c: [Aib] ¹⁰ →[Ala] ¹⁰	Leclerc et al. 1998 Krause et al. 2006b Brückner et al. 2002 Brückner et al. 2002 Krause et al. 2007 Huang et al. 1996 Mikkola et al. 2012 Kimonyo and Brückner 2013 Röhrich et al. 2013 Tamandegani et al. 2016
Pept-A-IIIc	1936	1959	991	1149	788	39.89	Ac	Aib	Ala	Aib	Ala	Aib	<i>Aib</i>	Gln	Aib	Vxx	Ala	Gly	<i>Vxx</i>	Aib	Pro	Vxx	Aib	<i>Vxx</i>	Gln	Gln	Pheol	New: Pept-1965-c-1, -2: [Lxx] ⁹ →[Vxx] ⁹ and [Lxx] ¹² →[Vxx] ¹² New: Hyporientalin A: [Aib] ¹⁰ →[Ala] ¹⁰	Tamandegani et al. 2016 Touati et al. 2018

Variable residues in table header and minor sequence variants in sequences are underlined. Amino acid exchanges in new compounds are set in italic.

TABLE 9 | Continued

Peptide	M	[M+Na] ⁺	[M+2Na] ²⁺	b ₁₃	y ₇	rt. (min)	R	R1	R2	<u>R3</u>	R4	R5	<u>R6</u>	R7	R8	<u>R9</u>	<u>R10</u>	<u>R11</u>	<u>R12</u>	R13	R14	R15	<u>R16</u>	<u>R17</u>	<u>R18</u>	R19	R20	Compound identical or positionally isomeric with	Reference
Pept-A-IVa	1936	1959	991	1163	774	40.21	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	Longibrachin A I (Positional isomer of Pept-A-VIIa) Trichoareocin 3 Trichobrachin II 05, 06 IIb A Trichokonin VI Trilongin BI Metanicin A Gliodeliquescin A Hypophellin 1 Longibrachin A I, Trilongin BI	Leclerc et al. 1998 Brückner et al. 2002 Krause et al. 2007 Huang et al. 1994 Mikkola et al. 2012 Kimonyo and Brückner 2013 Brückner et al. 1984 Röhrich et al. 2013 Tamandegani et al. 2016
Pept-A-IVb	1936	1959	991	1163	774	40.18	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	<u>Lxx</u>	<u>Ala</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	New: Suzukacillin A 11a, 09: [Aib] ¹⁰ →[Ala] ¹⁰ New: Trichocellin-TC-A-V, -VII: [Aib] ¹⁰ →[Ala] ¹⁰	Krause et al. 2006b Wada et al. 1994
Pept-A-Va	1950	1973	998	1177	774	40.73	Ac	Aib	Ala	Aib	Ala	Aib	<u>Aib</u>	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	Trichosporin TS-B-IVc (Position isomer of Pept-A-XVIa) Longibrachin A III Trichoareocin 5 Trichobrachin IIb C Trichokonin VIII Trilongin BIII Metanicin C Hypophellin 5 Longibrachin A III.	Iida et al. 1990 Leclerc et al. 1998 Brückner et al. 2002 Krause et al. 2007 Huang et al. 1996 Mikkola et al. 2012 Kimonyo and Brückner 2013 Röhrich et al. 2013 Tamandegani et al. 2016
Pept-A-Vb	1950	1973	998	1177	774	41.40	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	<u>Lxx</u>	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	Suzukacillin A 11a, A 09 Trichocellin TC-A-V, TC-A-VII	Krause et al. 2006b. Wada et al. 1994
Pept-A-VIa	1937	1960	991.5	1163	775	41.46	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	Aib	<u>Glu</u>	Gln	Pheol	Longibrachin B II Trilongin CI Hypophellin 2 Longibrachin B II., Trilongin CI.	Leclerc et al. 1998 Mikkola et al. 2012 Röhrich et al. 2013 Tamandegani et al. 2016
Pept-A-VIb	1937	1960	991.5	1163	775	41.50	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	<u>Lxx</u>	<u>Ala</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	Aib	<u>Glu</u>	Gln	Pheol	New: Longibrachin B II: [Val] ⁹ →[Lxx] ⁹ and [Aib] ¹⁰ →[Ala] ¹⁰ New: Trilongin CI: [Vxx] ⁹ →[Lxx] ⁹ and [Aib] ¹⁰ →[Ala] ¹⁰ New: Hypophellin 2: [Vxx] ⁹ →[Lxx] ⁹ and [Aib] ¹⁰ →[Ala] ¹⁰ New: Longibrachin B II., Trilongin CI.: [Vxx] ⁹ →[Lxx] ⁹ and [Aib] ¹⁰ →[Ala] ¹⁰ New: Trichocellin TC-B-I: [Aib] ¹⁰ →[Ala] ¹⁰ and [Aib] ¹² →[Lxx] ¹²	Leclerc et al. 1998 Mikkola et al. 2012 Röhrich et al. 2013 Tamandegani et al. 2016 Wada et al. 1994
Pept-A-VIIa	1936	1959	991	1163	774	41.00	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	(Positional isomer of Pept-A-IVa)	→ Pept-A-IVa
Pept-A-VIIb	1936	1959	991	1163	774	42.53	Ac	Aib	Ala	<u>Lxx</u>	Ala	Aib	Ala	Gln	Aib	Vxx	Ala	Gly	Lxx	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	New: Trichoareocin 1d: [Aib] ³ →[Lxx] ³	Brückner et al. 2002

TABLE 9 | Continued

Peptide	M	[M+Na] ⁺	[M+2Na] ²⁺	b ₁₃	y ₇	rt. (min)	R	R1	R2	<u>R3</u>	R4	R5	<u>R6</u>	R7	R8	<u>R9</u>	<u>R10</u>	<u>R11</u>	<u>R12</u>	R13	R14	R15	R16	<u>R17</u>	<u>R18</u>	R19	R20	Compound identical or positionally isomeric with	Reference
Pept-A-VIIIa	1950	1973	998	1177	774	42.29	Ac	Aib	Ala	<u>Vxx</u>	Ala	Aib	Ala	Gln	Aib	<u>Lxx</u>	Ala	Gly	Lxx	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	New: Suzukacillin A 11a, 09: [Aib] ³ →[Vxx] ³ and New: Trichocellin-TC-A-V, -VII: [Aib] ³ →[Vxx] ³ and [Aib] ¹⁰ →[Ala] ¹⁰ New: Trichoareocin 1d: [Aib] ³ →[Vxx] ³ and [Val] ⁹ →[Lxx] ⁹	Krause et al. 2006b Wada et al. 1994 Brückner et al. 2002
Pept-A-VIIIb	1950	1973	998	1177	774	42.46	Ac	Aib	Ala	<u>Vxx</u>	Ala	Aib	Ala	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	New: Longibrachin A I: [Aib] ³ →[Vxx] ³ New: Trichoareocin 3: [Ala] ³ →[Vxx] ³ New: Trichobrachin II 03: [Aib] ³ →[Vxx] ³ New: Trichobrachin II 05, 06 IIb A: [Aib] ³ →[Vxx] ³ New: Trichokonin IIc: [Ala] ³ →[Vxx] ³ New: Trichokonin VI: [Aib] ³ →[Vxx] ³ New: Trilongin BI: [Aib] ³ →[Vxx] ³ New: Metanicin A: [Aib] ³ →[Vxx] ³ New: Gliodeliquescin A: [Aib] ³ →[Vxx] ³ New: Hypophellin 1: [Aib] ³ →[Vxx] ³ New: Longibrachin A I, Trilongin BI: [Aib] ³ →[Vxx] ³	Leclerc et al. 1998 Brückner et al. 2002 Krause et al. 2007 Krause et al. 2007 Huang et al. 1996 Huang et al. 1994 Mikkola et al. 2012 Kimonyo and Brückner 2013 Brückner et al. 1984 Röhrich et al. 2013 Tamandegani et al. 2016
Pept-A-IXa	1950	1973	998	1163	788	42.76	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol	Longibrachin A II (Position isomer of Pept-A-XVa and Pept-A- XVIIb) Suzukacillin A 10a Trichoareocin 4 Trichobrachin II 07, 08, 09, IIb B Trichokonin VII Trilongin BII Metanicin B Hypophellin 3 Pept-1951-c Hyporientalin A	Leclerc et al. 1998 Krause et al. 2006b Brückner et al. 2002 Krause et al. 2007 Huang et al. 1996 Mikkola et al. 2012 Kimonyo and Brückner 2013 Röhrich et al. 2013 Tamandegani et al. 2016 Touati et al. 2018
Pept-A-IXb	1950	1973	998	1163	788	42.84	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	<u>Lxx</u>	Ala	Gly	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol	New: Suzukacillin A 10b, 11b, 13: [Aib] ¹⁰ →[Ala] ¹⁰ New: Trichocellin TC-A-VI, TC-A-VIII: [Aib] ¹⁰ →[Ala] ¹⁰	Krause et al. 2006b Wada et al. 1994

TABLE 9 | Continued

Peptide	M	[M+Na] ⁺	[M+2Na] ²⁺	b ₁₃	y ₇	rt. (min)	R	R1	R2	<u>R3</u>	R4	R5	<u>R6</u>	R7	R8	<u>R9</u>	<u>R10</u>	<u>R11</u>	<u>R12</u>	R13	R14	R15	<u>R16</u>	<u>R17</u>	<u>R18</u>	R19	R20	Compound identical or positionally isomeric with	Reference
Pept-A-Xa	1964	1987	1005	1177	788	43.28	Ac	Aib	Ala	Aib	Ala	Aib	<u>Aib</u>	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol	Longibrachin A IV (Position isomer of Pept-A-XIVb Pept-A-XXIa and Pept-XXVa) Trichoareocin 6 Trichobrachin II 10, IIb D Trichokonin IX Trilongin BIV Metanycin D Hypophellin 7	Pept-A-XVIIa, Leclerc et al. 1998 Brückner et al. 2002 Krause et al. 2007 Huang et al. 1995 Mikkola et al. 2012 Kimonyo and Brückner 2013 Röhrich et al. 2013
Pept-A-Xb	1964	1987	1005	1177	788	42.89	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	<u>Lxx</u>	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol	Suzukacillin A 10b, 11b, 13 Trichocellin TC-A-VI, TC-A-VIII	Krause et al. 2006b Wada et al. 1994
Pept-A-XIa	1951	1974	998.5	1177	775	43.60	Ac	Aib	Ala	<u>Ixx</u>	Ala	Aib	Ala	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	Aib	<u>Glu</u>	Gln	Pheol	New: Longibrachin B II: [Aib] ³ →[<i>Ixx</i>] ³ New: Trilongin CI: [Aib] ³ →[<i>Ixx</i>] ³ New: Hypophellin 2: [Aib] ³ →[<i>Ixx</i>] ³ New: Longibrachin B II., Trilongin CI.: [Aib] ³ →[<i>Ixx</i>] ³	Leclerc et al. 1998 Mikkola et al. 2012 Röhrich et al. 2013 Tamandegani et al. 2016
Pept-A-XIb	1951	1974	998.5	1177	775	43.60	Ac	Aib	Ala	Aib	Ala	Aib	<u>Aib</u>	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	Aib	<u>Glu</u>	Gln	Pheol	Trilongin CIII (Positional isomer of Pept-A-XIXa) Hypophellin 6 Longibrachin B III., Trilongin CIII.	Mikkola et al. 2012 Röhrich et al. 2013 Tamandegani et al. 2016
Pept-A-XIc	1951	1974	998.5	1177	775	43.62	Ac	Aib	Ala	<u>Ixx</u>	Ala	Aib	Ala	Gln	Aib	<u>Lxx</u>	<u>Ala</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	Aib	<u>Glu</u>	Gln	Pheol	New: Longibrachin B II: [Aib] ³ →[<i>Ixx</i>] ³ , [Val] ⁹ →[<i>Lxx</i>] ⁹ and [Aib] ¹⁰ →[<i>Ala</i>] ¹⁰ New: Trilongin CI: [Aib] ³ →[<i>Ixx</i>] ³ , [Vxx] ⁹ →[<i>Lxx</i>] ⁹ and [Aib] ¹⁰ →[<i>Ala</i>] ¹⁰ New: Hypophellin 2: [Aib] ³ →[<i>Ixx</i>] ³ , [Vxx] ⁹ →[<i>Lxx</i>] ⁹ and [Aib] ¹⁰ →[<i>Ala</i>] ¹⁰ New: Longibrachin B II., Trilongin CI.: [Aib] ³ →[<i>Ixx</i>] ³ , [Vxx] ⁹ →[<i>Lxx</i>] ⁹ and [Aib] ¹⁰ →[<i>Ala</i>] ¹⁰ New: Trichocellin TC-B-I: [Aib] ³ →[<i>Ixx</i>] ³ , [Aib] ¹⁰ →[<i>Ala</i>] ¹⁰ and [Aib] ¹² →[<i>Lxx</i>] ¹²	Leclerc et al. 1998 Mikkola et al. 2012 Röhrich et al. 2013 Tamandegani et al. 2016 Wada et al. 1994
Pept-A-XII	1937	1960	991.5	1163	775	42.81	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	<u>Lxx</u>	<u>Ala</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	Aib	<u>Glu</u>	Gln	Pheol	(Positional isomer of Pept-A-VIb)	→ Pept-A-VIb
Pept-A-XIIIa	1951	1974	998.5	1163	789	44.14	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	<u>Glu</u>	Gln	Pheol	Longibrachin B III Trilongin CII Hypophellin 4 Pept-1952-d Longibrachin A II., Trilongin BII.	Leclerc et al. 1998 Mikkola et al. 2012 Röhrich et al. 2013 Tamandegani et al. 2016 Tamandegani et al. 2016

TABLE 9 | Continued

Peptide	M	[M+Na] ⁺	[M+2Na] ²⁺	b ₁₃	y ₇	rt. (min)	R	R1	R2	<u>R3</u>	R4	R5	<u>R6</u>	R7	R8	<u>R9</u>	<u>R10</u>	<u>R11</u>	<u>R12</u>	<u>R13</u>	<u>R14</u>	<u>R15</u>	<u>R16</u>	<u>R17</u>	<u>R18</u>	R19	R20	Compound identical or positionally isomeric with	Reference
Pept-A-XIIIb	1951	1974	998.5	1163	789	44.16	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	<u>Lxx</u>	Ala	Gly	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	<u>Glu</u>	Gln	Pheol	New: Longibrachin B III: [Val] ⁹ →[Lxx] ⁹ and [Aib] ¹⁰ →[Ala] ¹⁰	Leclerc et al. 1998
																											New: Trilongin CII: [Vxx] ⁹ →[Lxx] ⁹ and [Aib] ¹⁰ →[Ala] ¹⁰	Mikkola et al. 2012	
																											New: Hypophellin 4: [Vxx] ⁹ →[Lxx] ⁹ and [Aib] ¹⁰ →[Ala] ¹⁰	Röhrich et al. 2013	
																											New: Pept-1952-d: [Vxx] ⁹ →[Lxx] ⁹ and [Aib] ¹⁰ →[Ala] ¹⁰	Tamandegani et al. 2016	
																											New: Longibrachin B II., Trilongin CI.: [Vxx] ⁹ →[Lxx] ⁹ and [Aib] ¹⁰ →[Ala] ¹⁰	Tamandegani et al. 2016	
																										New: Trichocellin TC-B-II: [Aib] ¹⁰ →[Ala] ¹⁰ and [Aib] ¹² →[Lxx] ¹²	Wada et al. 1994		
Pept-A-XIVa	1965	1988	1005.5	1177	789	44.22	Ac	Aib	Ala	Aib	Ala	Aib	<u>Aib</u>	Gln	Aib	<u>Lxx</u>	Ala	Gly	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	<u>Glu</u>	Gln	Pheol	Pept-1966-d	Tamandegani et al. 2016
Pept-A-XIVb	1964	1987	1005	1177	788	44.13	Ac	Aib	Ala	Aib	Ala	Aib	<u>Aib</u>	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol	(Position isomer of Pept-A-Xa, Pept-A-XVIIa, Pept-A-XXIa and Pept-XXVa)	→ Pept-A-Xa
Pept-A-XVa	1950	1973	998	1163	788	45.00	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol	(Position isomer of Pept-A-IXa and Pept-A-XVIIb)	→ Pept-A-IXa
Pept-A-XVb	1964	1987	1005	1177	788	44.74	Ac	Aib	Ala	Aib	Ala	Aib	<u>Aib</u>	Gln	Aib	<u>Lxx</u>	Ala	Gly	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol	Pept-1965-c-1, c-2 (Position isomer of Pept-A-XXIb)	Tamandegani et al. 2016
Pept-A-XVIa	1950	1973	998	1177	774	45.21	Ac	Aib	Ala	Aib	Ala	Aib	<u>Aib</u>	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	(Position isomer of Pept-A-Va)	→ Pept-A-Va
Pept-A-XVIIb	1950	1973	998	1177	774	45.33	Ac	Aib	Ala	Aib	Ala	Aib	<u>Aib</u>	Gln	Aib	<u>Lxx</u>	Ala	Gly	Lxx	Aib	Pro	Vxx	Aib	<i>Aib</i>	Gln	Gln	Pheol	New: Trichosporin TS-B-VIa: [Aib] ¹⁰ →[Ala] ¹⁰	Iida et al. 1990
																											New: <i>Trichoderma citrinoviride</i> sequence 7: [Aib] ¹⁰ →[Ala] ¹⁰	Maddau et al. 2009	
																										New: Pept-1965-c-1, c-2: [Vxx] ¹⁷ →[Aib] ¹⁷	Tamandegani et al. 2016		
Pept-A-XVIIa	1964	1987	1005	1177	788	46.21	Ac	Aib	Ala	Aib	Ala	Aib	<u>Aib</u>	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol	(Position isomer of Pept-A-Xa, Pept-A-XIVb, Pept-A-XXIa and Pept-XXVa)	→ Pept-A-Xa
Pept-A-XVIIb	1950	1973	998	1163	788	46.18	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol	(Position isomer of Pept-A-XVa and Pept-A-IXa)	→ Pept-A-IXa
Pept-A-XVIII	1978	2001	1012	1191	788	46.36	Ac	Aib	Ala	<u>Ixx</u>	Ala	Aib	<u>Aib</u>	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol	New: Trichosporin TS-B-IVd: [Ala] ³ →[Vxx] ³ (Position isomer of Pept-A-XXIV and Pept-XXVI)	Iida et al. 1990
																											New: Longibrachin A IV: [Aib] ³ →[Vxx] ³	Leclerc et al. 1998	
																											New: Trichoaureocin 6: [Aib] ³ →[Vxx] ³	Brückner et al. 2002	
																											New: Trichobrachin II 10, IIB D: [Aib] ³ →[Vxx] ³	Krause et al. 2007	
																											New: Trichokonin IX: [Aib] ³ →[Vxx] ³	Huang et al. 1995	
																											New: Trilongin BIV: [Aib] ³ →[Vxx] ³	Mikkola et al. 2012	
Pept-A-XIXa																										New: Metanicin D: [Aib] ³ →[Vxx] ³	Kimonyo and Brückner 2013		
																										New: Hypophellin 7: [Aib] ³ →[Vxx] ³	Röhrich et al. 2013		
Pept-A-XIXa	1951	1974	998.5	1177	775	46.67	Ac	Aib	Ala	Aib	Ala	Aib	<u>Aib</u>	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	Aib	<u>Glu</u>	Gln	Pheol	(Positional isomer of Pept-A-XIb)	→ Pept-A-XIb

TABLE 9 | Continued

Peptide	M	[M+Na] ⁺	[M+2Na] ²⁺	b ₁₃	y ₇	rt. (min)	R	R1	R2	<u>R3</u>	R4	R5	<u>R6</u>	R7	R8	<u>R9</u>	<u>R10</u>	<u>R11</u>	<u>R12</u>	<u>R13</u>	<u>R14</u>	<u>R15</u>	<u>R16</u>	<u>R17</u>	<u>R18</u>	<u>R19</u>	R20	Compound identical or positionally isomeric with	Reference
Pept-A-XIXb	1951	1974	998.5	1177	775	46.86	AcAib	Ala	Aib	Ala	Aib	Aib	Gln	Aib	Lxx	<u>Ala</u>	Gly	<u>Lxx</u>	Aib	Pro	Vxx	Aib	<u>Aib</u>	<u>Glu</u>	Gln	Pheol		New: Trichosporin TS-B-VIa: [Aib] ¹⁰ →[<i>Ala</i>] ¹⁰ and [Gln] ¹⁸ →[<i>Glu</i>] ¹⁸ New: <i>Trichoderma citrinoviride</i> sequence 7, [Aib] ¹⁰ →[<i>Ala</i>] ¹⁰ and [Gln] ¹⁸ →[<i>Glu</i>] ¹⁸ New: Pept-1965-c-1, c-2: [Vxx] ¹⁷ →[<i>Aib</i>] ¹⁷ and [Gln] ¹⁸ →[<i>Glu</i>] ¹⁸	Iida et al. 1990 Maddau et al. 2009 Tamandegani et al. 2016
Pept-A-XX	1964	1987	1005	1191	774	47.30	AcAib	Ala	<u>Ixx</u>	Ala	Aib	<u>Aib</u>	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol		New: Trichosporin TS-B-IVc: [Aib] ³ →[<i>Ixx</i>] ³ (Position isomer of Pept-A-XXIIa) New: Longibrachin A III: [Aib] ³ →[<i>Ixx</i>] ³ New: Trichoaureocin 5: [Aib] ³ →[<i>Ixx</i>] ³ New: Trichobrachin, IIb C: [Aib] ³ →[<i>Ixx</i>] ³ New: Trichokonin VIII: [Aib] ³ →[<i>Ixx</i>] ³ New: Trichosporin TS-B-IIId: [Ala] ³ →[<i>Ixx</i>] ³ New: Trilongin BIII: [Aib] ³ →[<i>Ixx</i>] ³ New: Metanicin C: [Aib] ³ →[<i>Ixx</i>] ³ New: Hypophellin 5: [Aib] ³ →[<i>Ixx</i>] ³ New: Longibrachin A III.: [Aib] ³ →[<i>Ixx</i>] ³	Iida et al. 1990 Leclerc et al. 1998 Brückner et al. 2002 Krause et al. 2007 Huang et al. 1996 Iida et al. 1990 Mikkola et al. 2012 Kimonyo and Brückner 2013 Röhrich et al. 2013 Tamandegani et al. 2016
Pept-A-XXIa	1964	1987	1005	1177	788	47.85	AcAib	Ala	Aib	Ala	Aib	<u>Aib</u>	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol		(Position isomer of Pept-A-Xa, Pept-A-XIVb, Pept-A-XVIIa and Pept-XXVa)	→ Pept-A-Xa
Pept-A-XXIb	1964	1987	1005	1177	788	47.75	AcAib	Ala	Aib	Ala	Aib	<u>Aib</u>	Gln	Aib	<u>Lxx</u>	Ala	Gly	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol		(Position isomer of Pept-A-XVb)	→ Pept-A-XVb
Pept-A-XXIIa	1964	1987	1005	1191	774	48.93	AcAib	Ala	<u>Ixx</u>	Ala	Aib	<u>Aib</u>	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol		New: (Position isomer of Pept-A-XX)	→ Pept-A-XX
Pept-A-XXIIb	1964	1987	1005	1191	774	48.79	AcAib	Ala	Aib	Ala	Aib	<u>Aib</u>	Gln	Aib	<u>Lxx</u>	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol		Trichosporin TS-B-VIa <i>Trichoderma citrinoviride</i> sequence 7	Iida et al. 1990 Maddau et al. 2009
Pept-A-XXIII	1965	1988	1005.5	1177	789	49.13	AcAib	Ala	Aib	Ala	Aib	<u>Aib</u>	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	<u>Glu</u>	Gln	Pheol		Trilongin CIV (Positional isomer of Pept-A-XXVIIa) Hypophellin 8	Mikkola et al. 2012 Röhrich et al. 2013
Pept-A-XXIV	1978	2001	1012	1191	788	49.89	AcAib	Ala	<u>Ixx</u>	Ala	Aib	<u>Aib</u>	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol		(Position isomer of Pept-A-XVIII and Pept-XXVI)	→ Pept-A-XVIII
Pept-A-XXVa	1964	1987	1005	1177	788	49.65	AcAib	Ala	Aib	Ala	Aib	<u>Aib</u>	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol		(Position isomer of Pept-A-Xa, Pept-A-XIVb, Pept-A-XVIIa and Pept-XXIa)	→ Pept-A-Xa
Pept-A-XXVb	1978	2001	1012	1191	788	49.72	AcAib	Ala	Aib	Ala	Aib	<u>Aib</u>	Gln	Aib	<u>Lxx</u>	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol		Suzukacillin A 12 (Position isomer of Pept-A-XXVIIb and Pept-XXVIIb)	Krause et al. 2006b
Pept-A-XXVIa	1978	2001	1012	1191	788	51.29	AcAib	Ala	<u>Ixx</u>	Ala	Aib	<u>Aib</u>	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol		(Position isomer of Pept-A-XVIII and Pept-XXIV)	→ Pept-A-XVIII
Pept-A-XXVIIb	1978	2001	1012	1191	788	50.85	AcAib	Ala	Aib	Ala	Aib	<u>Aib</u>	Gln	Aib	<u>Lxx</u>	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol		(Position isomer of Pept-A-XXVb and Pept-XXVIIb)	→ Pept-A-XVb
Pept-A-XXVIIa	1965	1988	1005.5	1177	789	51.44	AcAib	Ala	Aib	Ala	Aib	<u>Aib</u>	Gln	Aib	Vxx	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	<u>Glu</u>	Gln	Pheol		(Positional isomer of Pept-A-XXIII)	→ Pept-A-XXIII
Pept-A-XXVIIb	1978	2001	1012	1191	788	51.59	AcAib	Ala	Aib	Ala	Aib	<u>Aib</u>	Gln	Aib	<u>Lxx</u>	<u>Aib</u>	Gly	Lxx	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol		(Position isomer of Pept-A-XXVb and Pept-XXVIIb)	→ Pept-A-XVb

TABLE 10 | Sequences of the newly identified group B peptaibol compounds from *Trichoderma* species of clade Longibrachiatum and their similarities to known peptaibols available in the “Comprehensive Peptaibiotics Database”

Peptide	M	[M+Na] ⁺	[M+2Na] ²⁺	b ₁₃	y ₇	rt. (min)	R	R1	R2	<u>R3</u>	R4	<u>R5</u>	<u>R6</u>	R7	R8	<u>R9</u>	<u>R10</u>	<u>R11</u>	<u>R12</u>	<u>R13</u>	<u>R14</u>	<u>R15</u>	<u>R16</u>	<u>R17</u>	<u>R18</u>	<u>R19</u>	R20	Compound identical or positionally isomeric with	Reference
Pept-B-I	1908	1931	977	1135	774	22.59	AcAibAla	<u>Ala</u>	Ala	Aib	<u>Ala</u>	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol			New: Paracelsin B: [Aib] ³ →[Ala] ³ (Positional isomer of Pept-B-II, Pócsfalvi et al. 1997 III and V) New: Saturnisporin SA I: [Aib] ³ →[Ala] ³ New: Suzukacillin A 02, A 06: [Aib] ³ →[Ala] ³ New: Trichocellin TC-A-I, TC-A-III: [Aib] ³ →[Ala] ³	Rebuffat et al. 1993 Krause et al. 2006b Wada et al. 1994
Pept-B-II	1908	1931	977	1135	774	24.79	AcAibAla	<u>Ala</u>	Ala	Aib	<u>Ala</u>	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol			New: (Positional isomer of Pept-B-I, III and V)	→ Pept-B-I
Pept-B-III	1908	1931	977	1135	774	25.62	AcAibAla	<u>Ala</u>	Ala	Aib	<u>Ala</u>	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol			New: (Positional isomer of Pept-B-I, II and V)	→ Pept-B-I
Pept-B-IV	1922	1945	984	1135	788	25.72	AcAibAla	<u>Ala</u>	Ala	Aib	<u>Ala</u>	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol			New: Paracelsin H: [Aib] ³ →[Ala] ³ (Positional isomer of Pept-B-VII) New: Saturnisporin SA II: [Aib] ³ →[Ala] ³ New: Suzukacillin A 04, A 08: [Aib] ³ →[Ala] ³ New: Trichocellin TC-A-II, TC-A-IV: [Aib] ³ →[Ala] ³	Pócsfalvi et al. 1997 Rebuffat et al. 1993 Krause et al. 2006b Wada et al. 1994
Pept-B-V	1908	1931	977	1135	774	26.35	AcAibAla	<u>Ala</u>	Ala	Aib	<u>Ala</u>	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol			New: (Positional isomer of Pept-B-I, II and III)	→ Pept-B-I
Pept-B-VI	1922	1945	984	1149	774	27.22	AcAibAla	Aib	Ala	Aib	<u>Ala</u>	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol			Paracelsin B (Positional isomer of Pept-B-XII, XVIII and XXIII) Saturnisporin SA I Suzukacillin A 02, A 06 Trichocellin TC-A-I, TC-A-III	Pócsfalvi et al. 1997 Rebuffat et al. 1993 Krause et al. 2006b Wada et al. 1994
Pept-B-VII	1922	1945	984	1135	788	27.80	AcAibAla	<u>Ala</u>	Ala	Aib	<u>Ala</u>	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol			New: (Positional isomer of Pept-B-IV)	→ Pept-B-IV
Pept-B-VIII	1936	1959	991	1149	788	27.27	AcAibAla	Aib	Ala	Aib	<u>Ala</u>	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol			Paracelsin H (Positional isomer of Pept-B-XVII, XIX, XXII and XXIXb) Saturnisporin SA II Suzukacillin A 04, A 08 Trichocellin TC-A-II, TC-A-IV	Pócsfalvi et al. 1997 Rebuffat et al. 1993 Krause et al. 2006b Wada et al. 1994
Pept-B-IXa	1908	1931	977	1135	774	28.44	AcAibAla	Aib	Ala	Aib	<u>Ala</u>	Gln	Aib	<u>Vxx</u>	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol			Paracelsin A Suzukacillin A 01	Pócsfalvi et al. 1997 Krause et al. 2006b
Pept-B-IXb	1908	1931	977	1135	774	28.38	AcAibAla	Aib	Ala	Aib	<u>Ala</u>	Gln	Aib	Lxx	Aib	Gly	<u>Ala</u>	Aib	Pro	Vxx	Aib	<i>Aib</i>	Gln	Gln	Pheol			New: <i>Trichoderma citrinoviride</i> sequence 1: [Vxx] ¹⁷ →[Aib] ¹⁷	Maddau et al. 2009
Pept-B-X	1922	1945	984	1135	788	28.77	AcAibAla	Aib	Ala	Aib	<u>Ala</u>	Gln	Aib	<u>Vxx</u>	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol			Paracelsin F (Positional isomer of Pept-B-XI, XIII and XVa) Suzukacillin A 03	Pócsfalvi et al. 1997 Krause et al. 2006b
Pept-B-XI	1922	1945	984	1135	788	29.25	AcAibAla	Aib	Ala	Aib	<u>Ala</u>	Gln	Aib	<u>Vxx</u>	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol			(Positional isomer of Pept-B-X, XIII and XVa)	→ Pept-B-X
Pept-B-XII	1922	1945	984	1149	774	29.90	AcAibAla	Aib	Ala	Aib	<u>Ala</u>	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol			(Positional isomer of Pept-B-VI, XVIII and XXIII)	→ Pept-B-VI
Pept-B-XIII	1922	1945	984	1135	788	30.28	AcAibAla	Aib	Ala	Aib	<u>Ala</u>	Gln	Aib	<u>Vxx</u>	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol			(Positional isomer of Pept-B-X, XI and XVa)	→ Pept-B-X
Pept-B-XIVa	1923	1946	984.5	1149	775	31.36	AcAibAla	Aib	Ala	Aib	<u>Ala</u>	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	<u>Glu</u>	Gln	Pheol			Trichocellin TC-B-I	Wada et al. 1994
Pept-B-XIVb	1923	1946	984.5	1149	775	31.40	AcAibAla	Aib	Ala	Aib	<i>Aib</i>	Gln	Aib	<u>Vxx</u>	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	<u>Glu</u>	Gln	Pheol			New: Trichocellin TC-B-I: [Ala] ⁶ →[Aib] ⁶ and [Leu] ⁹ →[Vxx] ⁹	Wada et al. 1994

Variable residues in table header and minor sequence variants in sequences are underlined. Amino acid exchanges in new compounds are set in italic.

TABLE 10 | Continued

Peptide	M	[M+Na] ⁺	[M+2Na] ²⁺	b ₁₃	y ₇	rt. (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	Reference
Pept-B-XVa	1922	1945	984	1135	788	31.48	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	Vxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Vxx	Gln	Gln	Pheol	(Positional isomer of Pept-B-X, XI and -XIII)	→ Pept-B-X
Pept-B-XVb	1922	1945	984	1135	788	31.53	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	Lxx	Aib	Gly	Ala	Aib	Pro	Vxx	Aib	Vxx	Gln	Gln	Pheol	<i>Trichoderma citrinoviride</i> sequence 1	Maddau et al. 2009
Pept-B-XVI	1922	1945	984	1149	774	31.98	Ac	Aib	Ala	Aib	Ala	Aib	Aib	Gln	Aib	Vxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	Paracelsin C	Pócsfalvi et al. 1997
Pept-B-XVII	1936	1959	991	1149	788	32.67	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Vxx	Gln	Gln	Pheol	(Positional isomer of Pept-B-VIII, XIX, XXII and XXIXb)	→ Pept-B-VIII
Pept-B-XVIII	1922	1945	984	1149	774	33.49	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	(Positional isomer of Pept-B-VI, XII and XXIII)	→ Pept-B-VI
Pept-B-XIX	1936	1959	991	1149	788	33.55	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Vxx	Gln	Gln	Pheol	(Positional isomer of Pept-B-VIII, XVII, XXII and XXIXb)	→ Pept-B-VIII
Pept-B-XX	1936	1959	991	1163	774	34.41	Ac	Aib	Ala	Aib	Ala	Aib	Aib	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	Paracelsin D (Positional isomer of Pept-B-XXXIIIa, XXXVa, XLIIb, XLVIa and LVIII)	Pócsfalvi et al. 1997
																											Saturnisporin SA III	Rebuffat et al. 1993	
																											Suzukacillin A 05	Krause et al. 2006b	
Pept-B-XXI	1937	1960	991.5	1149	789	34.15	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Vxx	Glu	Gln	Pheol	Trichocellin TC-B-II	Wada et al. 1994
Pept-B-XXII	1936	1959	991	1149	788	34.59	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Vxx	Gln	Gln	Pheol	(Positional isomer of Pept-B-VIII, XVII, XIX and XXIXb)	→ Pept-B-VIII
Pept-B-XXIII	1922	1945	984	1149	774	35.25	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	(Positional isomer of Pept-B-VI, XII and XVIII)	→ Pept-B-VI
Pept-B-XXIV	1950	1973	998	1163	788	35.59	Ac	Aib	Ala	Aib	Ala	Aib	Aib	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Vxx	Gln	Gln	Pheol	Saturnisporin SA IV (Positional isomer of Pept-B-XXVII, XXXIIa, XXXVIII and XLVa)	Rebuffat et al. 1993
																											Suzukacillin A 07	Krause et al. 2006b	
																											Longibrachin B II., Trilongin CI.	Tamandegani et al. 2016	
Pept-B-XXV	1937	1960	991.5	1163	775	35.97	Ac	Aib	Ala	Aib	Ala	Aib	Aib	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	Glu	Gln	Pheol	New: Trichocellin TC-B-I: [Ala] ⁶ →[Aib] ⁶ (Positional isomer of Pept-B-XXXVII)	Wada et al. 1994
																											New: Paracelsin D: [Gln] ¹⁸ →[Glu] ¹⁸	Pócsfalvi et al. 1997	
																											New: Saturnisporin SA III: [Gln] ¹⁸ →[Glu] ¹⁸	Rebuffat et al. 1993	
																											New: Suzukacillin A 05: [Gln] ¹⁸ →[Glu] ¹⁸	Krause et al. 2006b	
Pept-B-XXVI	1950	1973	998	1177	774	36.65	Ac	Aib	Ala	Ixx	Ala	Aib	Aib	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	New: Paracelsin D: [Aib] ³ →[Vxx] ³	Pócsfalvi et al. 1997
																											New: Saturnisporin SA III: [Aib] ³ →[Vxx] ³	Rebuffat et al. 1993	
																											New: Suzukacillin A 05: [Aib] ³ →[Vxx] ³	Krause et al. 2006b	
Pept-B-XXVII	1950	1973	998	1163	788	37.31	Ac	Aib	Ala	Aib	Ala	Aib	Aib	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Vxx	Gln	Gln	Pheol	(Positional isomer of Pept-B-XXIV, XXXIIa, XXXVIII and XLVa)	→ Pept-B-XXIV
Pept-B-XXVIII	1950	1973	998	1177	774	37.89	Ac	Aib	Ala	Aib	Ala	Ixx	Aib	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	New: Paracelsin D: [Aib] ⁵ →[Vxx] ⁵	Pócsfalvi et al. 1997
																											New: Saturnisporin SA III: [Aib] ⁵ →[Vxx] ⁵	Rebuffat et al. 1993	
																											New: Suzukacillin A 05: [Aib] ⁵ →[Vxx] ⁵	Krause et al. 2006b	
Pept-B-XXIXa	1936	1959	991	1149	788	38.30	Ac	Aib	Ala	Aib	Ala	Aib	Aib	Gln	Aib	Lxx	Ala	Gly	Aib	Aib	Pro	Vxx	Aib	Vxx	Gln	Gln	Pheol	New: Paracelsin D: [Aib] ¹⁰ →[Ala] ¹⁰	Pócsfalvi et al. 1997
																											New: Saturnisporin SA III: [Aib] ¹⁰ →[Ala] ¹⁰	Rebuffat et al. 1993	
																											New: Suzukacillin A 05: [Aib] ¹⁰ →[Ala] ¹⁰	Krause et al. 2006b	

TABLE 10 | Continued

Peptide	M	[M+Na] ⁺	[M+2Na] ²⁺	b ₁₃	y ₇	rt. (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	Reference
Pept-B-XXIXb	1936	1959	991	1149	788	37.80	Ac	Aib	Ala	Aib	Ala	Aib	<u>Ala</u>	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol	(Positional isomer of Pept-B-VIII, XVII, XIX and XXII)	→ Pept-B-VIII
Pept-B-XXX	1950	1973	998	1177	774	38.51	Ac	Aib	Ala	Aib	Ala	Aib	Aib	Gln	Aib	Lxx	Aib	Gly	<u>Vxx</u>	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	<i>Trichoderma citrinoviride</i> sequence 4 (Positional isomer of Pept-B-XXXIIIc, XLIIa, XLVIb and LIII)	Maddau et al. 2009
Pept-B-XXXI	1951	1974	998.5	1163	789	39.13	Ac	Aib	Ala	Aib	Ala	Aib	<i>Aib</i>	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	<u>Vxx</u>	<u>Glu</u>	Gln	Pheol	New: Trichocellin TC-B-II: [Ala] ⁶ →[Aib] ⁶ (Positional isomer of Pept-B-XXXIVb and LII) New: Saturnisporin SA IV: [Gln] ¹⁸ →[Glu] ¹⁸ New: Suzukacillin A 07: [Gln] ¹⁸ →[Glu] ¹⁸ New: Pept-1965-c-1, c-2: [Vxx] ¹⁷ →[Aib] ¹⁷	Wada et al. 1994 Rebuffat et al. 1993 Krause et al. 2006b Tamandegani et al. 2016
Pept-B-XXXIIa	1950	1973	998	1163	788	39.15	Ac	Aib	Ala	Aib	Ala	Aib	Aib	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol	(Positional isomer of Pept-B-XXIV, XXVII, XXXVIII and XLVa)	→ Pept-B-XXIV
Pept-B-XXXIIb	1964	1987	1005	1177	788	39.20	Ac	Aib	Ala	Aib	Ala	Aib	<u>I_{xxx}</u>	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol	New: Paracelsin H: [Ala] ⁶ →[Vxx] ⁶ (Positional isomer of Pept-B-XLIb) New: Saturnisporin SA II: [Ala] ⁶ →[Vxx] ⁶ New: Saturnisporin SA IV: [Aib] ⁶ →[Vxx] ⁶ New: Suzukacillin A 04, 08: [Ala] ⁶ →[Vxx] ⁶ New: Suzukacillin A 07: [Ala] ⁶ →[Vxx] ⁶ New: Trichocellin TC-A-II, TC-A-IV: [Ala] ⁶ →[Vxx] ⁶	Pócsfalvi et al. 1997 Rebuffat et al. 1993 Rebuffat et al. 1993 Krause et al. 2006b Krause et al. 2006b Wada et al. 1994
Pept-B-XXXIIIa	1936	1959	991	1163	774	38.98	Ac	Aib	Ala	Aib	Ala	Aib	Aib	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	(Positional isomer of Pept-B-XX, XXXVa, XLIIb, XLVIa and LVIII)	→ Pept-B-XX
Pept-B-XXXIIIb	1936	1959	991	1163	774	39.25	Ac	Aib	Ala	Aib	Ala	Aib	<u>Ala</u>	Gln	Aib	Lxx	Aib	Gly	<u>Vxx</u>	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	<i>Trichoderma citrinoviride</i> sequence 2 (Positional isomer of Pept-XXXVb)	Maddau et al. 2009
Pept-B-XXXIIIc	1950	1973	998	1177	774	39.20	Ac	Aib	Ala	Aib	Ala	Aib	Aib	Gln	Aib	Lxx	Aib	Gly	<u>Vxx</u>	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	(Positional isomer of Pept-B-XXX, XLIIa, XLVIb and LIII)	→ Pept-B-XXX
Pept-B-XXXIIId	1951	1974	998.5	1177	775	39.31	Ac	Aib	Ala	Aib	Ala	<u>I_{xxx}</u>	Aib	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	<u>Glu</u>	Gln	Pheol	New: Paracelsin D: [Aib] ⁵ →[I _{xxx}] ⁵ and [Aib] ¹⁸ →[Glu] ¹⁸ New: Saturnisporin SA III: [Aib] ⁵ →[I _{xxx}] ⁵ and [Aib] ¹⁸ →[Glu] ¹⁸ New: Suzukacillin A 05: [Aib] ⁵ →[I _{xxx}] ⁵ and [Aib] ¹⁸ →[Glu] ¹⁸	Pócsfalvi et al. 1997 Rebuffat et al. 1993 Krause et al. 2006b
Pept-B-XXXIVa	1937	1960	991.5	1149	789	39.59	Ac	Aib	Ala	Aib	Ala	Aib	Aib	Gln	Aib	Lxx	<u>Ala</u>	Gly	<i>Aib</i>	Aib	Pro	Vxx	Aib	<u>Vxx</u>	<u>Glu</u>	Gln	Pheol	New: Pept-1966-d: [Lxx] ¹² →[Aib] ¹²	Tamandegani et al. 2016
Pept-B-XXXIVb	1951	1974	998.5	1163	789	39.13	Ac	Aib	Ala	Aib	Ala	Aib	<i>Aib</i>	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	<u>Vxx</u>	<u>Glu</u>	Gln	Pheol	New: (Positional isomer of Pept-B-XXXI and LII)	→ Pept-B-XXXI

TABLE 10 | Continued

Peptide	M	[M+Na] ⁺	[M+2Na] ²⁺	b ₁₃	y ₇	rt. (min)	R R1 R2 R3 R4 R5 R6 R7 R8 R9 R10R11R12R13R14R15R16R17R18R19 R20	Compound identical or positionally isomeric with	Ref.
Pept-B-XXXVa	1936	1959	991	1163	774	39.17	AcAibAlaAibAlaAibAibGlnAibLxxAib Gly Aib Aib Pro Vxx Aib Aib Gln Gln Pheol	(Positional isomer of Pept-B-XX, XXXIIIa, XLIIb, XLVIa and LVIII)	→ Pept-B-XX
Pept-B-XXXVb	1936	1959	991	1163	774	39.78	AcAibAlaAibAlaAibAibAlaGlnAibLxxAib Gly Vxx Aib Pro Vxx Aib Aib Gln Gln Pheol	(Positional isomer of Pept-B-XXXIIIb)	→ Pept-B-XXXIIIb
Pept-B-XXXVI	1964	1987	1005	1191	774	39.85	AcAibAlaVxxAlaVxxAibGlnAibLxxAib Gly Aib Aib Pro Vxx Aib Aib Gln Gln Pheol	New: Paracelsin D: [Aib] ³ →[Vxx] ³ and [Aib] ⁵ →[Vxx] ⁵ New: Saturnisporin SA III: [Aib] ³ →[Vxx] ³ and [Aib] ⁵ →[Vxx] ⁵ New: Suzukacillin A 05: [Aib] ³ →[Vxx] ³ and [Aib] ⁵ →[Vxx] ⁵	Pócsfalvi et al. 1997 Rebuffat et al. 1993 Krause et al. 2006b
Pept-B-XXXVII	1937	1960	991.5	1163	775	40.54	AcAibAlaAibAlaAibAibGlnAibLxxAib Gly Aib Aib Pro Vxx Aib Aib Glu	Gln Pheol New: (Positional isomer of Pept-B-XXV)	→ Pept-B-XXV
Pept-B-XXXVIII	1950	1973	998	1163	788	40.13	AcAibAlaAibAlaAibAibGlnAibLxxAib Gly Aib Aib Pro Vxx Aib Vxx	Gln Gln Pheol (Positional isomer of Pept-B-XXIV, XXVII, XXXIIa and XLVa)	→ Pept-B-XXIV
Pept-B-XXXIX	1964	1987	1005	1177	788	39.57	AcAibAlaAibAlaVxxAibGlnAibLxxAib Gly Aib Aib Pro Vxx Aib Vxx	Gln Gln Pheol New: Saturnisporin SA IV: [Aib] ⁵ →[Vxx] ⁵ New: Suzukacillin A 07: [Aib] ⁵ →[Vxx] ⁵	Rebuffat et al. 1993 Krause et al. 2006b
Pept-B-XL	1950	1973	998	1163	788	40.55	AcAibAlaAibAlaAibAlaGlnAibLxxAib Gly Vxx Aib Pro Vxx Aib Vxx	Gln Gln Pheol New: Trichoderma citrinoviride sequence 5: [Aib] ⁶ →[Ala] ⁶ (Positional isomer of Pept-B-XLVIIIa) New: Trichoderma citrinoviride sequence 6: [Aib] ⁶ →[Ala] ⁶ New: Trichoderma citrinoviride sequence 8: [Aib] ⁶ →[Ala] ⁶	Maddau et al. 2009 Maddau et al. 2009 Maddau et al. 2009
Pept-B-XLIa	1964	1987	1005	1177	788	40.98	AcAibAlaAibAlaAibAibGlnAibLxxAib Gly Vxx Aib Pro Vxx Aib Vxx	Gln Gln Pheol Trichoderma citrinoviride sequence 5 (Positional isomer of Pept-B-XLVIIIb, LV, LVI, LXb and LXI) Trichoderma citrinoviride sequence 6 Trichoderma citrinoviride sequence 8	Maddau et al. 2009 Maddau et al. 2009 Maddau et al. 2009
Pept-B-XLIb	1964	1987	1005	1177	788	40.82	AcAibAlaAibAlaAibVxxGlnAibLxxAib Gly Aib Aib Pro Vxx Aib Vxx	Gln Gln Pheol New: (Positional isomer of Pept-B-XXXIIb)	→ Pept-B-XXXIIb
Pept-B-XLIIa	1950	1973	998	1177	774	41.54	AcAibAlaAibAlaAibAibGlnAibLxxAib Gly Vxx Aib Pro Vxx Aib Aib Gln Gln Pheol	(Positional isomer of Pept-B-XXX, XXXIIIc, XLVIb and LIII)	→ Pept-B-XXX
Pept-B-XLIIb	1936	1959	991	1163	774	41.64	AcAibAlaAibAlaAibAibGlnAibLxxAib Gly Aib Aib Pro Vxx Aib Aib Gln Gln Pheol	Paracelsin D (Positional isomer of Pept-B-XX, XXXIIIa, XXXVa, XLVIa and LVIII)	→ Pept-B-XX
Pept-B-XLIII	1964	1987	1005	1191	774	41.26	AcAibAlaAibAlaVxxVxxGlnAibLxxAib Gly Aib Aib Pro Vxx Aib Aib Gln Gln Pheol	New: Paracelsin B: [Aib] ⁵ →[Vxx] ⁵ and [Ala] ⁶ →[Vxx] ⁶ New: Paracelsin D: [Aib] ⁵ →[Vxx] ⁵ and [Aib] ⁶ →[Vxx] ⁶ New: Saturnisporin SA I: [Aib] ⁵ →[Vxx] ⁵ and [Ala] ⁶ →[Vxx] ⁶ New: Saturnisporin SA III: [Aib] ⁵ →[Vxx] ⁵ and [Aib] ⁶ →[Vxx] ⁶ New: Suzukacillin A 02, A 06: [Aib] ⁵ →[Vxx] ⁵ and [Ala] ⁶ →[Vxx] ⁶ New: Suzukacillin A 05: [Aib] ⁵ →[Vxx] ⁵ and [Aib] ⁶ →[Vxx] ⁶ New: Trichocellin TC-A-I, TC-A-III: [Aib] ⁵ →[Vxx] ⁵ and [Ala] ⁶ →[Vxx] ⁶	Pócsfalvi et al. 1997 Pócsfalvi et al. 1997 Rebuffat et al. 1993 Rebuffat et al. 1993 Krause et al. 2006b Krause et al. 2006b Wada et al. 1994
Pept-B-XLIV	1965	1988	1005.5	1191	775	41.65	AcAibAlaAibAlaVxxVxxGlnAibLxxAib Gly Aib Aib Pro Vxx Aib Aib Glu	Gln Pheol New: Trichocellin TC-B-I: [Aib] ³ →[Vxx] ³ and [Ala] ⁶ →[Vxx] ⁶ (Positional isomer of Pept-B-L)	Wada et al. 1994
Pept-B-XLVa	1950	1973	998	1163	788	41.92	AcAibAlaAibAlaAibAibGlnAibLxxAib Gly Aib Aib Pro Vxx Aib Vxx	Gln Gln Pheol (Positional isomer of Pept-B-XXIV, XXVII, XXXIIa and XXXVIII)	→ Pept-B-XXIV

TABLE 10 | Continued

Peptide	M	[M+Na] ⁺	[M+2Na] ²⁺	b ₁₃	y ₇	rt. (min)	R	R1	R2	<u>R3</u>	R4	R5	<u>R6</u>	R7	R8	<u>R9</u>	<u>R10</u>	<u>R11</u>	<u>R12</u>	<u>R13</u>	<u>R14</u>	<u>R15</u>	<u>R16</u>	<u>R17</u>	<u>R18</u>	<u>R19</u>	<u>R20</u>	Compound identical or positionally isomeric with	Ref.
Pept-B-XLVb	1978	2001	1012	1191	788	42.47	Ac	Aib	Ala	<u>Ixx</u>	Ala	Aib	<u>Ixx</u>	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol	New: Paracelsin H: [Aib] ³ →[<i>Ixx</i>] ³ and [Ala] ⁵ →[<i>Ixx</i>] ⁵ (Positional isomer of Pept-B-XLIX) New: Saturnisporin SA II: [Aib] ³ →[<i>Ixx</i>] ³ and [Ala] ⁶ →[<i>Ixx</i>] ⁶ New: Saturnisporin SA IV: [Aib] ³ →[<i>Ixx</i>] ³ and [Aib] ⁶ →[<i>Ixx</i>] ⁶ New: Suzukacillin A 04, 08: [Aib] ³ →[<i>Ixx</i>] ³ and [Ala] ⁶ →[<i>Ixx</i>] ⁶ New: Suzukacillin A 07: [Aib] ³ →[<i>Ixx</i>] ³ and [Aib] ⁶ →[<i>Ixx</i>] ⁶ New: Trichocellin TC-A-II, A-IV: [Aib] ³ →[<i>Ixx</i>] ³ and [Ala] ⁶ →[<i>Ixx</i>] ⁶	Pócsfalvi et al. 1997 Rebuffat et al. 1993 Rebuffat et al. 1993 Krause et al. 2006b Krause et al. 2006b Wada et al. 1994
Pept-B-XLVla	1936	1959	991	1163	774	42.42	Ac	Aib	Ala	Aib	Ala	Aib	Aib	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	(Positional isomer of Pept-B-XX, XXXIIIa, XXXVa, XLIIb and LVIII)	→ Pept-B-XX
Pept-B-XLVlb	1950	1973	998	1177	774	42.42	Ac	Aib	Ala	Aib	Ala	Aib	Aib	Gln	Aib	Lxx	Aib	Gly	<u>Vxx</u>	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	(Positional isomer of Pept-B-XXX, XXXIIIc, XLIIa and LIII)	→ Pept-B-XXX
Pept-B-XLVII	1965	1988	1005.5	1177	789	42.13	Ac	Aib	Ala	Aib	Ala	<u>Ixx</u>	<i>Aib</i>	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	<u>Vxx</u>	<u>Glu</u>	Gln	Pheol	New: Trichocellin TC-B-II: [Aib] ⁵ →[<i>Ixx</i>] ⁵ and [Gln] ¹⁸ →[<i>Glu</i>] ¹⁸ New: Saturnisporin SA IV: [Aib] ⁵ →[<i>Ixx</i>] ⁵ and [Gln] ¹⁸ →[<i>Glu</i>] ¹⁸ New: Suzukacillin A 07: [Aib] ⁵ →[<i>Ixx</i>] ⁵ and [Gln] ¹⁸ →[<i>Glu</i>] ¹⁸	Wada et al. 1994 Rebuffat et al. 1993 Krause et al. 2006b
Pept-B-XLVIIIa	1950	1973	998	1163	788	43.00	Ac	Aib	Ala	Aib	Ala	Aib	<u>Ala</u>	Gln	Aib	Lxx	Aib	Gly	<u>Vxx</u>	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol	New: (Positional isomer of Pept-B-XL)	→ Pept-B-XL
Pept-B-XLVIIIb	1964	1987	1005	1177	788	42.85	Ac	Aib	Ala	Aib	Ala	Aib	Aib	Gln	Aib	Lxx	Aib	Gly	<u>Vxx</u>	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol	(Positional isomer of Pept-B-XLIa, LV, LVI, LXb and LXI)	→ Pept-B-XLIa
Pept-B-XLIX	1978	2001	1012	1191	788	42.56	Ac	Aib	Ala	<u>Ixx</u>	Ala	Aib	<u>Ixx</u>	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol	New: (Positional isomer of Pept-B-XLVb)	→ Pept-B-XLVb
Pept-B-L	1965	1988	1005.5	1191	775	42.67	Ac	Aib	Ala	Aib	Ala	<u>Ixx</u>	<u>Ixx</u>	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	<u>Glu</u>	Gln	Pheol	New: (Positional isomer of Pept-B-XLIV) Longibrachin B III., Trilongin CIII.	→ Pept-B-XLIV Tamandegani et al. 2016
Pept-B-LI	1978	2001	1012	1205	774	43.24	Ac	Aib	Ala	<u>Ixx</u>	Ala	<u>Ixx</u>	<u>Ixx</u>	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	New: Paracelsin B: [Aib] ³ →[<i>Ixx</i>] ³ , [Aib] ⁵ →[<i>Ixx</i>] ⁵ and [Ala] ⁶ →[<i>Ixx</i>] ⁶ New: Paracelsin D: [Aib] ³ →[<i>Ixx</i>] ³ , [Aib] ⁵ →[<i>Ixx</i>] ⁵ and [Aib] ⁶ →[<i>Ixx</i>] ⁶ New: Saturnisporin SA I: [Aib] ³ →[<i>Ixx</i>] ³ , [Aib] ⁵ →[<i>Ixx</i>] ⁵ and [Ala] ⁶ →[<i>Ixx</i>] ⁶ New: Saturnisporin SA III: [Aib] ³ →[<i>Ixx</i>] ³ , [Aib] ⁵ →[<i>Ixx</i>] ⁵ and [Aib] ⁶ →[<i>Ixx</i>] ⁶ New: Suzukacillin A 02, A 06: [Aib] ³ →[<i>Ixx</i>] ³ , [Aib] ⁵ →[<i>Ixx</i>] ⁵ and [Ala] ⁶ →[<i>Ixx</i>] ⁶ New: Suzukacillin A 05: [Aib] ³ →[<i>Ixx</i>] ³ , [Aib] ⁵ →[<i>Ixx</i>] ⁵ and [Aib] ⁶ →[<i>Ixx</i>] ⁶ New: Trichocellin TC-A-I, TC-A-III : [Aib] ³ →[<i>Ixx</i>] ³ , [Aib] ⁵ →[<i>Ixx</i>] ⁵ and [Ala] ⁶ →[<i>Ixx</i>] ⁶	Pócsfalvi et al. 1997 Pócsfalvi et al. 1997 Rebuffat et al. 1993 Rebuffat et al. 1993 Krause et al. 2006b Krause et al. 2006b Wada et al. 1994
Pept-B-LII	1951	1974	998.5	1163	789	43.38	Ac	Aib	Ala	Aib	Ala	Aib	<i>Aib</i>	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	<u>Vxx</u>	<u>Glu</u>	Gln	Pheol	New: (Positional isomer of Pept-B-XXXI and XXXIVb)	→ Pept-B-XXXI

TABLE 10 | Continued

Peptide	M	[M+Na] ⁺	[M+2Na] ²⁺	b ₁₃	y ₇	rt. (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	Reference
Pept-B-LIII	1950	1973	998	1177	774	43.99	Ac	Aib	Ala	Aib	Ala	Aib	Aib	Gln	Aib	Lxx	Aib	Gly	V _{xx}	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	(Positional isomer of Pept-B-XXX, XXXIIIc, XLIIa and XLVIb) Pept-1952-d Longibrachin A II., Trilongin BII.	→ Pept-B-XXX Tamandegani et al. 2016 Tamandegani et al. 2016
Pept-B-LIV	1978	2001	1012	1191	788	44.00	Ac	Aib	Ala	Aib	Ala	V _{xx}	V _{xx}	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	V _{xx}	Gln	Gln	Pheol	New: Paracelsin H: [Aib] ⁵ →[V _{xx}] ⁵ and [Ala] ⁶ →[V _{xx}] ⁶ New: Saturnisporin SA II: [Aib] ⁵ →[V _{xx}] ⁵ and [Aib] ⁶ →[V _{xx}] ⁶ New: Saturnisporin SA IV: [Aib] ⁵ →[V _{xx}] ⁵ and [Ala] ⁶ →[V _{xx}] ⁶ New: Suzukacillin A 04, 08: [Aib] ⁵ →[V _{xx}] ⁵ and [Ala] ⁶ →[V _{xx}] ⁶ New: Suzukacillin A 07: [Aib] ⁵ →[V _{xx}] ⁵ and [Aib] ⁶ →[V _{xx}] ⁶ New: Trichocellin TC-A-II, A-IV: [Aib] ⁵ →[V _{xx}] ⁵ and [Ala] ⁶ →[V _{xx}] ⁶	Pöcsfalvi et al. 1997 Rebuffat et al. 1993 Rebuffat et al. 1993 Krause et al. 2006b Krause et al. 2006b Wada et al. 1994
Pept-B-LV	1964	1987	1005	1177	788	44.44	Ac	Aib	Ala	Aib	Ala	Aib	Aib	Gln	Aib	Lxx	Aib	Gly	V _{xx}	Aib	Pro	Vxx	Aib	V _{xx}	Gln	Gln	Pheol	(Positional isomer of Pept-B-XLIa, XLVIIIb, LVI, LXb and LXI)	→ Pept-B-XLIa
Pept-B-LVI	1950	1973	998	1163	788	45.07	Ac	Aib	Ala	Aib	Ala	Aib	Ala	Gln	Aib	Lxx	Aib	Gly	V _{xx}	Aib	Pro	Vxx	Aib	V _{xx}	Gln	Gln	Pheol	(Positional isomer of Pept-B-XLIa, XLVIIIb, LV, LXb and LXI)	→ Pept-B-XLIa
Pept-B-LVII	1979	2002	1012.5	1191	789	45.36	Ac	Aib	Ala	Aib	Ala	V _{xx}	V _{xx}	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	V _{xx}	Glu	Gln	Pheol	New: Trichocellin TC-B-II: [Aib] ⁵ →[V _{xx}] ⁵ and [Ala] ⁶ →[V _{xx}] ⁶	Wada et al. 1994
Pept-B-LVIII	1936	1959	991	1163	774	45.81	Ac	Aib	Ala	Aib	Ala	Aib	Aib	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	(Positional isomer of Pept-B-XX, XXXIIIa, XXVc, XLIIb and XLVIa)	→ Pept-B-XX
Pept-B-LIX	1992	2015	1019	1205	788	45.74	Ac	Aib	Ala	V _{xx}	Ala	V _{xx}	V _{xx}	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	V _{xx}	Gln	Gln	Pheol	New: Paracelsin H: [Aib] ³ →[V _{xx}] ³ , [Aib] ⁵ →[V _{xx}] ⁵ and [Ala] ⁶ →[V _{xx}] ⁶ New: Saturnisporin SA II: [Aib] ³ →[V _{xx}] ³ , [Aib] ⁵ →[V _{xx}] ⁵ and [Ala] ⁶ →[V _{xx}] ⁶ New: Saturnisporin SA IV: [Aib] ³ →[V _{xx}] ³ , [Aib] ⁵ →[V _{xx}] ⁵ and [Aib] ⁶ →[V _{xx}] ⁶ New: Suzukacillin A 04, 08: [Aib] ³ →[V _{xx}] ³ , [Aib] ⁵ →[V _{xx}] ⁵ and [Ala] ⁶ →[V _{xx}] ⁶ New: Suzukacillin A 07: [Aib] ³ →[V _{xx}] ³ , [Aib] ⁵ →[V _{xx}] ⁵ and [Aib] ⁶ →[V _{xx}] ⁶ New: Trichocellin TC-A-II, A-IV: [Aib] ³ →[V _{xx}] ³ , [Aib] ⁵ →[V _{xx}] ⁵ and [Ala] ⁶ →[V _{xx}] ⁶	Pöcsfalvi et al. 1997 Rebuffat et al. 1993 Rebuffat et al. 1993 Krause et al. 2006b Krause et al. 2006b Wada et al. 1994
Pept-B-LXa	1964	1987	1005	1177	788	46.50	Ac	Aib	Ala	Aib	Ala	Aib	Aib	Gln	Aib	V _{xx}	Aib	Gly	L _{xx}	Aib	Pro	Vxx	Aib	V _{xx}	Gln	Gln	Pheol	(Positional isomer of Pept-B-XLIXa)	→ Pept-B-XLIXa
Pept-B-LXb	1964	1987	1005	1177	788	46.29	Ac	Aib	Ala	Aib	Ala	Aib	Aib	Gln	Aib	Lxx	Aib	Gly	V _{xx}	Aib	Pro	Vxx	Aib	V _{xx}	Gln	Gln	Pheol	(Positional isomer of Pept-B-XLIa, XLVIIIb, LV, LVI and LXI)	→ Pept-B-XLIa
Pept-B-LXI	1964	1987	1005	1177	788	48.35	Ac	Aib	Ala	Aib	Ala	Aib	Aib	Gln	Aib	Lxx	Aib	Gly	V _{xx}	Aib	Pro	Vxx	Aib	V _{xx}	Gln	Gln	Pheol	(Positional isomer of Pept-B-XLIa, XLVIIIb, LV, LVIII and LXb)	→ Pept-B-XLIa

TABLE 11 | Sequences of the newly identified brevicelsins (group C) from *Trichoderma* species of clade Longibrachiatum and their similarities to known peptaibols available in the “Comprehensive Peptaibiotics Database”

Peptide	M	[M+Na] ⁺	[M+2Na] ²⁺	b ₁₃	y ₇	rt. (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	Reference
Brevicelsin-I	1851	1874	948.5	1078774	28.72		Ac	Aib	Ala	Aib	Ala	Aib	-	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	New: Hypophellin 18, 35, 39: [Lxx] ¹¹ →[Aib] ¹¹ New: Paracelsin B, D: [Ala] ⁶ , [Aib] ⁶ New: Saturnisporin SA I., SA III: [Ala] ⁶ , [Aib] ⁶ New: Suzukacillin A 02, A 06, A 05: [Ala] ⁶ , [Aib] ⁶ New: Trichocellin TC-A-I, -III: [Ala] ⁶	Röhrich et al. 2013 Pócsfalvi et al. 1997 Rebuffat et al. 1993 Krause et al. 2006b Wada et al. 1994
Brevicelsin-II	1865	1888	955.5	1092774	29.98		Ac	Aib	Ala	<u>Lxx</u>	Ala	Aib	-	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	New: HPV-9, -12, -20b: [Ala] ³ →[Vxx] ³ , [Lxx] ¹¹ →[Aib] ¹¹ New: HPV-3: [Ser] ³ →[Vxx] ³ , [Lxx] ¹¹ →[Aib] ¹¹ New: HPV-5: [Ala] ³ →[Vxx] ³ , [Vxx] ¹¹ →[Aib] ¹¹ New: Hypophellin 17, 34: [Ala] ³ →[Vxx] ³ , [Lxx] ¹¹ →[Aib] ¹¹ New: Hypophellin 20, 40: [Aib] ³ →[Vxx] ³ , [Lxx] ¹¹ →[Aib] ¹¹ New: Hypophellin 18, 35, 39: [Aib] ³ →[Vxx] ³ , [Lxx] ¹¹ →[Aib] ¹¹ New: Hypophellin 31: [Ser] ³ →[Vxx] ³ , [Lxx] ¹¹ →[Aib] ¹¹ New: Paracelsin B, D: [Aib] ³ →[Vxx] ³ [Ala] ⁶ , [Aib] ⁶ New: Saturnisporin SA I, SA III: [Aib] ³ →[Vxx] ³ , [Ala] ⁶ , [Aib] ⁶ New: Suzukacillin A 02, A 06, A 05: [Aib] ³ →[Vxx] ³ , [Ala] ⁶ , [Aib] ⁶ New: Trichocellin TC-A-I, -III: [Aib] ³ →[Vxx] ³ , [Ala] ⁶	Röhrich et al. 2012 Röhrich et al. 2012 Röhrich et al. 2012 Röhrich et al. 2013 Röhrich et al. 2013 Röhrich et al. 2013 Röhrich et al. 2013 Pócsfalvi et al. 1997 Rebuffat et al. 1993 Krause et al. 2006b Wada et al. 1994
Brevicelsin -III	1852	1875	949	1078775	30.56		Ac	Aib	Ala	Aib	Ala	Aib	-	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	<u>Glu</u>	Gln	Pheol	New: Hypophellin 38: [Lxx] ¹¹ →[Aib] ¹¹ New: Trichocellin TC-A-I, -III: [Ala] ⁶ , [Aib] ⁶	Röhrich et al. 2013 Wada et al. 1994
Brevicelsin -IV	1865	1888	955.5	1078788	31.82		Ac	Aib	Ala	Aib	Ala	Aib	-	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol	New: Hypophellin 20, 40: [Lxx] ¹¹ →[Aib] ¹¹ New: Paracelsin H: [Ala] ⁶ New: Saturnisporin SA II, IV: [Ala] ⁶ , [Aib] ⁶ New: Suzukacillin A 04, A 08, 07: [Ala] ⁶ , [Aib] ⁶ New: Trichocellin TC-A-II, -IV: [Ala] ⁶	Röhrich et al. 2013 Pócsfalvi et al. 1997 Rebuffat et al. 1993 Krause et al. 2006b Wada et al. 1994
Brevicelsin -V	1879	1902	962.5	1092788	32.82		Ac	Aib	Ala	Aib	Ala	<u>Lxx</u>	-	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol	New: Hypophellin 22, 45: [Lxx] ¹¹ →[Aib] ¹¹ (Positional isomer of Brevicelsin VIII) New: Paracelsin H: [Aib] ⁵ →[Vxx] ⁵ , [Ala] ⁶ New: Saturnisporin SA II, IV: [Aib] ⁵ →[Vxx] ⁵ , [Ala] ⁶ , [Aib] ⁶ New: Suzukacillin A 04, A 08, 07: [Aib] ⁵ →[Vxx] ⁵ , [Ala] ⁶ , [Aib] ⁶ New: Trichocellin TC-A-II, -IV: [Aib] ⁵ →[Vxx] ⁵ , [Ala] ⁶	Röhrich et al. 2013 Pócsfalvi et al. 1997 Rebuffat et al. 1993 Krause et al. 2006b Wada et al. 1994
Brevicelsin -VI	1865	1888	955.5	1092774	33.20		Ac	Aib	Ala	Aib	Ala	<u>Lxx</u>	-	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	Aib	Gln	Gln	Pheol	New: Hypophellin 4: [Lxx] ¹¹ →[Aib] ¹¹ New: Paracelsin B, D: [Aib] ⁵ →[Vxx] ⁵ , [Ala] ⁶ , [Aib] ⁶ New: Saturnisporin SA I., SA III: [Aib] ⁵ →[Vxx] ⁵ , [Ala] ⁶ , [Aib] ⁶ New: Suzukacillin A 02, A 06, A 05: [Aib] ⁵ →[Vxx] ⁵ , [Ala] ⁶ , [Aib] ⁶ New: Trichocellin TC-A-I, -III: [Aib] ⁵ →[Vxx] ⁵ , [Ala] ⁶	Röhrich et al. 2013 Pócsfalvi et al. 1997 Rebuffat et al. 1993 Krause et al. 2006b Wada et al. 1994
Brevicelsin -VII	1866	1889	956	1078789	33.65		Ac	Aib	Ala	Aib	Ala	Aib	-	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	<u>Vxx</u>	<u>Glu</u>	Gln	Pheol	New: Hypophellin 21, 43: [Lxx] ¹¹ →[Aib] ¹¹ New: Hypocitrinin-7: [Lxx] ¹¹ →[Aib] ¹¹	Röhrich et al. 2013 Röhrich et al. 2014
Brevicelsin -VIII	1879	1902	962.5	1092788	36.19		Ac	Aib	Ala	Aib	Ala	<u>Lxx</u>	-	Gln	Aib	Lxx	Aib	Gly	Aib	Aib	Pro	Vxx	Aib	<u>Vxx</u>	Gln	Gln	Pheol	New: (Positional isomer of Brevicelsin V)	→ Brevicelsin V

Variable residues in table header and minor sequence variants in sequences are underlined. Amino acid exchanges in new compounds are set in italic.

Of the 51 sequences from group A, containing exclusively of 20-residue-long peptaibols, 32 have been previously described in the literature, and 19 were new, differing by 1–3 amino acids from known sequences (Table 9). Group B also comprises 20-residue sequences (Table 10). 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. 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 subterminal positions like R3 and R17. Out of the 77 group B sequences, 34 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). This is an interesting observation because only voglmayrin-16 has the same high mass among the known molecules (Röhrich et al. 2014). Furthermore, only 9 other, completely different peptaibiotic compounds have higher masses according to the literature: aibellin, texenomycin A, B, polysporin D, SCH 643432, septocylindrin A and B, as well as *Clonostachys rosea* sequences #2 and #3 (Kumazawa et al. 1994, Grigoriev et al. 2002, New et al. 1996, Degenkolb et al. 2007, Summers et al. 2007, Rodríguez et al. 2011).

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 11). They are not N-terminally truncated derivatives of their full-length precursors – like it is the case for the 16-residue-long brevikindins deriving from 18-residue-long 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). A full genome sequence is available for *T. parareesei*, analysis of this sequence, however, revealed no extra 19-modules NRPS synthetases but only a 20-modules enzyme (Marik et al. 2019). The 19-residue-long peptaibols could be produced by the same, 20-modules 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-long peptaibols were proposed by Degenkolb et al. (2012). R6 is also skipped in *T. phellinicola* 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.

6.5.2 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 15). 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.

Pept-B-XX and Pept-B-XXVII were produced by all strains examined in all Group B, whereas the other compounds were produced only by certain strains (Supplementary Table 16). Five peptaibol compounds (Pept-B-VII, Pept-B-XVII, Pept-B-XX, Pept-B-XXVII, and Pept-XLV-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-XXXIIIa, Pept-A-IVb, Pept-XLIb, Pept-XLIII, Pept-B-XLVa, Pept-B-LI, Pept-B-LIV, and Pept-B-LVIb, 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). The analysis of the *T. longibrachiatum* SZMC 1775 and the three *T. bissettii* strains (SZMC 1773, 1776, 12546) revealed similar, but still different, profiles (Supplementary Table 16). The environmental isolates of *T. longibrachiatum* SZMC 1775 and *T. bissettii* (SZMC 1773, 1776) produced more similar profiles, whereas the peptaibol profile of the clinical isolate of *T. bissettii* (SZMC 12546) was different from those of the three environmental strains. Clinical and environmental strains could not be differentiated based on ITS1, ITS2, *act1*, *cal1*, *tef1*, *hfb4* and *rpb2* sequences of 17 investigated strains of *T. longibrachiatum* and *T. bissettii* (proposed as *T. longibrachiatum* f. *bissettii*) (Hatvani et al. 2019). Therefore, the investigation of more *T. longibrachiatum* and *T. bissettii* strains in terms of peptaibol production would be interesting. 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 peptaibols in addition to the group B sequences.

We carried out a cluster analysis of the peptaibol diversity profiles in different *Trichoderma* species of clade Longibrachiatum based on the production levels of different peptaibols by various fungal producers (Supplementary Tables 15 and 16). According to their peptaibol profiles, members of clade Longibrachiatum were divided into two main clusters (Figure 8). The first cluster involves species producing exclusively group A peptaibols. Among them, *T. novae-zelandiae* is characterised with a relatively poor, but sharply distinct, profile of abundantly produced peptaibol compounds from group A, like Pept-A-XXIb, XVIb, XII, Vb, Ib, and IIIc. It may be related to the geographical origin of this species, which is endemic to New Zealand, and to the basal position occupied by it within the clade Longibrachiatum (Samuels et al. 2012b). This species has tuberculate conidia, a trait also found in clade Viride (Jaklitsch et al. 2006), and it may be an ancestral trait of clade Longibrachiatum (Druzhinina et al. 2012). Our results suggest that the production of group A peptaibols may be another ancestral trait of clade Longibrachiatum, 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 production of group A to group B peptaibols has not been completed fully in certain species: wild-type *T. reesei* as well as *T. saturnisporum* and *T.*

konilangbra also produce some group A compounds in addition to group B peptaibols. 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 9). This cluster consists 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 corresponds to the subclade Citrinoviride/Pseudokoningii (Table 9, Figure 8). 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 9, Figure 8). All three examined members of this subclade produced the entirely new compound Pept-B-LIX (1992 Da).

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. 2012b). 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 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 clade Longibrachiatum.

Figure 8 | 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 colour, species attributed to single phylogenetic lineages are marked with a star, while mutant strains are indicated with a filled pentagon. The colour scale denotes production level increasing from zero (deep blue) to high (deep red).

6.5.3 Bioactivity of *T. reesei* peptaibols

T. reesei is a popular, well-characterised species and its strains are widely used for their cellulase production in the biotechnological industry, therefore this species was selected to answer the question whether peptaibols can be used in plant protection. The bioactivity of the peptaibols produced by *T. reesei* QM9414 against plants and several human and phytopathogenic filamentous fungi were investigated.

6.5.3.1 Antifungal effect of *T. reesei* peptaibols on filamentous fungi

The purified peptaibol extracts (98% purity, containing the mixture of the peptaibols) of *T. reesei* QM9414 (shown in Table 10 and Supplementary Table 16) was tested on human and phytopathogenic filamentous fungi, furthermore, the producer strain itself, as well as its *ΔlaeI* mutant (Table 12). 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 ALM. A stronger inhibition was observed in the case of the *ΔlaeI* mutant of *T. reesei* than in the case of the other strains, suggesting that the mutation in the methyl transferase gene – known as a global epigenetic regulator of gene expression – may also affect tolerance to these metabolites. The previous results, in which crude peptaibol extracts were tested on several bacterial, yeast and filamentous fungal strains against peptaibols produced by the species belonging to clade Viride showed similar results. The inhibitory effect of peptaibols to bacteria and filamentous fungi has previously been reviewed (Szekeres et al. 2005, Daniel and Rodrigues Filho 2007). It has also been demonstrated that purified trichokonin VI triggers a change of fungal membrane permeability and disintegration of subcellular structures, has an effect on mitochondrial membrane permeabilisation and intracellular reactive oxygen species (ROS) production, induces phosphatidylserine exposure and eventually triggers metacaspase-independent apoptosis in *F. oxysporum* (Shi et al. 2012).

TABLE 12 | Antifungal activity of the purified peptaibol extract from *T. reesei* QM9414 to filamentous fungi

Tested filamentous fungal strain	MIC of purified peptaibol extract (mg ml ⁻¹)	MIC of ALM * standard (mg ml ⁻¹)	MIC of nystatin standard (mg ml ⁻¹)
<i>Alternaria alternata</i> SZMC 16085	0.1	0.05	0.003125
<i>Aspergillus fumigatus</i> SZMC 23245	0.1	0.1	0.0125
<i>Fusarium falciforme</i> SZMC 11407	0.05	0.05	0.025
<i>Fusarium keratoplasticum</i> SZMC 11414	0.1	0.1	0.05
<i>Fusarium solani</i> SC SZMC 11467	0.1	0.1	0.05
<i>Phoma cucurbitacearum</i> SZMC 16088	0.05	0.05	0.1
<i>Trichoderma reesei</i> QM9414	0.1	0.05	0.00625
<i>Trichoderma reesei</i> QM9414 G2 <i>Δlae1</i>	0.05	0.05	0.00625

SC: species complex

*Harzianum A contamination could not be detected in the alametichin standard based on the exact mass of its deprotonated molecular ion ($[M-H]^-$, $m/z = 399.1808$).

6.5.3.2 Bioactivity of *T. reesei* peptaibols on *A. thaliana* plants

In order to evaluate the potential of peptaibols as antifungal agents for plant protection, the purified (98%) microheterogeneous mixture of the 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. The plants treated with methanol did not show any difference compared to the absolute control plants, therefore, these plants were used as controls during the subsequent experiments. All of the treated plants were completely inhibited after treatment with the peptaibol extract at concentrations of 50, 10, and 5 mg ml⁻¹, which eventually

resulted in the death of the plants. Primary root growth was only detected at concentrations $\leq 1 \text{ mg ml}^{-1}$, however, inhibited growth could be observed till concentrations of 0.1 mg ml^{-1} (Figure 9). Treatment with 1 mg ml^{-1} peptaibol solution resulted in a hook formation of the primary roots. Chlorophyll-a, -b and carotenoid levels were decreased after the treatment with extracts of $\geq 0.3 \text{ mg ml}^{-1}$ (Figure 10). 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 11).

Figure 9 | Primary root growth of 6 (A), 7 (B), 8 (C), and 9 (D) day-old *A. thaliana* plants after treatment with peptaibol extract from *T. 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: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ and **** $P \leq 0.0001$.

Figure 10 | Pigment content of 15-day-old *A. thaliana* leaves after treatment with peptaibol extract from *T. reesei* QM9414: chlorophyll-a (**A**), chlorophyll-b (**B**), carotenoids (**C**) and anthocyanins (**D**). Methanol was used for the control plants. Significance is assessed based on *P*-values: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ and **** $P \leq 0.0001$.

Figure 11 | Biomass of 15-day-old *A. thaliana* plants after treatment with peptaibol extract from *T. reesei* QM9414. Methanol was used for the control plants. Significance is assessed based on *P*-values: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ and **** $P \leq 0.0001$.

ALM, the most studied peptaibol was shown to induce resistance in plants (Engelberth et al. 2000, Engelberth et al. 2001, Maischak et al. 2010, Li et al. 2016), although it can also cause lesions on *Arabidopsis* leaves (Rippa et al. 2010). At higher concentration, it induces rRNA cleavage-associated rapid death (Rippa et al. 2007). ALM 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-specific ALM permeabilisation of *A. thaliana* roots. Engelberth et al. (2001) successfully demonstrated the high biological activity of ALM 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 trichorzins 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).

It was hypothesized long ago that small secondary metabolites of *Trichoderma* may act as auxin-like compounds between the optimum concentrations of 10^{-5} and 10^{-6} M, but have inhibitory effects at higher concentrations (Thimann, 1937, Cleland, 1972, Brenner, 1981). Secondary metabolites of *T. koningii* (koninginin A) and *T. harzianum* (6-pentyl- α -pyrone) inhibited the growth of etiolated wheat coleoptiles at relatively high concentrations ($\geq 10^{-3}$ M), but lower doses (10^{-4} – 10^{-3} M) had no effect (Cutler et al. 1986, Cutler et al. 1989). In another study, the same compound mentioned as 6-pentyl-2H-pyran-2-on showed inhibitory effects at high concentration, but interestingly a growth induction could also be detected in wheat seedlings after the treatment with low concentrations. Furthermore, tomato plants showed more intensive growth and better root system development after the foliar spray treatment with the same compound at a concentration of 0.166 ml l^{-1} (Vinale et al. 2008). Another secondary metabolite, harzianolide was tested on rapeseed and tomato seedlings and had a positive effect on growth and defense response of the plants after the treatment with the concentration of 1 mg l^{-1} (Vinale et al. 2008). The treatment of the seeds at the concentrations of 100 and $10 \text{ }\mu\text{g/seed}$ resulted in stem length reduction, while at the concentration of 100, 10 and 1 ng/seed the stem length was increased in comparison to the control (Vinale et al. 2009).

6.5.3.3 Bioactivity of *T. reesei* peptaibols on mammalian cells

The endpoint of toxic concentration – the last dilution step of the purified peptaibol solution, which is still toxic – was determined for the peptaibol extract of *T. reesei* QM9414 on mammalian cells (Table 13). After 20 min incubation at $37 \text{ }^{\circ}\text{C}$ or 24 h at room temperature, the sperm motility inhibition end point was detected after treatment with $3 \text{ }\mu\text{g ml}^{-1}$ 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 PK-15 cells was observed at a concentration of $8 \text{ }\mu\text{g ml}^{-1}$ peptaibol solution. Boar sperm cells are frequently used for the detection of toxins, which affect plasma membranes (Castagnoli et al. 2018, Vicente-Carrillo, 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, Andersson et al. 2010). Similar measurements of peptaibol extracts produced by *T. longibrachiatum* Thb have been reported, and a mixture of trilongins proved to be a stronger 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 (trichokonin VI) could inhibit HepG2 cancer cells by inducing autophagy and apoptosis through an influx of Ca^{2+} , which triggered the activation of μ -calpain and proceeded to the translocation of Bcl-2-associated X protein (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 PK-15 cells at 0.1 mg ml^{-1} , which raises the question of a possible *in vivo* toxicity. Degenkolb et al. (2008a) 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).

TABLE 13 | Toxicity of the purified peptaibol extract from *T. reesei* QM9414 to boar sperm and porcine kidney cells

	Sperm motility inhibition		Acrosome reaction	Inhibition of proliferation of PK-15 cells
	20 min	24 h		
	EC ₅₀ (μg ml ⁻¹)			
Purified peptaibol extract	3	3	3	8
Reference (ALM)	5	0.2	0.2	8

The values are the median of three measurements, represented by four microscopic fields. The variation between measurements was one dilution step of the 2-fold dilution series.

7. CONCLUSIONS

Several new compounds of known and novel peptaibol groups, such as koningiopsins, tripleurins and brevicelsins have been identified in this work, broadening the already wide diversity of peptaibol compounds produced by *Trichoderma* strains. The phylogenetic relationships between the producer species are less reflected by the clustering based on peptaibol profiles, than based on genes, but their peptaibol profiles may also provide an insight into their evolution. The results also provide a better understanding of their interactions with several organisms. Peptaibols were shown to be toxic towards Gram-positive bacteria and filamentous fungi and less effective against Gram-negative bacteria. Yeasts were not affected by peptaibol treatment. These results suggest that the type of cell wall of the host organism may play role in the sensitivity to peptaibols. The peptaibol production of *Trichoderma* can be affected by the host organisms during infections and the different organisms respond variously to peptaibol treatments. Although, they are toxic to bacteria and fungi, no negative effects could be detected on plants below a certain concentration. This finding can be useful especially in tropical agricultural environments, as peptaibol solutions with biocontrol abilities can replace the use of those *Trichoderma* strains, which can also grow at human body temperature and cause infections. This can lead to the use of peptaibols as biocontrol agents in plant protection.

8. SUMMARY

Trichoderma species are ecologically and agriculturally beneficial, but certain species can cause damage in the commercial production of mushrooms or may be the causal agents of human infections. Among the bioactive secondary metabolites produced by *Trichoderma* species, studying the peptaibols is important for the better understanding of the processes that occur during their interactions with phytopathogens throughout their biocontrol activity, or during infections of mushrooms, or even humans. Species of the clade Viride including *T. koningiopsis* and *T. gamsii* are beneficial organisms of industrial, agricultural, and medicinal fields. *T. aggressivum* f. *europaeum* and *T. pleuroti* belong to clade Harzianum/Virens and can cause green mould diseases in the commercial production of white button mushroom (*A. bisporus*) and oyster mushroom (*P. ostreatus*), respectively. Clade Longibrachiatum contains species such as *T. longibrachiatum*, *T. bissettii* or *T. citrinoviride*, which can cause infections in immunocompromised humans. Certain species of this clade are beneficial and widely used in the industry, e.g. *T. reesei* for its cellulase production. This clade is ecologically highly versatile, some of its species can be found worldwide like the opportunistic human pathogens *T. longibrachiatum* and *T. bissettii*, whereas others are ecologically restricted, like the biotechnologically important cellulase producer *T. reesei*.

Based on using alamethicin (ALM) as standard, the high-performance liquid chromatography (HPLC) mass spectrometry (MS) instrument was optimized to identify peptaibol compounds. In this work, a total of 215 peptaibol compounds were identified from various *Trichoderma* strains, out of which 131 sequences proved to be new to science. The majority of the new compounds belong to already described peptaibol subgroups, such as hypomurocins, trikoningins, trichobrachsins, paracelsins, suzukacillins, saturnisporins, trichoaureocins, trichocellins, longibrachsins, hyporientalins, trichokonins, trilongins, metanicins, trichosporins, gliodeliquescins, hypophellins and *T. citrinoviride* sequences. Among the new sequences, three completely novel peptaibol subgroups could also be identified and described as koningiopsins, tripleurins and brevicelsins with 11, 24 and 8 sequences, respectively. These novel sequences showed homology to the peptaibol subgroups of trichorzianins, trichokindins, tricholongins, trichostrigocins and the ones mentioned above.

The study of the peptaibiome composition of *T. koningiopsis* and *T. gamsii* from clade Viride revealed a total of 30 peptaibol sequences (26 new and 4 known). Certain peptaibol compounds produced by *T. gamsii* were positionally isomeric with the sequence of

trikoningin KA V known from the literature, and 15 new compounds were homologous with tricholongin B and LB, trichostrigocin TSG-A and TSG-B, trichorzianin TA, TB and Tap sequences. The novel peptaibol group of koningiopsins was described with 11 new sequences, which were produced by *T. koningiopsis*, and showed similarities to trichorzianin TA, TB and Tap sequences. The examination of their antibiotic activity against a broad spectrum of different microorganisms showed that Gram-positive bacteria were strongly inhibited, while Gram-negative bacteria seemed to be less sensitive to the peptaibol treatment. No inhibitory effects of the studied peptaibol extracts could be observed to yeasts, while filamentous fungi showed considerable sensitivity, suggesting that the peptaibol sensitivity of an organism may depend on its cell wall type.

During the study of the peptaibol production of *Trichoderma* species causing green mould diseases, 20 new and 5 already known hypomurocin-like compounds were identified from *T. aggressivum* f. *europaeum*. In the case of *T. pleuroti*, the production of a completely new peptaibol group could be detected and named as tripleurins that were found to be related to trichokindins and trichorzins PA. The results also indicate that the peptaibols produced by *T. aggressivum* f. *europaeum* and *T. pleuroti* are potential growth inhibitors of mushroom mycelia, therefore their contribution to the antagonistic arsenal of mushroom green mould species can be suggested. Furthermore, the changes detected in the quantity of peptaibol compounds during *in vitro* confrontation of *Trichoderma* species with *Agaricus* and *Pleurotus* implicate the impact of the host mushroom on *Trichoderma* peptaibol metabolism.

Structural diversity examination of peptaibol compounds produced by *Trichoderma* species from clade Longibrachiatum revealed a total of 128 20-residue-long peptaibols from the 17 species examined, including 53 new and 75 recurrent compounds, as well as 8 new 19-residue sequences. These peptaibols could be categorized into groups A, B and C based on their primary structure, where groups A and B consist of 20-residue peptaibols, while group C comprises exclusively of eight new, 19-residue-long sequences. Group C was found to be novel and named as brevicelsins. The peptaibols belonging to group A were positionally isomeric or showed similarity to paracelsin, suzukacillin A, trichocellin-TC, trichoaureocin, longibrachin A and B, trilongin B and C, hypophellin, trichobrachin, trichokonin, trichosporin TS-B, metanicin, gliodeliquescin A, or hyporientalin A sequences. The peptaibols belonging to group B were positionally isomeric or showed similarity to paracelsin, suzukacillin A, trichocellin-TC, saturnisporin SA, or *T. citrinoviride* sequences. Brevicelsins were similar to the 19-residue-long hypophellins and differed from the other peptaibols produced by strains

of clade Longibrachiatum in the absence of the 6th amino acid residue. They also showed similarities to the 20-residue-long paracelsin, suzukacillin A, trichocellin TC and saturnisporin SA sequences bearing in mind the lack of the 6th position in these sequences. We also carried out a cluster analysis of the peptaibol diversity profiles based on the production levels of different peptaibols by various fungal producers of clade Longibrachiatum. According to their peptaibol profiles, members of clade Longibrachiatum were divided into two main clusters (group A and B). Our results suggest that the production of group A peptaibols may be an ancestral trait of clade Longibrachiatum, 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 has not been completed fully in certain species, which are able to produce some group A compounds in addition to group B peptaibols. However, the phylogenetic relationships between the subclades are less reflected by the clustering based on peptaibol profiles.

The purified (98%) extract containing the microheterogeneous mixture of peptaibols produced by *T. reesei* QM9414 was selected for testing its effects on *Arabidopsis* seedlings. Negative effects on the plants could not be detected below 0.1 mg ml⁻¹ concentration of the purified peptaibol extract from *T. reesei* QM9414, but it could still inhibit phytopathogenic filamentous fungal growth. This observation suggests that purified peptaibol extracts may have potential in plant disease management. Direct treatment with these peptaibols was highly toxic on boar semen and porcine kidney tubular epithelial PK-15 cells at 0.1 mg ml⁻¹, which raises the question of a possible *in vivo* toxicity. On the other hand, several studies have 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. *T. reesei* is a well-characterised, widely used cellulase producer in the biotechnological industry, therefore its peptaibols could be produced as valuable by-products or even the main products of fermentation.

Altogether, this work highlights the great peptaibiome diversity within the genus *Trichoderma* and provides new informations about the biological activity of peptaibols against various organisms and opens an avenue to their potential exploration in plant protection.

9. ÖSSZEFOGLALÓ

A *Trichoderma* nemzetségbe tartozó fajok mind ökológiai, mind mezőgazdasági szempontból fontos szerepet töltenek be, néhány *Trichoderma* faj azonban képes jelentős károkat okozni a gombatermesztésben, továbbá egyes fajok akár humán fertőzések kórokozói is lehetnek. A *Trichoderma* fajok által termelt másodlagos anyagcseretermékek közül a peptaibolok vizsgálata kiemelkedően fontos lehet annak érdekében, hogy jobban megértsük a termelő fajok biokontroll tulajdonságait, illetve a gombatermesztésben okozott károk, vagy akár a humán fertőzések hátterében zajló folyamatokat. A *Trichoderma* nemzetség Viride kládjába tartozó fajok, mint például a *T. koningiopsis* és a *T. gamsii*, előnyös tulajdonságaik miatt közkedveltek a mezőgazdaságban, iparban és gyógyszeriparban. A Harzianum kládba tartozó *T. aggressivum* f. *europaeum* és *T. pleuroti* fajok képesek zöldpenészes megbetegedéseket okozni termesztett gombákon, mint például a csiperkén (*A. bisporus*), vagy a laskagombán (*P. ostreatus*). A Longibrachiatum kládba tartozó *T. longibrachiatum*, *T. bissettii* és *T. citrinoviride* fajok legyengült immunrendszerű emberekben fertőzéseket okozhatnak. A klád egyes fajai gazdaságilag előnyösek is lehetnek, ilyen például a *T. reesei*, melyet széles körben használnak kiváló celluláz termelő képessége miatt. A Longibrachiatum klád ökológiai szempontból rendkívül változatos, egyes fajaik világszerte megtalálhatóak, mint például az opportunista humán patogén *T. longibrachiatum* és *T. bissettii*, míg más fajok csak egyes területeken izolálhatóak, mint például a biotechnológiai szempontból jelentős celluláztermelő *T. reesei*.

A nagy teljesítményű folyadékromatográfiás (high-performance liquid chromatography, HPLC) tömegspektrométer (mass spectrometry, MS) készüléket alamethicin (ALM) standard segítségével peptaibol-vegyületek azonosítása céljából optimalizáltuk. Munkám során összesen 215 peptaibol-vegyületet sikerült azonosítani *Trichoderma* fajokból, melyek közül 131 szekvencia bizonyult újnak. Ezek az új peptidek sok esetben már létező peptaibol-csoportokba tartoznak, melyek a hypomurocinok, a trikoninginek, a trichobrachinok, a paracelsinek, a suzukacillinek, a saturnisporinok, a trichoaureocinek, a trichocellinek, a longibrachinok, a hyporientalinok, a trichokoninok, a trilinginok, a metanicinek, a trichosporinok, a gliodeliquescinek, a hypophellinek és a *T. citrinoviride* szekvenciák. Az új szekvenciák közt sikerült 3 teljesen új peptaibolcsoportot is azonosítanunk, melyek a koningiopsinok 11 új peptaibollal, a tripleurinok 24 új szekvenciával és a brevicelsinek 8 új peptiddel. Ezek az új peptaibolcsoportok a trichorzianinokkal, a

trichokindinekkal, a tricholonginokkal, a trichostrogocinokkal továbbá a fentebb felsorolt peptaibol-csoportokkal mutattak homológiát.

A Viride kládba tartozó *T. koningiopsis* and *T. gamsii* fajok peptaibiomjának feltérképezése során 30 (26 új és 4 ismert) peptaibol-szekvenciát azonosítottunk. A *T. gamsii* által termelt peptaibolok közül egyes szekvenciák a már ismert trikoningin KA V peptaibol pozicionális izomerjei, azonban 15 új peptidet is sikerült azonosítanunk, melyek a tricholongin B, LB, trichostrogocin TSG-A, TSG-B, trichorzianins TA, TB and Tap szekvenciákkal mutattak homológiát. A *T. koningiopsis* új, általunk felfedezett, koningiopsin peptaibolcsoportba tartozó 11 új peptidet termelt melyek a trichorzianin TA, TB és Tap szekvenciákkal voltak homológok. A bioaktivitási tesztek során kiderült, hogy a Gram-pozitív baktériumok érzékenyebben reagálnak peptaibol-kezelésre, mint a Gram-negatív baktériumok. Élesztőkkel szemben gátló hatást nem figyeltünk meg, viszont fonalas gombákon jól detektálható növekedésgátlást tapasztaltunk. Ezek alapján elmondható, hogy a megtámadott mikroorganizmusok sejtfalszerkezete befolyással lehet a peptaibolok toxicitására.

A zöldpenészes megbetegedéseket okozó *Trichoderma* fajok peptaibol-termelésének tanulmányozása során 20 új és 5 ismert hypomurocin-szerű peptaibolt azonosítottunk, melyeket a *T. aggressivum* f. *europaeum* termelt. A *T. pleuroti* egy teljesen új csoportba tartozó általunk felfedezett peptaibolokat, a tripleurinokat termelte, melyek leginkább a trichokindins és trichorzins PA peptaibolokhoz hasonlítottak. Eredményeink alapján elmondhatjuk, hogy a *T. aggressivum* f. *europaeum* és *T. pleuroti* által termelt peptaibolok a termesztett gombák micéliumának potenciális növekedésgátlói, tehát ezek is szerepet játszhatnak a zöldpenész tünetegyüttes kialakulásában. Csiperkegomba (*Agaricus*) és laskagomba (*Pleurotus*) jelenlétében további mennyiségi változásokat is megfigyeltünk a *Trichoderma* fajok peptaibol-termelésben, ami arra utal, hogy a gazdaszervezetnek hatása van a *Trichoderma* fajok peptaibol-metabolizmusára.

A Longibrachiatum kládba tartozó 17 faj által termelt peptaibol-diverzitásának vizsgálata során 128 (53 új és 75 már azonosított) 20 aminosav hosszú, valamint 8 új, általunk felfedezett, 19 aminosav hosszú peptaibol-szekvenciát sikerült azonosítanunk. Ezeket a peptaibolokat az elsődleges szerkezetük alapján 3 csoportba tudtuk besorolni, melyeket A, B és C csoportoknak neveztük el. Az A és B csoportok 20 aminosav hosszú szekvenciákat tartalmaznak, míg a C csoportba az új, 19 aminosav hosszú peptaibolok tartoztak. A C csoport

teljesen új peptaibolokat tartalmazott, melyeket brevicelsineknek neveztünk el. Az A csoportba tartozó szekvenciák pozicionális izomerei, vagy hasonlóságot mutatnak már ismert paracelsin, suzukacillin A, trichocellin-TC, trichoaureocin, longibrachin A, B, trilongin B, C, hypophellin, trichobrachin, trichokonin, trichosporin TS-B, metanicin, gliodeliquescin A, vagy hyporientalin A szekvenciákkal. A B csoportba tartozó peptaibolok számos paracelsin, suzukacillin A, trichocellin-TC, saturnisporin SA vagy *T. citrinoviride* szekvenciákkal mutattak hasonlóságot, vagy bizonyos esetekben pozicionális izomerei voltak azoknak. A brevicelsinek a 19 aminosav hosszú hypophellinekhez hasonlítottak leginkább, valamint a többi, Longibrachium kládba tartozó peptaiboloktól a 6. pozíció kiesése miatt tértek el. Szekvencia-hasonlóságot továbbá a 20 aminosav hosszú paracelsin, suzukacillin A, trichocellin TC, saturnisporin SA szekvenciákkal mutatnak, ez esetekben is a felsorolt csoportok szekvenciáiban lévő 6. pozíció hiányát figyelembe véve. A peptaibolok termelési szintje alapján készített peptaibol-profilokkal klaszteranalízist is végeztünk a Longibrachium kládba tartozó termelő fajokról. A peptaibolprofilok alapján a klád tagjait két fő klaszterbe tudtuk elkülöníteni (A és B csoport). Eredményeink alapján az A csoportba tartozó peptaibolok termelése a Longibrachium klád egy ősi tulajdonsága lehet, míg a B csoportba tartozó peptaibolok termelésére való váltás többször is megtörtént, mely konvergens evolúció eredménye lehet. Ez a váltás egyes fajok esetében nem történt meg teljes mértékben, ugyanis ezek a fajok képesek a B csoportba tartozó peptaibolok termelése mellett az A csoportba tartozó peptaibolok termelésére is. A peptaibolprofilok alapján történő klaszterezés azonban kevésbé tükrözi a szubkládok közötti filogenetikai kapcsolatokat.

A *T. reesei* QM9414 által termelt, tisztított, peptaibolok mikroheterogén keverékét tartalmazó (98%) kivonatot teszteltük *Arabidopsis* növényeken is. Negatív hatást nem azonosítottunk 0,1 mg ml⁻¹ töménységű oldat alkalmazása esetén, mely koncentráció képes volt számos növénypatogén gomba növekedésének gátlására. Ez a megfigyelés megerősíti, hogy a tisztított peptaibol-kivonatok biokontroll alkalmazásának potenciális értéke lehet a jövőben. Ezen készítmények erősen toxikusnak bizonyultak vaddisznósperma és sertésvese epiteliális sejtvonal (porcine kidney tubular epithelial cell line, PK-15) sejtekre nézve, mely a peptaibolok lehetséges *in vivo* toxicitásának kérdését teheti fel. Azonban számos tanulmány bizonyította azt, hogy a peptaibolok toxicitása jóval az emberi tűréshatár alatt van, a toxicitás csak közvetlenül a sejtmembránnal érintkezve jelentkezik, hasonlóan, mint az általános amfifil vegyületek, mosószerek esetében. A *T. reesei* egy részletesen feltérképezett, széles

körben használt, a biotechnológiai ipar által használt celluláztermelő faj, ezért az általa termelt peptaibolok mellék-, de akár főtermékként is könnyen kivonhatók lennének.

Kutatásaim eredményei kiemelik a *Trichoderma* nemzetség képviselőire jellemző peptaibomok változatosságát és új információkkal szolgálnak a peptaibolok különböző organizmusokra gyakorolt bioaktivitásairól, valamint új utat nyitnak a növényvédelemben történő alkalmazásuk felé.

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12.SUPPLEMENTARY MATERIAL

Figure 1 | Typical b ion series of described peptaibols ranged from b_{12} at m/z 1106 (A), m/z 1108 (B), m/z 1122 (C), m/z 1136 (D) resulting from the full scan measurements of crude peptaibol extracts prepared from *T. gamsii* SZMC 1656 performed on Varian-MS system

Figure 2 | Typical b ion series of described peptaibols ranging from b_{12} at m/z 1120 (Koningiopsin IIb, IIIb - A), m/z 1120 (Koningiopsins VIa, VIb - B), m/z 1122 (Koningiopsins Ia, Ib- C), m/z 1122 (Koningiopsins IIIa - D), m/z 1136 (Koningiopsins IIa, IV, Va, Vb - E) resulting from the full scan measurements of crude peptaibol extracts prepared from *T. koningiopsis* SZMC 12500 performed on Varian-MS system

Figure 3 | Typical b ion series of six selected compounds: Hypomurocin B1 (A), Pept-1733-a-1 (B), Hypomurocin B 3a (C), Pept-1717-a-1 (D), Pept-1747-a-2 (E) and Pept-1761-a-3 (F) resulting from the full scan measurements of crude peptaibol extracts prepared from *T. aggressivum* f. *europaeum* CBS 433.95 (SZMC 1811) performed on Varian-MS system

Figure 4 | Typical b ion series of six selected compounds: Tripleurin IIIa (A), Tripleurin IV (B), Tripleurin VII (C), Tripleurin VIIIa (D), Tripleurin XIIc (E) and Tripleurin XIV (F) resulting from the full scan measurements of crude peptaibol extracts prepared from *T. pleuroti* TPhu1 (SZMC 12454) performed on Varian-MS system

Figure 5 | Typical b-type ion series of three selected compounds: Pept-A-IVa (A), Pept-B-XXVII (B) from b_{13} at m/z 1163 and Brevicelsin I (C) ranged from b_{13} at m/z 1078 resulting from the full scan measurements of crude peptaibol extracts performed on Varian MS system and prepared from *T. orientale* SZMC 12556, *T. saturnisporum* SZMC 22606 and *T. flagellatum* SZMC 22608, respectively.

Figure 6 | Typical MS² spectra of y-ions 754.5 (A), 755.5 (B), 768.5 (C) and 769.5 (D) resulting from the full scan measurements of crude peptaibol extracts of *T. gamsii* SZMC 1656 and *T. koningiopsis* SZMC 12500 performed on Varian-MS system. The MS² patterns originating from the same *m/z* values were identical.

Figure 7 | Typical MS² spectra of y-ions 612.5 (A), 626.5 (B), 660.5 (C) and 674.5 (D) resulting from the full scan measurements of crude peptaibol extracts of A, B: *T. aggressivum* f. *europaeum* CBS 433.95 (SZMC 1811) and C, D: *T. pleuroti* TPhu1 (SZMC 12454) performed on Varian-MS system. The MS² patterns originating from the same m/z values were identical.

Figure 8 | Typical MS² spectra of y-type ions m/z 774.4 (A), m/z 775.4 (B), m/z 788.4 (C) and m/z 789.4 (D) resulting from the full scan measurements of crude peptaibol extracts of the strains belonging to Longibrachiatum clade. Analyses were performed on Varian MS system. MS² spectra obtained from precursor ions of the same m/z ratio were identical.

Figure 9 | Typical MS² spectra of y-type ions m/z 774.4 (A) and m/z 788.4 (B) resulting from the full scan measurements of crude peptaibol extracts of the strains belonging to Longibrachiatum clade. Analyses were performed on Orbitrap-MS system. MS² spectra obtained from precursor ions of the same m/z ratio were identical.

Figure 10 | EIC resulting from full scan measurements of crude extracts from *T. gamsii* SZMC 1656. The coeluted components within some peaks were identified based on Varian-MS² examinations.

Figure 11 | EIC resulting from full scan measurements of crude extracts prepared from *T. koningiopsis* SZMC 12500

Figure 12 | TICs and EICs resulting from full scan measurements of peptaibol molecules prepared from *T. aggressivum* f. *europaeum* CBS 433.95 (SZMC 1811) performed on Varian-MS system.

Figure 13 | TIC and EIC resulting from full scan measurements of peptaibol molecules prepared from *T. pleuroti* TPhu1 (SZMC 12454) performed on Varian-MS system. The co-eluted components within some peaks were identified based on Varian-MS² examinations.

Figure 14 | EIC resulting from full scan measurements of crude extracts from *T. pinnatum* SZMC 22603 performed on Varian-MS system. The coeluting components within some peaks were identified based on MS² experiments.

Figure 15 | EIC resulting from full scan measurements of crude extracts from *T. aethiopicum* SZMC 22602 performed on Varian-MS system. The coeluting components within some peaks were identified based on MS² experiments.

Figure 16 | EIC resulting from full scan measurements of crude extracts from *T. ghanense* SZMC 22604 performed on Varian-MS system. The coeluting components within some peaks were identified based on MS² experiments.

Figure 17 | EIC resulting from full scan measurements of crude extracts from *T. citrinoviride* SZMC 22618 performed on Varian-MS system. The coeluting components within some peaks were identified based on MS² experiments.

Figure 18 | EIC resulting from full scan measurements of crude extracts from *T. pseudokoningii* SZMC 22613 performed on Varian-MS system. The coeluting components within some peaks were identified based on MS² experiments.

Figure 19 | EIC resulting from full scan measurements of crude extracts from *T. orientale* SZMC 12556 performed on Varian-MS system. The coeluting components within some peaks were identified based on MS² experiments.

Figure 20 | EIC resulting from full scan measurements of crude extracts from *T. bissettii* SZMC 1776 performed on Varian-MS system. The coeluting components within some peaks were identified based on MS² experiments.

Figure 21 | EIC resulting from full scan measurements of crude extracts from *T. bissettii* SZMC 1773 performed on Varian-MS system. The coeluting components within some peaks were identified based on MS² experiments.

Figure 22 | EIC resulting from full scan measurements of crude extracts from *T. longibrachiatum* SZMC 1775 performed on Varian-MS system. The coeluting components within some peaks were identified based on MS² experiments.

Figure 23 | EIC resulting from full scan measurements of crude extracts from *T. bissettii* SZMC 12546 performed on Varian-MS system. The coeluting components within some peaks were identified based on MS² experiments.

Figure 24 | EIC resulting from full scan measurements of crude extracts from *T. capillare* SZMC 22605 performed on Varian-MS system. The coeluting components within some peaks were identified based on MS² experiments.

Figure 25 | EIC resulting from full scan measurements of crude extracts from *T. novae-zelandiae* SZMC 22612 performed on Varian-MS system. The coeluting components within some peaks were identified based on MS² experiments.

Figure 26 | EIC resulting from full scan measurements of crude extracts from *T. parareesei* SZMC 22615 performed on Varian-MS system. The coeluting components within some peaks were identified based on MS² experiments.

Figure 27 | EIC resulting from full scan measurements of crude extracts from *T. reesei* SZMC 22614 performed on Varian-MS system. The coeluting components within some peaks were identified based on MS² experiments.

Figure 28 | EIC resulting from full scan measurements of crude extracts from *T. reesei* SZMC 22616 performed on Varian-MS system. The coeluting components within some peaks were identified based on MS² experiments.

Figure 29 | EIC resulting from full scan measurements of crude extracts from *T. reesei* SZMC 22617 performed on Varian-MS system. The coeluting components within some peaks were identified based on MS² experiments.

Figure 30 | EIC resulting from full scan measurements of crude extracts from *T. saturnisporum* SZMC 22606 performed on Varian-MS system. The coeluting components within some peaks were identified based on MS² experiments.

Figure 31 | EIC resulting from full scan measurements of crude extracts from *T. effusum* SZMC 22611 performed on Varian-MS system. The coeluting components within some peaks were identified based on MS² experiments.

Figure 32 | EIC resulting from full scan measurements of crude extracts from *T. andinense* SZMC 22610 performed on Varian-MS system. The coeluting components within some peaks were identified based on MS² experiments.

Figure 33 | EIC resulting from full scan measurements of crude extracts from *T. sinense* SZMC 22609 performed on Varian-MS system. The coeluting components within some peaks were identified based on MS² experiments.

Figure 34 | EIC resulting from full scan measurements of crude extracts from *T. flagellatum* SZMC 22608 performed on Varian-MS system. The coeluting components within some peaks were identified based on MS² experiments.

Figure 35 | EIC resulting from full scan measurements of crude extracts from *T. konilangbra* SZMC 22607 performed on Varian-MS system. The coeluting components within some peaks were identified based on MS² experiments.

Table 1 | Diagnostic fragment ions of peptaibols of clade Longibrachiatum peptaibol compounds of group A detected with full scan Orbitrap-MS measurement

Peptide	M	[M+H] ⁺	b ₁	b ₂	b ₃	b ₄	b ₅	b ₆	b ₇	b ₈	b ₉	b ₁₀	b ₁₁	b ₁₂	b ₁₃	y ₇
Pept-A-Ia	1922.0989	1923.1048	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	823.4642	894.5065	951.5278	1064.6081	1149.6611	774.4469
Pept-A-Ib	1922.0989	1923.1048	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	837.4860	908.5167	965.5449	1064.6081	1149.6611	774.4469
Pept-A-IIa	1923.0829	1924.0999	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	823.4642	908.5167	965.5449	1064.6081	1149.6611	775.4322
Pept-A-IIb	1923.0829	1924.0999	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	823.4642	894.5065	951.5278	1064.6081	1149.6611	775.4322
Pept-A-IIIa	1936.1146	1937.1206	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	823.4642	908.5167	965.5449	1064.6081	1149.6611	788.4616
Pept-A-IIIb	1936.1146	1937.1206	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	823.4642	894.5065	951.5278	1064.6081	1149.6611	788.4616
Pept-A-IIIC	1936.1146	1937.1206	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	837.4860	908.5167	965.5449	1064.6081	1149.6611	788.4616
Pept-A-IVa	1936.1146	1937.1206	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	823.4642	908.5167	965.5449	1078.6224	1163.6769	774.4469
Pept-A-IVb	1936.1146	1937.1206	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	837.4860	908.5167	965.5449	1078.6224	1163.6769	774.4469
Pept-A-Va	1950.1302	1951.1368	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	837.4860	922.5319	979.5613	1092.6402	1177.6921	774.4469
Pept-A-Vb	1950.1302	1951.1368	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	837.4860	922.5319	979.5613	1092.6402	1177.6921	774.4469
Pept-A-VIa	1937.0986	1938.0984	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	823.4642	908.5167	965.5449	1078.6224	1163.6769	775.4322
Pept-A-VIb	1937.0986	1938.0984	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	837.4860	908.5167	965.5449	1078.6224	1163.6769	775.4322
Pept-A-VIIa	1936.1146	1937.1206	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	823.4642	908.5167	965.5449	1078.6224	1163.6769	774.4469
Pept-A-VIIb	1936.1146	1937.1206	128.0704	199.1071	298.1597	369.2134	454.2668	525.3029	n.d.	738.4129	837.4860	908.5167	965.5449	1078.6224	1163.6769	774.4469
Pept-A-VIIIa	1950.1302	1951.1368	128.0704	199.1071	298.1597	369.2134	454.2668	525.3029	n.d.	738.4129	851.4953	922.5319	979.5613	1092.6402	1177.6921	774.4469
Pept-A-VIIIb	1950.1302	1951.1368	128.0704	199.1071	298.1597	369.2134	454.2668	525.3029	n.d.	738.4129	837.4860	922.5319	979.5613	1092.6402	1177.6921	774.4469
Pept-A-IXa	1950.1302	1951.1368	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	823.4642	908.5167	965.5449	1078.6224	1163.6769	788.4616
Pept-A-IXb	1950.1302	1951.1368	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	837.4860	908.5167	965.5449	1078.6224	1163.6769	788.4616
Pept-A-Xa	1964.1459	1965.1522	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	837.4860	922.5319	979.5613	1092.6402	1177.6921	788.4616
Pept-A-Xb	1964.1459	1965.1522	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	837.4860	922.5319	979.5613	1092.6402	1177.6921	788.4616
Pept-A-XIa	1951.1143	1952.1302	128.0704	199.1071	298.1597	369.2134	454.2668	525.3029	n.d.	738.4129	837.4860	922.5319	979.5613	1092.6402	1177.6921	775.4322
Pept-A-XIb	1951.1143	1952.1302	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	837.4860	922.5319	979.5613	1092.6402	1177.6921	775.4322
Pept-A-XIc	1951.1143	1952.1302	128.0704	199.1071	298.1597	369.2134	454.2668	525.3029	n.d.	738.4129	851.4953	922.5319	979.5613	1092.6402	1177.6921	775.4322
Pept-A-XII	1937.0986	1938.0984	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	837.4860	908.5167	965.5449	1078.6224	1163.6769	775.4322
Pept-A-XIIIa	1951.1143	1952.1302	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	823.4642	908.5167	965.5449	1078.6224	1163.6769	789.4509

n.d.: not detected

Table 1 | continued

Peptide	M	[M+H] ⁺	b ₁	b ₂	b ₃	b ₄	b ₅	b ₆	b ₇	b ₈	b ₉	b ₁₀	b ₁₁	b ₁₂	b ₁₃	y ₇
Pept-A-XIIIb	1951.1143	1952.1302	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	837.4860	908.5167	965.5449	1078.6224	1163.6769	789.4509
Pept-A-XIVa	1965.1299	1966.1444	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	922.5319	979.5613	1092.6402	1177.6921	789.4509
Pept-A- XIVb	1964.1459	1965.1522	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	837.4860	922.5319	979.5613	1092.6402	1177.6921	788.4616
Pept-A-XVa	1950.1302	1951.1368	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	823.4642	908.5167	965.5449	1078.6224	1163.6769	788.4616
Pept-A-XVb	1964.1459	1965.1522	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	922.5319	979.5613	1092.6402	1177.6921	788.4616
Pept-A-XVIa	1950.1302	1951.1368	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	837.4860	922.5319	979.5613	1092.6402	1177.6921	774.4469
Pept-A-XVIb	1950.1302	1951.1368	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	922.5319	979.5613	1092.6402	1177.6921	774.4469
Pept-A-XVIIa	1964.1459	1965.1522	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	837.4860	922.5319	979.5613	1092.6402	1177.6921	788.4616
Pept-A-XVIIb	1950.1302	1951.1368	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	823.4642	908.5167	965.5449	1078.6224	1163.6769	788.4616
Pept-A-XVIII	1978.1615	1979.1713	128.0704	199.1071	298.1597	369.2134	454.2668	539.3186	n.d.	752.4301	851.4953	936.5563	993.5684	1106.6547	1191.7080	788.4616
Pept-A-XIXa	1951.1143	1952.1302	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	837.4860	922.5319	979.5613	1092.6402	1177.6921	775.4322
Pept-A-XIXb	1951.1143	1952.1302	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	922.5319	979.5613	1092.6402	1177.6921	775.4322
Pept-A-XX	1964.1459	1965.1522	128.0704	199.1071	298.1597	369.2134	454.2668	539.3186	n.d.	752.4301	851.4953	936.5563	993.5867	1106.6547	1191.7080	774.4469
Pept-A-XXIa	1964.1459	1965.1522	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	837.4860	922.5319	979.5613	1092.6402	1177.6921	788.4616
Pept-A-XXIb	1964.1459	1965.1522	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	922.5319	979.5613	1092.6402	1177.6921	788.4616
Pept-A-XXIIa	1964.1459	1965.1522	128.0704	199.1071	298.1597	369.2134	454.2668	539.3186	n.d.	752.4301	851.4953	936.5563	993.5684	1106.6547	1191.7080	774.4469
Pept-A-XXIIb	1964.1459	1965.1522	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1106.6547	1191.7080	774.4469
Pept-A-XXIII	1965.1299	1966.1444	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	837.4860	922.5319	979.5613	1092.6402	1177.6921	789.4509
Pept-A-XXIV	1978.1615	1979.1713	128.0706	199.1072	298.1597	369.2134	454.2668	539.3186	n.d.	752.4301	851.4953	936.5563	993.5684	1106.6547	1191.7080	788.4616
Pept-A-XXVa	1964.1459	1965.1522	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	837.4860	922.5319	979.5613	1092.6402	1177.6921	788.4616
Pept-A-XXVb	1978.1615	1979.1713	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1106.6547	1191.7080	788.4616
Pept-A-XXVIa	1978.1615	1979.1713	128.0704	199.1071	298.1597	369.2134	454.2668	539.3186	n.d.	752.4301	851.4953	936.5563	993.5684	1106.6547	1191.7080	788.4616
Pept-A-XXVIb	1978.1615	1979.1713	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1106.6547	1191.7080	788.4616
Pept-A-XXVIIa	1965.1299	1966.1444	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	837.4860	922.5319	979.5613	1092.6402	1177.6921	789.4509
Pept-A-XXVIIb	1978.1615	1979.1713	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1106.6547	1191.7080	788.4616

n.d.: not detected

Table 2 | Diagnostic fragment ions of peptaibols of clade Longibrachiatum peptaibols of group B detected with full scan Orbitrap-MS measurement

Peptide	M	[M+H] ⁺	b ₁	b ₂	b ₃	b ₄	b ₅	b ₆	b ₇	b ₈	b ₉	b ₁₀	b ₁₁	b ₁₂	b ₁₃	y ₇
Pept-B-I	1908.0833	1909.0897	128.0704	199.1071	270.1443	341.1819	426.2344	497.2745	n.d.	710.3871	823.4642	908.5167	965.5449	1050.5927	1135.6458	774.4469
Pept-B-II	1908.0833	1909.0897	128.0704	199.1071	270.1443	341.1819	426.2344	497.2745	n.d.	710.3871	823.4642	908.5167	965.5449	1050.5927	1135.6458	774.4469
Pept-B-III	1908.0833	1909.0897	128.0704	199.1071	270.1443	341.1819	426.2344	497.2745	n.d.	710.3871	823.4642	908.5167	965.5449	1050.5927	1135.6458	774.4469
Pept-B-IV	1922.0989	1923.1048	128.0704	199.1071	270.1443	341.1819	426.2344	497.2745	n.d.	710.3871	823.4642	908.5167	965.5449	1050.5927	1135.6458	788.4616
Pept-B-V	1908.0833	1909.0897	128.0704	199.1071	270.1443	341.1819	426.2344	497.2745	n.d.	710.4143	823.4719	908.5167	965.5449	1050.5927	1135.6458	774.4469
Pept-B-VI	1922.0989	1923.1048	128.0704	199.1071	284.1598	355.1985	440.2505	511.2871	n.d.	724.3967	837.4860	922.5308	979.5613	1064.6081	1149.6611	774.4469
Pept-B-VII	1922.0989	1923.1048	128.0704	199.1071	270.1443	341.1819	426.2344	497.2745	n.d.	710.3871	823.4642	908.5167	965.5449	1050.5927	1135.6458	788.4616
Pept-B-VIII	1936.1146	1937.1206	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	837.4860	922.5319	979.5613	1064.6081	1149.6611	788.4616
Pept-B-IXa	1908.0833	1909.0897	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	823.4642	908.5167	965.5449	1050.5927	1135.6458	774.4469
Pept-B-IXb	1909.0986	1909.0897	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	837.4860	922.5319	979.5613	1050.5927	1135.6458	774.4469
Pept-B-X	1922.0989	1923.1048	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	823.4642	908.5167	965.5449	1050.5927	1135.6458	788.4616
Pept-B-XI	1922.0989	1923.1048	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	823.4642	908.5167	965.5449	1050.5927	1135.6458	788.4616
Pept-B-XII	1922.0989	1923.1048	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	837.4860	922.5319	979.5613	1064.6081	1149.6611	774.4469
Pept-B-XIII	1922.0989	1923.1048	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	823.4642	908.5167	965.5449	1050.5927	1135.6458	788.4616
Pept-B-XIVa	1923.0829	1924.0999	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	837.4860	922.5319	979.5613	1064.6081	1149.6611	775.4322
Pept-B-XIVb	1923.0829	1924.0999	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4078	837.4860	922.5319	979.5613	1064.6081	1149.6611	775.4322
Pept-B-XVa	1922.0989	1923.1048	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	823.4642	908.5167	965.5449	1050.5927	1135.6458	788.4616
Pept-B-XVb	1922.0989	1923.1048	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	837.4860	922.5319	979.5613	1050.5927	1135.6458	788.4616
Pept-B-XVI	1922.0989	1923.1048	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	837.4860	922.5319	979.5613	1064.6081	1149.6611	774.4469
Pept-B-XVII	1936.1146	1937.1206	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	837.4860	922.5319	979.5613	1064.6081	1149.6611	788.4616
Pept-B-XVIII	1923.1067	1924.0999	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	837.4860	922.5319	979.5613	1064.6081	1149.6611	774.4469
Pept-B-XIX	1936.1146	1937.1206	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	837.4860	922.5319	979.5613	1064.6081	1149.6611	788.4616
Pept-B-XX	1936.1146	1937.1206	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5465	993.5684	1078.6224	1163.6769	774.4469
Pept-B-XXI	1937.0986	1938.0984	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	837.4860	922.5319	979.5613	1064.6081	1149.6611	789.4509
Pept-B-XXII	1936.1146	1937.1206	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	837.4860	922.5319	979.5613	1064.6081	1149.6611	788.4616
Pept-B-XXIII	1922.0989	1923.1048	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	837.4860	922.5319	979.5613	1064.6081	1149.6611	774.4469

n.d.: not detected

Table 2 | continued

Peptide	M	[M+H] ⁺	b ₁	b ₂	b ₃	b ₄	b ₅	b ₆	b ₇	b ₈	b ₉	b ₁₀	b ₁₁	b ₁₂	b ₁₃	y ₇
Pept-B-XXIV	1950.1302	1951.1368	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.5267	936.5563	993.5684	1078.6224	1163.6755	788.4616
Pept-B-XXV	1937.0986	1938.0984	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1078.6224	1163.6769	775.4322
Pept-B-XXVI	1950.1302	1951.1368	128.0704	199.1071	298.1756	369.2134	454.2668	539.3186	n.d.	752.4368	865.5170	950.5699	1007.5874	1092.6402	1177.6921	774.4469
Pept-B-XXVII	1950.1302	1951.1368	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1078.6224	1163.6769	788.4616
Pept-B-XXVIII	1950.1302	1951.1368	128.0704	199.1071	284.1598	355.1985	454.2668	539.3186	n.d.	752.4301	865.5170	950.5699	1007.5874	1092.6402	1177.6921	774.4469
Pept-B-XXIXa	1936.1146	1937.1206	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	922.5319	979.5613	1064.6081	1149.6611	788.4616
Pept-B-XXIXb	1936.1146	1937.1206	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	837.4860	922.5319	979.5613	1064.6081	1149.6611	788.4616
Pept-B-XXX	1950.1302	1951.1368	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1092.6402	1177.6921	774.4469
Pept-B-XXXI	1951.1143	1952.1302	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1078.6224	1163.6769	789.4509
Pept-B-XXXIIa	1950.1302	1951.1368	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1078.6224	1163.6769	788.4616
Pept-B-XXXIIb	1964.1459	1965.1522	128.0704	199.1071	284.1598	355.1985	440.2528	539.3186	n.d.	752.4301	865.5170	950.5699	1007.5874	1092.6402	1177.6921	788.4616
Pept-B-XXXIIIa	1936.1146	1937.1206	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1078.6224	1163.6769	774.4469
Pept-B-XXXIIIb	1936.1146	1937.1206	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	837.4860	922.5319	979.5613	1078.6224	1163.6769	774.4469
Pept-B-XXXIIIc	1950.1302	1951.1368	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1092.6402	1177.6921	774.4469
Pept-B-XXXIIId	1951.1143	1952.1302	128.0704	199.1071	284.1598	355.1985	454.2668	539.3186	n.d.	752.4301	865.5170	950.5699	1007.5874	1092.6402	1177.6921	775.4322
Pept-B-XXXIVa	1937.0986	1938.0984	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	922.5319	979.5613	1064.6081	1149.6611	789.4509
Pept-B-XXXIVb	1951.1143	1952.1302	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1078.6224	1163.6769	789.4509
Pept-B-XXXVa	1936.1146	1937.1206	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1078.6224	1163.6769	774.4469
Pept-B-XXXVb	1936.1146	1937.1206	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	837.4860	922.5319	979.5613	1078.6224	1163.6769	774.4469
Pept-B-XXXVI	1964.1459	1965.1522	128.0704	199.1071	298.1756	369.2134	468.2668	553.3335	n.d.	766.4425	879.5344	964.5869	1021.6084	1106.7307	1191.7080	774.4469
Pept-B-XXXVII	1937.0986	1938.0984	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1078.6224	1163.6769	775.4322
Pept-B-XXXVIII	1950.1302	1951.1368	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1078.6224	1163.6769	788.4616
Pept-B-XXXIX	1964.1459	1965.1522	128.0704	199.1071	284.1598	355.1985	454.2668	539.3186	n.d.	752.4301	865.5170	950.5699	1007.5874	1092.6402	1177.6921	788.4616
Pept-B-XL	1950.1302	1951.1368	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	837.4860	922.5319	979.5613	1078.6224	1163.6769	788.4616
Pept-B- XLIa	1964.1459	1965.1522	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1092.6402	1177.6921	788.4616
Pept-B- XLIb	1964.1459	1965.1522	128.0704	199.1071	284.1598	355.1985	440.2528	539.3186	n.d.	752.4301	865.5170	950.5699	1007.5874	1092.6402	1177.6921	788.4616

n.d.: not detected

Table 2 | continued

Peptide	M	[M+H] ⁺	b ₁	b ₂	b ₃	b ₄	b ₅	b ₆	b ₇	b ₈	b ₉	b ₁₀	b ₁₁	b ₁₂	b ₁₃	y ₇
Pept-B- XLIIa	1950.1302	1951.1368	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1092.6402	1177.6921	774.4469
Pept-B- XLIIb	1936.1146	1937.1206	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1078.6224	1163.6769	774.4469
Pept-B- XLIII	1964.1459	1965.1522	128.0704	199.1071	284.1598	355.1985	454.2668	553.3335	n.d.	766.4425	879.5344	964.5869	1021.6084	1106.7307	1191.7080	774.4469
Pept-B- XLIV	1965.1299	1966.1444	128.0704	199.1071	284.1598	355.1985	454.2668	553.3337	n.d.	766.4421	879.5344	964.5869	1021.6047	1106.6547	1191.7080	775.4322
Pept-B- XLVa	1950.1302	1951.1368	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1078.6224	1163.6769	788.4616
Pept-B- XLVb	1978.1615	1979.1713	128.0704	199.1071	284.1598	369.2134	454.2668	553.3335	n.d.	766.4425	879.5344	964.5869	1021.6084	1106.6547	1191.7080	788.4616
Pept-B-XLVIa	1936.1146	1937.1206	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1078.6224	1163.6769	774.4469
Pept-B-XLVIb	1950.1302	1951.1368	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1092.6402	1177.6921	774.4469
Pept-B-XLVII	1964.1459	1965.1522	128.0704	199.1071	284.1598	355.1985	454.2668	539.3186	n.d.	752.4301	865.5170	950.5699	1007.5874	1092.6402	1177.6921	789.4509
Pept-B- XLVIIIa	1950.1302	1951.1368	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	837.4860	922.5319	979.5613	1078.6224	1163.6769	788.4616
Pept-B- XLVIIIb	1964.1459	1965.1522	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1092.6402	1177.6921	788.4616
Pept-B-XLIX	1978.1615	1979.1713	128.0704	199.1071	298.1756	369.2134	454.2668	553.3335	n.d.	766.4425	879.5344	964.5869	1021.6084	1106.6547	1191.7080	788.4616
Pept-B-L	1965.1299	1966.1444	128.0704	199.1071	284.1598	355.1985	454.2668	553.3335	n.d.	766.4425	879.5344	964.5869	1021.6084	1106.6547	1191.7080	775.4322
Pept-B-LI	1978.1615	1979.1713	128.0704	199.1071	284.1598	369.2134	468.2816	567.3482	n.d.	780.4582	893.5424	978.6031	1035.6162	1120.6686	1205.7234	774.4469
Pept-B-LII	1951.1143	1952.1302	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1078.6224	1163.6769	789.4509
Pept-B-LIII	1950.1302	1951.1368	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1092.6402	1177.6921	774.4469
Pept-B-LIV	1978.1615	1979.1713	128.0704	199.1071	284.1598	355.1985	454.2668	553.3335	n.d.	766.4425	879.5344	964.5869	1021.6084	1106.6547	1191.7080	788.4616
Pept-B-LV	1964.1459	1965.1522	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1092.6402	1177.6921	788.4616
Pept-B-LVI	1950.1302	1951.1368	128.0704	199.1071	284.1598	355.1985	440.2528	511.2871	n.d.	724.3967	837.4860	922.5319	979.5613	1078.6224	1163.6769	788.4616
Pept-B-LVII	1979.1456	1980.1604	128.0704	199.1071	284.1598	355.1985	454.2668	553.3335	n.d.	766.4425	879.5344	964.5869	1021.6084	1106.6547	1191.7080	789.4509
Pept-B-LVIII	1936.1146	1937.1206	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1078.6224	1163.6769	774.4469
Pept-B-LIX	1992.1772	1993.1845	128.0704	199.1071	298.1756	369.2134	468.2816	567.3482	n.d.	780.4582	893.5424	978.6031	1035.6162	1120.6686	1205.7234	788.4616
Pept-B-LXa	1964.1459	1965.1522	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	837.4860	922.5319	979.5613	1092.6402	1177.6921	788.4616
Pept-B-LXb	1964.1459	1965.1522	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1092.6402	1177.6921	788.4616
Pept-B-LXI	1964.1459	1965.1522	128.0704	199.1071	284.1598	355.1985	440.2528	525.3029	n.d.	738.4129	851.4953	936.5563	993.5684	1092.6402	1177.6921	788.4616

n.d.: not detected

Table 3 | Diagnostic fragment ions of peptaibols of brevicelsins (group C) from clade Longibrachiatum detected with the full scan Orbitrap-MS measurement

Peptide	M	[M+H] ⁺	b ₁	b ₂	b ₃	b ₄	b ₅	b ₆	b ₇	b ₈	b ₉	b ₁₀	b ₁₁	b ₁₂	b ₁₃	y ₇
Brevicelsin I	1851.0618	1852.0668	128.0704	199.1071	284.1598	355.1985	440.2528	-	n.d.	653.3604	766.4414	851.4953	908.5167	993.5684	1078.6224	774.4469
Brevicelsin II	1865.0775	1866.0851	128.0704	199.1071	298.1756	369..2134	454.2668	-	n.d.	667.4159	780.4582	865.5097	922.5319	1007.5874	1092.6402	774.4469
Brevicelsin III	1852.0458	1853.0613	128.0704	199.1071	284.1598	355.1985	440.2528	-	n.d.	653.3604	766.4425	851.4953	908.5167	993.5684	1078.6224	775.4322
Brevicelsin IV	1865.0775	1866.0851	128.0704	199.1071	284.1598	355.1985	440.2528	-	n.d.	653.3604	766.4425	851.4953	908.5167	993.5684	1078.6224	788.4616
Brevicelsin V	1879.0931	1880.1007	128.0704	199.1071	284.1598	355.1985	454.2668	-	n.d.	667.3751	780.4582	865.5170	922.5319	1007.5874	1092.6402	788.4616
Brevicelsin VI	1865.0775	1866.0851	128.0704	199.1071	284.1598	355.1985	454.2668	-	n.d.	667.3751	780.4582	865.5170	922.5319	1007.5874	1092.6402	774.4469
Brevicelsin VII	1866.0615	1867.0745	128.0704	199.1071	284.1598	355.1985	440.2528	-	n.d.	653.3604	766.4425	851.4953	908.5167	993.5684	1078.6224	789.4509
Brevicelsin VIII	1879.0931	1880.1007	128.0704	199.1071	284.1598	355.1985	454.2668	-	n.d.	667.3751	780.4582	865.5170	922.5319	1007.5874	1092.6402	788.4616

n.d.: not detected

Table 4 | Diagnostic fragment ions of acylium ion (y_7) detected with Orbitrap-MS² measurements of peptaibol compounds from group A

Peptide	y_7	$y_7 - H_2O$ *	$y_7 - AA$ (19)	$y_7 - AA$ (19-18)	$y_7 - AA$ (19-17)	$y_7 - AA$ (19-16)	$y_7 - AA$ (19-15)
Pept-A-Ia	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-A-Ib	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-A-IIa	775.4322	757.4	624.3356	496.2768	367.2330	282.1803	197.1279
Pept-A-IIb	775.4322	757.4	624.3356	496.2768	367.2330	282.1803	197.1279
Pept-A-IIIa	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-A-IIIb	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-A-IIIC	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-A-IVa	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-A-IVb	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-A-Va	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-A-Vb	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-A-VIa	775.4322	757.4	624.3356	496.2768	367.2330	282.1803	197.1279
Pept-A-VIb	775.4322	757.4	624.3356	496.2768	367.2330	282.1803	197.1279
Pept-A-VIIa	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-A-VIIb	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-A-VIIIa	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-A-VIIIb	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-A-IXa	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-A-IXb	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-A-Xa	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-A-Xb	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-A-XIa	775.4322	757.4	624.3356	496.2768	367.2330	282.1803	197.1279
Pept-A-XIb	775.4322	757.4	624.3356	496.2768	367.2330	282.1803	197.1279
Pept-A-XIc	775.4322	757.4	624.3356	496.2768	367.2330	282.1803	197.1279
Pept-A-XII	775.4322	757.4	624.3356	496.2768	367.2330	282.1803	197.1279
Pept-A-XIIIa	789.4509	771.3	638.3518	510.2926	381.2485	282.1804	197.1280
Pept-A-XIIIb	789.4509	771.3	638.3518	510.2926	381.2485	282.1804	197.1280
Pept-A-XIVa	789.4509	771.3	638.3518	510.2926	381.2485	282.1804	197.1280
Pept-A- XIVb	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-A-XVa	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-A-XVb	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-A-XVIa	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-A-XVIb	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-A-XVIIa	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-A-XVIIb	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-A-XVIII	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-A-XIXa	775.4322	757.4	624.3356	496.2768	367.2330	282.1803	197.1279
Pept-A-XIXb	775.4322	757.4	624.3356	496.2768	367.2330	282.1803	197.1279
Pept-A-XX	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-A-XXIa	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-A-XXIb	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280

* determined on Varian-MS system

Table 4 | continued

Peptide	y ₇	y ₇ - H ₂ O *	y ₇ - AA (19)	y ₇ - AA (19-18)	y ₇ - AA (19-17)	y ₇ - AA (19-16)	y ₇ - AA (19-15)
Pept-A-XXIIa	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-A-XXIIb	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-A-XXIII	789.4509	771.3	638.3518	510.2926	381.2485	282.1804	197.1280
Pept-A-XXIV	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-A-XXVa	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-A-XXVb	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-A-XXVIa	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-A-XXVIb	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-A-XXVIIa	789.4509	771.3	638.3518	510.2926	381.2485	282.1804	197.1280
Pept-A-XXVIIb	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280

* determined on Varian-MS system

Table 5 | Diagnostic fragment ions of acylium ion (y₇) detected with Orbitrap-MS² measurements of peptaibol compounds from group B

Peptide	y ₇	y ₇ - H ₂ O *	y ₇ - AA (19)	y ₇ - AA (19-18)	y ₇ - AA (19-17)	y ₇ - AA (19-16)	y ₇ - AA (19-15)
Pept-B-I	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-II	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-III	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-IV	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-V	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-VI	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-VII	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-VIII	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-IXa	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-IXb	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-X	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-XI	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-XII	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-XIII	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-XIVa	775.4322	757.4	624.3356	496.2768	367.2330	282.1803	197.1279
Pept-B-XIVb	775.4322	757.4	624.3356	496.2768	367.2330	282.1803	197.1279
Pept-B-XVa	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-XVb	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-XVI	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-XVII	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-XVIII	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-XIX	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-XX	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279

* determined on Varian-MS system

Table 5 | continued

Peptide	y ₇	y ₇ - H ₂ O *	y ₇ - AA (19)	y ₇ - AA (19-18)	y ₇ - AA (19-17)	y ₇ - AA (19-16)	y ₇ - AA (19-15)
Pept-B-XXI	789.4	771.3	638.3518	510.2926	381.2485	282.1804	197.1280
Pept-B-XXII	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-XXIII	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-XXIV	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-XXV	775.4322	757.4	624.3356	496.2768	367.2330	282.1803	197.1279
Pept-B-XXVI	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-XXVII	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-XXVIII	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-XXIXa	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-XXIXb	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-XXX	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-XXXI	789.4509	771.3	638.3518	510.2926	381.2485	282.1804	197.1280
Pept-B-XXXIIa	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-XXXIIb	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-XXXIIIa	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-XXXIIIb	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-XXXIIIc	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-XXXIIId	775.4322	757.4	624.3356	496.2768	367.2330	282.1803	197.1279
Pept-B-XXXIVa	789.4509	771.3	638.3518	510.2926	381.2485	282.1804	197.1280
Pept-B-XXXIVb	789.4509	771.3	638.3518	510.2926	381.2485	282.1804	197.1280
Pept-B-XXXVa	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-XXXVb	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-XXXVI	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-XXXVII	775.4322	757.4	624.3356	496.2768	367.2330	282.1803	197.1279
Pept-B-XXXVIII	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-XXXIX	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-XL	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B- XLIa	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B- XLIb	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B- XLIIa	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B- XLIIb	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B- XLIII	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B- XLIV	775.4322	757.4	624.3356	496.2768	367.2330	282.1803	197.1279
Pept-B- XLVa	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B- XLVb	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-XLVa	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-XLVb	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-XLVII	789.4509	771.3	638.3518	510.2926	381.2485	282.1804	197.1280

* determined on Varian-MS system

Table 5 | continued

Peptide	y ₇	y ₇ - H ₂ O *	y ₇ - AA (19)	y ₇ - AA (19-18)	y ₇ - AA (19-17)	y ₇ - AA (19-16)	y ₇ - AA (19-15)
Pept-B- XLVIIIa	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B- XLVIIIb	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-XLIX	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-L	775.4322	757.4	624.3356	496.2768	367.2330	282.1803	197.1279
Pept-B-LI	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-LII	789.4509	771.3	638.3518	510.2926	381.2485	282.1804	197.1280
Pept-B-LIII	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-LIV	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-LV	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-LVI	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-LVII	789.4509	771.3	638.3518	510.2926	381.2485	282.1804	197.1280
Pept-B-LVIII	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Pept-B-LIX	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-LXa	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-LXb	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Pept-B-LXI	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280

* determined on Varian-MS system

Table 6 | Diagnostic fragment ions of acylium ion (y₇) detected with Orbitrap-MS² measurements of brevicelsins (group C)

Peptide	y ₇	y ₇ - H ₂ O *	y ₇ - AA (19)	y ₇ - AA (19-18)	y ₇ - AA (19-17)	y ₇ - AA (19-16)	y ₇ - AA (19-15)
Brevicelsin I	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Brevicelsin II	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Brevicelsin III	775.4322	757.4	624.3356	496.2768	367.2330	282.1803	197.1279
Brevicelsin IV	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Brevicelsin V	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280
Brevicelsin VI	774.4469	756.4	623.3480	495.2911	367.2330	282.1803	197.1279
Brevicelsin VII	789.4509	771.3	638.3518	510.2926	381.2485	282.1804	197.1280
Brevicelsin VIII	788.4616	770.3	637.3637	509.3106	381.2485	282.1804	197.1280

* determined on Varian-MS system

Table 7 | Diagnostic fragment ions of peptaibols detected with the full scan Varian-MS measurement of peptaibol extracts from plate cultures of *T. gamsii* SZMC 1656

Peptide	Area size	M	[M+Na] ⁺	[M+2Na] ²⁺	b ₁	b ₂	b ₃	b ₄	b ₅	b ₆	b ₇	b ₈	b ₉	b ₁₀	b ₁₁	b ₁₂	y ₇
Pept-Ia	0.28%	1861.1	1884.1	953.55	128.0	185.0	256.0	341.0	440.1	n.d.	653.4	738.4	823.5	910.5	1023.8	1108.6	754.5
Pept-Ib	1.51%	1876.2	1899.2	961.1	128.0	185.0	256.0	341.0	440.1	n.d.	653.4	738.4	823.5	910.5	1023.8	1108.6	768.5
Pept-IIa	1.66%	1876.1	1899.1	961.05	128.0	185.0	256.0	341.1	454.2	n.d.	667.4	752.5	837.5	924.5	1037.3	1122.6	754.5
Pept-IIb	1.99%	1875.2	1898.2	960.6	128.0	185.0	256.0	341.0	440.1	n.d.	653.4	738.4	823.5	910.5	1023.8	1108.6	768.5
Pept-IIIa	0.56%	1876.2	1899.2	961.1	128.0	185.0	256.0	341.1	454.2	n.d.	667.4	752.5	837.5	924.5	1037.3	1122.6	754.5
Pept-IIIb	2.96%	1875.3	1898.3	960.65	128.0	185.0	256.0	341.0	440.1	n.d.	653.4	738.4	823.5	910.5	1023.8	1108.6	768.5
Pept-IVa	3.70%	1875.2	1898.2	960.6	128.0	185.0	256.0	341.1	454.2	n.d.	667.4	752.5	837.5	924.5	1037.3	1122.6	754.5
Pept-IVb	9.21%	1875.2	1898.2	960.6	128.0	185.0	256.0	341.0	440.1	n.d.	653.4	738.4	823.5	910.5	1023.8	1108.6	768.5
Pept-Va	4.49%	1876.2	1899.2	961.1	128.0	185.0	256.0	341.1	454.2	n.d.	667.4	752.5	837.5	924.5	1037.3	1122.6	754.5
Pept-Vb	5.41%	1890.2	1913.2	968.1	128.0	185.0	256.0	341.1	454.2	n.d.	667.4	752.5	837.5	924.5	1037.3	1122.6	768.5
Pept-VIa	0.84%	1876.2	1899.2	961.1	128.0	185.0	256.0	341.1	454.2	n.d.	667.4	752.5	837.5	924.5	1037.3	1122.6	754.5
Pept-VIb	23.32%	1890.2	1913.2	968.1	128.0	185.0	256.0	341.1	454.2	n.d.	667.4	752.5	837.5	924.5	1037.3	1122.6	768.5
Pept-VII	4.16%	1889.3	1912.3	967.65	128.0	185.0	256.0	341.1	454.2	n.d.	667.4	752.5	837.5	924.5	1037.3	1122.6	768.5
Pept-VIIIa	0.49%	1874.2	1897.2	960.1	128.0	185.0	256.0	341.1	454.2	n.d.	667.4	752.5	837.5	924.5	1037.3	1122.6	754.5
Pept-VIIIb	27.99%	1890.2	1913.2	968.1	128.0	185.0	256.0	341.1	454.2	n.d.	667.4	752.5	837.5	924.5	1037.3	1122.6	768.5
Pept-IX	5.92%*	1874.5	1897.5	960.25	128.0	184.9	256.0	341.3	454.2	n.d.	667.4	752.4	837.5	908.6	1021.7	1106.7	768.5
Pept-X		1904.2	1927.2	975.1	128.0	199.0	270.0	355.0	467.9	n.d.	681.4	766.5	851.5	938.5	1051.0	1136.8	
Pept-XI	4.82%	1903.3	1926.3	974.65	128.0	199.0	270.0	355.0	467.9	n.d.	681.4	766.5	851.5	938.5	1051.0	1136.8	768.5
Pept-XII	0.69%	1904.2	1927.2	975.1	128.0	199.0	270.0	355.0	467.9	n.d.	681.4	766.5	851.5	938.5	1051.0	1136.8	768.5

n.d.: not detected, *value belonging to both peptides (result of the same peak of y₇ ion)

Table 8 | Diagnostic fragment ions of peptaibols detected with the full scan Varian-MS measurement of peptaibol extracts from plate cultures of *T. koningiopsis* SZMC 12500

Peptide	Area size	M	[M+Na] ⁺	[M+2Na] ²⁺	b ₁	b ₂	b ₃	b ₄	b ₅	b ₆	b ₇	b ₈	b ₉	b ₁₀	b ₁₁	b ₁₂	y ₇
Koningiopsin Ia	0.91%	1875.5	1898.5	960.75	128.0	199.1	270.0	355.1	453.3	n.d.	666.4	751.5	836.6	923.5	1037.6	1122.2	754.5
Koningiopsin Ib	5.96%	1889.5	1912.5	967.75	128.0	199.1	270.0	355.1	453.3	n.d.	666.4	751.5	836.6	923.5	1037.6	1122.2	768.5
Koningiopsin IIa	8.13%	1890.5	1913.5	968.25	128.0	199.0	270.0	355.0	440.2	n.d.	653.4	738.4	850.9	938.6	1050.9	1136.8	754.5
Koningiopsin IIb	2.88%	1889.6	1912.6	967.8	128.1	199.1	270.0	355.1	440.1	n.d.	653.4	738.4	822.5	922.5	1035.8	1120.8	768.5
Koningiopsin IIIa	0.97%	1891.6	1914.6	968.8	128.1	199.0	270.1	355.1	426.0	n.d.	639.3	724.5	836.4	923.5	1037.1	1122.6	755.5
Koningiopsin IIIb	2.03%	1873.6	1896.6	959.8	128.1	199.1	270.0	355.1	440.1	n.d.	653.4	738.4	822.5	922.5	1035.8	1120.8	754.5
Koningiopsin IV	12.27%	1903.6	1926.6	974.8	128.0	199.0	270.0	355.0	440.2	n.d.	653.4	738.4	850.9	938.6	1050.9	1136.8	768.5
Koningiopsin Va	47.94%	1903.6	1926.6	974.8	128.0	199.0	270.0	355.0	440.2	n.d.	653.4	738.4	850.9	938.6	1050.9	1136.8	768.5
Koningiopsin Vb	5.95%	1905.6	1928.6	975.8	128.0	199.0	270.0	355.0	440.2	n.d.	653.4	738.4	850.9	938.6	1050.9	1136.8	769.5
Koningiopsin VIa	11.54%	1888.6	1911.6	967.3	128.1	199.1	270.1	355.0	440.2	n.d.	653.3	738.4	851.6	921.6	1035.8	1120.7	768.5
Koningiopsin VIb	1.41%	1888.7	1911.7	967.35	128.1	199.1	270.1	355.0	440.2	n.d.	653.3	738.4	851.6	921.6	1035.8	1120.7	769.5

n.d.: not detected

Table 9 | Diagnostic fragment ions of acylium ion (y_7) detected with Varian-MS² measurements of peptaibol extracts from plate cultures of *T. gamsii* SZMC 1656

Peptide	y_7	$y_7 - H_2O$	$y_7 - AA (19)$	$y_7 - AA (19-18)$	$y_7 - AA (19-17)$	$y_7 - AA (19-16)$
Pept-Ia	754.5	736.5	637.4	509.4	381.4	282.3
Pept-Ib	768.5	750.5	651.4	523.4	395.4	282.4
Pept-IIa	754.5	736.5	637.4	509.4	381.4	282.3
Pept-IIb	768.5	750.5	651.4	523.4	395.4	282.4
Pept-IIIa	754.5	736.5	637.4	509.4	381.4	282.3
Pept-IIIb	768.5	750.5	651.4	523.4	395.4	282.4
Pept-IVa	754.5	736.5	637.4	509.4	381.4	282.3
Pept-IVb	768.5	750.5	651.4	523.4	395.4	282.4
Pept-Va	754.5	736.5	637.4	509.4	381.4	282.3
Pept-Vb	768.5	750.5	651.4	523.4	395.4	282.4
Pept-VIa	754.5	736.5	637.4	509.4	381.4	282.3
Pept-VIb	768.5	750.5	651.4	523.4	395.4	282.4
Pept-VII	768.5	750.5	651.4	523.4	395.4	282.4
Pept-VIIIa	754.5	736.5	637.4	509.4	381.4	282.3
Pept-VIIIb	768.5	750.5	651.4	523.4	395.4	282.4
Pept-IX	768.5	750.5	651.4	523.4	395.4	282.4
Pept-X	768.5	750.5	651.4	523.4	395.4	282.4
Pept-XI	768.5	750.5	651.4	523.4	395.4	282.4
Pept-XII	768.5	750.5	651.4	523.4	395.4	282.4

Table 10 | Diagnostic fragment ions of acylium ion (y_7) detected with Varian-MS² measurements of peptaibol extracts from plate cultures of *T. koningiopsis* SZMC 12500

Peptide	y_7	$y_7 - H_2O$	$y_7 - AA (19)$	$y_7 - AA (19-18)$	$y_7 - AA (19-17)$	$y_7 - AA (19-16)$
Koningiopsin Ia	754.5	736.5	637.4	509.4	381.4	282.3
Koningiopsin Ib	768.5	750.5	651.4	523.4	395.4	282.4
Koningiopsin IIa	754.5	736.5	637.4	509.4	381.4	282.3
Koningiopsin IIb	768.5	750.5	651.4	523.4	395.4	282.4
Koningiopsin IIIa	755.5	737.5	638.4	509.4	381.4	282.3
Koningiopsin IIIb	754.5	736.5	637.4	509.4	381.4	282.3
Koningiopsin IV	768.5	750.5	651.4	523.4	395.4	282.4
Koningiopsin Va	768.5	750.5	651.4	523.4	395.4	282.4
Koningiopsin Vb	769.5	751.5	652.4	523.4	395.3	282.3
Koningiopsin VIa	768.5	750.5	651.4	523.4	395.4	282.4
Koningiopsin VIb	769.5	751.5	652.4	523.4	395.3	282.3

Table 11 | Diagnostic fragment ions of peptaibols detected with the full scan Varian-MS measurement in plate cultures of *T. aggressivum* f. *europaeum* CBS 433.95 (SZMC 1811)

Name of compound	Area size	M	[M+H] ⁺	[M+Na] ⁺	[M+2H] ²⁺	[M+Na+H] ²⁺	[M+2Na] ²⁺	b ₁	b ₂	b ₃	b ₄	b ₅	b ₆	b ₇	b ₈	b ₉	b ₁₀	b ₁₁	b ₁₂	y ₆
Pept-1719-a-1	5.6%	1719	1720.4	1742.4	860.7	871.7	882.7	128	215	286	399	484	n.d.	697	796	881	938	1023	1108.5	612.4
Pept-1719-a-2	0.6%	1719	1720.4	1742.4	860.7	871.7	882.7	128	215	286	399	484	n.d.	697	796	881	938	1023	1107.7	612.4
Pept-1733-a-1	7.6%	1733	1734.4	1756.4	867.7	878.7	889.7	128	215	286	399	498	n.d.	711	810	895	952	1037	1122.1	612.4
Pept-1703-a-1	4.0%	1703	1704.4	1726.3	852.7	863.7	874.7	128	199	270	383	468	n.d.	681	780	865	922	1007	1092	612.3
Pept-1733-a-2	4.0%	1733	1734.4	1756.4	867.7	878.7	889.7	128	215	286	399	484	n.d.	711	810	895	952	1037	1121.9	612.4
Pept-1717-a-1	0.4%	1717	1718.4	1740.4	859.7	870.7	881.7	128	199	270	383	482	n.d.	695	794	879	936	1021	1105.9	612.4
Pept-1733-a-3	12.5%	1733	1734.4	1756.4	867.7	878.7	889.7	128	215	286	399	484	n.d.	697	796	881	938	1037	1122.1	612.4
Pept-1717-a-2	2.9%	1717	1718.4	1740.4	859.7	870.7	881.7	128	199	270	383	482	n.d.	695	794	879	936	1021	1106	612.4
Pept-1747-a-1	2.4%	1747	1748.4	1770.4	874.7	885.7	896.7	128	215	286	399	498	n.d.	711	810	895	952	1051	1135.9	612.4
Pept-1747-a-2	4.2%	1747	1748.4	1770.4	874.7	885.7	896.7	128	215	286	399	498	n.d.	711	810	895	952	1051	1135.9	612.4
Pept-1747-a-3	13.5%	1747	1748.4	1770.4	874.7	885.7	896.7	128	215	286	399	498	n.d.	711	810	895	952	1051	1136	612.4
Pept-1761-a-1	0.6%	1762	1763.4	1785.4	881.7	892.7	904.2	128	215	286	399	498	n.d.	725	824	909	966	1065	1150.1	612.4
Pept-1717-a-3	5.6%	1717	1718.4	1740.4	859.7	870.7	881.7	128	199	270	383	468	n.d.	681	780	865	922	1021	1105.9	612.4
Pept-1731-a-1	1.4%	1731	1732.4	1754.4	866.7	877.7	888.7	128	199	270	383	468	n.d.	695	794	879	936	1035	1119.9	612.4
Pept-1747-a-4	8.1%	1747	1748.4	1770.4	874.7	885.7	896.7	128	215	286	399	484	n.d.	711	810	895	952	1051	1136.6	612.4
Pept-1731-a-2	1.3%	1731	1732.4	1754.4	866.7	877.7	888.7	128	199	270	383	482	n.d.	695	794	879	936	1035	1120.9	612.4
Pept-1761-b-1	2.2%	1761	1762.4	1784.4	881.7	892.7	903.7	128	215	286	399	498	n.d.	711	810	895	952	1051	1136	626.4
Pept-1761-a-2	1.0%	1761	1762.4	1784.4	881.7	892.7	903.7	128	215	286	399	498	n.d.	725	824	909	966	1065	1149.9	612.4
Pept-1731-a-3	6.9%	1731	1732.4	1754.4	866.7	877.7	888.7	128	199	270	383	482	n.d.	695	794	879	936	1035	1120.2	612.4
Pept-1761-a-3	8.7%	1761	1762.4	1784.4	881.7	892.7	903.7	128	215	286	399	498	n.d.	725	824	909	966	1065	1150	612.4
Pept-1731-a-4	2.5%	1731	1732.4	1754.4	866.7	877.7	888.7	128	199	270	383	468	n.d.	695	794	879	936	1035	1119.8	612.4
Pept-1745-a-1	0.5%	1745	1746.4	1768.4	873.7	884.7	895.7	128	199	270	383	482	n.d.	709	808	893	950	1049	1134.3	612.4
Pept-1775-b-1	0.4%	1775	1776.4	1798.4	888.7	899.7	910.7	128	215	286	399	498	n.d.	725	824	909	966	1065	1149.6	626.4
Pept-1775-b-2	0.3%	1775	1776.4	1798.4	888.7	899.7	910.7	128	215	286	399	498	n.d.	725	824	909	966	1065	1149.7	626.4
Pept-1745-a-2	2.8%	1745	1746.4	1768.4	873.7	884.7	895.7	128	199	270	383	482	n.d.	709	808	893	950	1049	1133.9	612.4

n.d.: not detected

Table 12 | Diagnostic fragment ions of acylium ion (y_6) detected with Varian-MS² measurements in plate cultures of *T. aggressivum* f. *europaeum* CBS 433.95 (SZMC 1811)

Name of compound	M	y_6	$y_6\text{-H}_2\text{O}$	$y_6\text{-AA (18)}$	$y_6\text{-AA (18-17)}$	$y_6\text{-AA (18-16)}$	$y_6\text{-AA (18-15)}$
Pept-1719-a-1	1719	612.4	594.4	509.4	381.4	296.4	211.4
Pept-1719-a-2	1719	612.4	594.4	509.4	381.4	296.4	211.4
Pept-1733-a-1	1733	612.4	594.4	509.4	381.4	296.4	211.4
Pept-1703-a-1	1703	612.3	594.4	509.4	381.4	296.4	211.4
Pept-1733-a-2	1733	612.4	594.4	509.4	381.4	296.4	211.4
Pept-1717-a-1	1717	612.4	594.4	509.4	381.4	296.4	211.4
Pept-1733-a-3	1733	612.4	594.4	509.4	381.4	296.4	211.4
Pept-1717-a-2	1717	612.4	594.4	509.4	381.4	296.4	211.4
Pept-1747-a-1	1747	612.4	594.4	509.4	381.4	296.4	211.4
Pept-1747-a-2	1747	612.4	594.4	509.4	381.4	296.4	211.4
Pept-1747-a-3	1747	612.4	594.4	509.4	381.4	296.4	211.4
Pept-1761-a-1	1762	612.4	594.4	509.4	381.4	296.4	211.4
Pept-1717-a-3	1717	612.4	594.4	509.4	381.4	296.4	211.4
Pept-1731-a-1	1731	612.4	594.4	509.4	381.4	296.4	211.4
Pept-1747-a-4	1747	612.4	594.4	509.4	381.4	296.4	211.4
Pept-1731-a-2	1731	612.4	594.4	509.4	381.4	296.4	211.4
Pept-1761-b-1	1761	626.4	608.4	509.4	381.4	296.4	211.4
Pept-1761-a-2	1761	612.4	594.4	509.4	381.4	296.4	211.4
Pept-1731-a-3	1731	612.4	594.4	509.4	381.4	296.4	211.4
Pept-1761-a-3	1761	612.4	594.4	509.4	381.4	296.4	211.4
Pept-1731-a-4	1731	612.4	594.4	509.4	381.4	296.4	211.4
Pept-1745-a-1	1745	612.4	594.4	509.4	381.4	296.4	211.4
Pept-1775-b-1	1775	626.4	608.4	509.4	381.4	296.4	211.4
Pept-1775-b-2	1775	626.4	608.4	509.4	381.4	296.4	211.4
Pept-1745-a-2	1745	612.4	594.4	509.4	381.4	296.4	211.4

Table 13 | Diagnostic fragment ions of peptaibols detected with the full scan Varian-MS measurement in plate cultures of *T. pleuroti* TPhu1 (SZMC 12454)

Name of compound	Area size	M	[M+H] ⁺	[M+Na] ⁺	[M+2H] ²⁺	[M+Na+H] ²⁺	[M+2Na] ²⁺	b ₁	b ₂	b ₃	b ₄	b ₅	b ₆	b ₇	b ₈	b ₉	b ₁₀	b ₁₁	b ₁₂	y ₆
Tripleurin Ia	0.6%	1767	1768.0	1790.0	884.5	895.5	906.5	128	215	286	371	456	n.d.	669	768	853	924	1023	1107.9	660.3
Tripleurin II	2.8%	1767	1768.0	1790.0	884.5	895.5	906.5	128	215	286	371	456	n.d.	669	768	853	924	1009	1094.5	674.3
Tripleurin Ib	6.5%	1767	1768.0	1790.0	884.5	895.5	906.5	128	215	286	371	456	n.d.	669	768	853	924	1023	1107.8	660.3
Tripleurin IIIa	4.6%	1782	1783.0	1805.0	891.5	902.5	914.0	128	215	286	371	470	n.d.	683	782	867	938	1023	1107.9	674.4
Tripleurin IIIb	6.3%	1782	1783.0	1805.0	891.5	902.5	914.0	128	215	286	371	470	n.d.	683	782	867	938	1023	1108	674.4
Tripleurin IV	1.4%	1752	1753.4	1775.4	876.5	887.5	899.2	128	199	270	355	440	n.d.	653	752	837	908	993	1077.9	674.4
Tripleurin V	7.0%	1782	1783.0	1805.0	891.5	902.5	914.0	128	215	286	371	456	n.d.	669	768	867	938	1037	1122.5	660.3
Tripleurin VI	7.9%	1782	1783.0	1805.0	891.5	902.5	914.0	128	215	286	371	470	n.d.	669	768	853	924	1023	1107.9	674.3
Tripleurin VII	1.2%	1752	1753.4	1775.4	876.5	887.5	899.2	128	199	270	355	440	n.d.	653	752	837	908	1007	1092.5	660.4
Tripleurin VIIa	4.0%	1796	1797.0	1819.0	898.5	909.5	921.0	128	215	286	371	470	n.d.	697	796	881	952	1037	1121.8	674.3
Tripleurin IXa	0.8%	1765	1766.0	1788.0	883.5	894.5	905.5	128	199	270	355	454	n.d.	667	766	865	936	1021	1106.6	660.4
Tripleurin X	1.2%	1765	1766.0	1788.0	883.5	894.5	905.5	128	199	270	355	454	n.d.	667	766	851	922	1007	1091.8	674.4
Tripleurin XI	7.4%	1796	1797.0	1819.0	898.5	909.5	921.0	128	215	286	371	456	n.d.	683	782	881	952	1051	1135.8	660.3
Tripleurin VIIIb	10.0%	1796	1797.0	1819.0	898.5	909.5	921.0	128	215	286	371	470	n.d.	683	782	867	938	1037	1122.2	674.3
Tripleurin IXb	5.9%	1765	1766.0	1788.0	883.5	894.5	905.5	128	199	270	355	454	n.d.	667	766	865	936	1021	1105.8	660.4
Tripleurin XIIa	0.9%	1810	1811.0	1833.0	905.5	916.5	928.0	128	215	286	371	470	n.d.	697	782	881	952	1051	1135.8	674.4
Tripleurin XIIb	1.3%	1810	1811.0	1833.0	905.5	916.5	928.0	128	215	286	371	470	n.d.	697	782	881	952	1051	1135.9	674.4
Tripleurin XIIc	12.0%	1810	1811.0	1833.0	905.5	916.5	928.0	128	215	286	371	470	n.d.	697	782	881	952	1051	1135.9	674.3
Tripleurin XIIIa	0.8%	1780	1781.0	1803.0	890.5	901.5	913.0	128	199	270	355	454	n.d.	695	780	865	936	1021	1106	674.3
Tripleurin XIIIb	3.1%	1780	1781.0	1803.0	890.5	901.5	913.0	128	199	270	355	454	n.d.	695	780	851	922	1021	1106.4	674.4
Tripleurin XIIIc	7.4%	1780	1781.0	1803.0	890.5	901.5	913.0	128	199	270	355	454	n.d.	695	780	851	922	1021	1105.9	674.4
Tripleurin XIV	2.2%	1824	1825.0	1847.0	912.5	923.5	935.0	128	215	286	371	470	n.d.	697	782	895	966	1065	1149.8	674.3
Tripleurin XV	4.6%	1794	1795.0	1817.0	897.5	908.5	920.0	128	199	270	355	454	n.d.	681	780	865	936	1035	1120.6	674.3
Tripleurin XVI	0.3%	1808	1809.0	1831.0	904.5	915.5	927.0	128	199	270	355	454	n.d.	695	780	879	950	1049	1133.8	674.3

n.d.: not detected

Table 14 | Diagnostic fragment ions of acylium ion (y_6) detected with Varian-MS² measurements in plate cultures of *T. pleuroti* TPhu1 (SZMC 12454)

Name of compound	M	y_6	y_6 -H ₂ O	y_6 -AA (18)	y_6 -AA (18-17)	y_6 -AA (18-16)	y_6 -AA (18-15)
Tripleurin Ia	1767	660.3	642.3	509.3	381.3	296.3	211.3
Tripleurin II	1767	674.3	656.3	523.3	395.3	296.3	211.3
Tripleurin Ib	1767	660.3	642.3	509.3	381.3	296.3	211.3
Tripleurin IIIa	1782	674.4	656.4	523.4	395.4	296.4	211.4
Tripleurin IIIb	1782	674.4	656.4	523.4	395.4	296.4	211.4
Tripleurin IV	1752	674.4	656.4	523.4	395.4	296.4	211.4
Tripleurin V	1782	660.3	642.3	509.3	381.3	296.3	211.3
Tripleurin VI	1782	674.3	656.3	523.3	395.3	296.3	211.3
Tripleurin VII	1752	660.4	642.4	509.4	381.4	296.4	211.4
Tripleurin VIIIa	1796	674.3	656.3	523.3	395.3	296.3	211.3
Tripleurin IXa	1765	660.4	642.4	509.4	381.4	296.4	211.4
Tripleurin X	1765	674.4	656.4	523.4	395.4	296.4	211.4
Tripleurin XI	1796	660.3	642.3	509.3	381.3	296.3	211.3
Tripleurin VIIIb	1796	674.3	656.3	523.3	395.3	296.3	211.3
Tripleurin IXb	1765	660.4	642.4	509.4	381.4	296.4	211.4
Tripleurin XIIa	1810	674.4	656.4	523.4	395.4	296.4	211.4
Tripleurin XIIb	1810	674.4	656.4	523.4	395.4	296.4	211.4
Tripleurin XIIc	1810	674.3	656.3	523.3	395.3	296.3	211.3
Tripleurin XIIIa	1780	674.3	656.3	523.3	395.3	296.3	211.3
Tripleurin XIIIb	1780	674.4	656.4	523.4	395.4	296.4	211.4
Tripleurin XIIIc	1780	674.4	656.4	523.4	395.4	296.4	211.4
Tripleurin XIV	1824	674.3	656.3	523.3	395.3	296.3	211.3
Tripleurin XV	1794	674.3	656.3	523.3	395.3	296.3	211.3
Tripleurin XVI	1808	674.3	656.3	523.3	395.3	296.3	211.3

Table 15 | Relative amounts of the identified peptaibol compounds in the peptaibol profiles of *T. aethiopicum*, *T. pinnatum*, *T. capillare*, *T. pseudokoningii*, *T. citrinoviride*, *T. longibrachiatum*, *T. bissettii*, *T. orientale* and *T. novae-zelandiae*. The colours represent the extent of the production (percentage) of each strain from the lowest (yellow) to the highest (red).

	<i>T. aethiopicum</i>	<i>T. pinnatum</i>	<i>T. ghanense</i>	<i>T. capillare</i>	<i>T. pseudokoningii</i>	<i>T. citrinoviride</i>	<i>T. bissettii</i>	<i>T. longibrachiatum</i>	<i>T. bissettii</i>	<i>T. bissettii</i>	<i>T. orientale</i>	<i>T. novae-zelandiae</i>
Peptaibol	22602	22603	22604	22605	22613	22618	1773	1775	1776	12546	12556	22612
Pept-A-Ia										2.01%	5.19%	
Pept-A-Ib												3.19%
Pept-A-IIa							2.20%	1.58%	1.15%			
Pept-A-IIb										0.40%		
Pept-A-IIIa					1.24%							
Pept-A-IIIb											2.06%	
Pept-A-IIIc												1.99%
Pept-A-IVa	27.09%	31.98%		29.78%	16.71%	8.97%	25.40%	42.77%	14.52%	43.21%	47.08%	26.43%
Pept-A-IVb			19.67%									
Pept-A-Va					5.30%							
Pept-A-Vb												3.65%
Pept-A-VIa				9.94%	2.14%	0.78%	16.36%	14.57%	23.34%	5.81%	1.90%	
Pept-A-VIb	6.89%	10.39%	28.06%									
Pept-A-VIIa						0.73%						
Pept-A-VIIb	3.77%											
Pept-A-VIIIa		10.50%										
Pept-A-VIIIb								3.04%				
Pept-A-IXa	3.05%			21.94%	30.27%	24.51%	12.00%	1.98%	7.79%	16.72%	34.48%	
Pept-A-IXb		1.60%	22.55%									47.01%
Pept-A-Xa					8.69%							
Pept-A-XIa	2.05%							1.29%	0.35%			
Pept-A-XIb					1.44%		0.69%					
Pept-A-XIc		3.76%										
Pept-A-XII												0.54%

Table 15 | continued

	<i>T. aethiopicum</i>	<i>T. pinnatum</i>	<i>T. ghanense</i>	<i>T. capillare</i>	<i>T. pseudo-koningii</i>	<i>T. citrinoviride</i>	<i>T. longibrachiatum</i> f. <i>bissettii</i>	<i>T. longibrachiatum</i>	<i>T. bissettii</i>	<i>T. longibrachiatum</i> f. <i>bissettii</i>	<i>T. orientale</i>	<i>T. novae-zelandiae</i>
Peptaibol	22602	22603	22604	22605	22613	22618	1773	1775	1776	12546	12556	22612
Pept-A-XIIIa				6.78%			5.01%	1.42%	7.70%	0.79%		
Pept-A-XIIIb			17.97%									
Pept-A-XIVa	0.48%	0.36%										3.48%
Pept-A-XIVb					2.82%	1.99%						
Pept-A-XVa	0.29%			2.88%	2.68%	2.55%	1.86%	0.31%	1.96%	1.68%	6.67%	
Pept-A-XVb		0.56%										4.83%
Pept-A-XVIa	36.48%	24.23%	1.61%	12.08%	8.19%	14.79%	15.26%	23.21%	11.18%	19.80%	1.45%	
Pept-A-XVIb												3.00%
Pept-A-XVIIa		0.16%			5.10%		0.15%					
Pept-A-XVIIb						1.24%			0.27%			
Pept-A-XVIII	0.32%											
Pept-A-XIXa	6.75%	5.61%	1.97%	3.45%	0.61%	0.61%	8.44%	6.72%	13.19%	1.91%		
Pept-A-XX	4.85%	6.31%						1.47%	0.40%			
Pept-A-XXIa	3.51%	1.39%	2.54%	8.80%	14.80%	35.47%	7.28%	0.93%	6.06%	6.80%	1.16%	
Pept-A-XXIb												5.87%
Pept-A-XXIIa	3.01%	2.23%										
Pept-A-XXIIb						0.50%						
Pept-A-XXIII	0.31%	0.26%	1.49%	2.54%			2.84%	0.49%	4.23%	0.23%		
Pept-A-XXIV	0.75%											
Pept-A-XXVa		0.64%		1.80%			1.76%		7.51%	0.65%		
Pept-A-XXVb						4.24%		0.19%				
Pept-A-XXVIa	0.40%											
Pept-A-XXVIb						2.47%						
Pept-A-XXVIIa									0.34%			
Pept-A-XXVIIb						1.14%						

Table 16 | Relative amounts of the identified peptaibol compounds in the peptaibol profiles of *T. reesei*, *T. saturnisporum*, *T. andinense*, *T. effusum*, *T. parareesei*, *T. flagellatum*, *T. sinense* and *T. konilangbra*. The colours represent the extent of the production (percentage) of each strain from the lowest (yellow) to the highest (red).

	<i>T. reesei</i>	<i>T. reesei</i>	<i>T. reesei</i> Δ	<i>T. saturnisporum</i>	<i>T. andinense</i>	<i>T. effusum</i>	<i>T. parareesei</i>	<i>T. flagellatum</i>	<i>T. sinense</i>	<i>T. konilangbra</i>
Peptaibol	22614	22616	22617	22606	22610	22611	22615	22608	22609	22607
Pept-B-I	0.62%									
Pept-B-II	0.55%									
Pept-B-III	0.37%									
Pept-B-IV	0.78%									
Pept-B-V	0.58%									
Pept-B-VI	0.98%									
Pept-B-VII	0.88%									
Pept-B-VIII		1.47%	2.33%							
Pept-B-IXa	3.61%	4.23%	3.59%							
Pept-B-IXb							0.92%			
Pept-B-X	0.44%									
Pept-B-XI	0.65%									
Pept-B-XII	10.85%	11.96%	16.31%	6.88%	4.23%	10.83%	5.00%	2.69%		
Pept-B-XIII	0.54%									
Pept-B-XIVa				0.44%	1.85%	0.87%	1.08%	0.41%		
Pept-B-XIVb	0.52%	0.64%	1.09%							
Pept-B-XVa	4.67%	5.80%	6.38%							
Pept-B-XVb							0.95%			
Pept-B-XVI	0.19%									
Pept-B-XVII	13.80%	17.22%	21.61%	27.43%	9.41%	19.57%	7.10%	1.20%		
Pept-B-XVIII	0.65%									
Pept-B-XIX	1.02%							0.29%		
Pept-B-XX	15.04%	15.88%	17.04%	9.33%	13.51%	16.03%	11.55%	9.54%	7.41%	4.75%
Pept-B-XXI				0.53%	1.25%	1.21%	0.69%			

Table 16 | continued

	<i>T. reesei</i>	<i>T. reesei</i>	<i>T. reesei</i> Δ	<i>T. saturnisporum</i>	<i>T. andinense</i>	<i>T. effusum</i>	<i>T. parareesei</i>	<i>T. flagellatum</i>	<i>T. sinense</i>	<i>T. konilangbra</i>
Peptaibol	22614	22616	22617	22606	22610	22611	22615	22608	22609	22607
Pept-B-XXII	1.01%									
Pept-B-XXIII					4.01%		3.26%			
Pept-B-XXIV	0.50%									
Pept-B-XXV	1.08%	1.11%	1.48%	0.60%	4.15%	0.67%	4.12%	1.76%		
Pept-B-XXVI								1.38%	3.59%	
Pept-B-XXVII	22.12%	24.75%	19.40%	40.50%	21.07%	36.54%	17.23%	5.60%	1.96%	3.70%
Pept-B-XXVIII								9.50%	14.33%	7.97%
Pept-B-XXIXa					8.69%					
Pept-B-XXIXb							7.10%			
Pept-B-XXX	1.49%	2.40%	0.70%							
Pept-B-XXXI					2.61%			1.63%		
Pept-B-XXXIIa	2.24%	2.08%	1.80%							
Pept-B-XXXIIb								1.12%	2.10%	
Pept-B-XXXIIIa					7.04%		8.55%			
Pept-B-XXXIIIb	0.90%									
Pept-B-XXXIIIc		0.56%	0.81%							
Pept-B-XXXIIId								3.18%		
Pept-B-XXXIVa					1.66%					
Pept-B-XXXIVb						1.05%	1.89%			
Pept-A-IVa				3.49%						
Pept-A-IVb										22.06%
Pept-B-XXXVa		0.82%	1.47%			2.63%				
Pept-B-XXXVb	0.93%				2.78%					

Table 16 | continued

	<i>T. reesei</i>	<i>T. reesei</i>	<i>T. reesei</i> Δ	<i>T. saturnisporum</i>	<i>T. andinense</i>	<i>T. effusum</i>	<i>T. parareesei</i>	<i>T. flagellatum</i>	<i>T. sinense</i>	<i>T. konilangbra</i>
Peptaibol	22614	22616	22617	22606	22610	22611	22615	22608	22609	22607
Pept-B-XXXVI								2.08%	9.12%	
Pept-B-XXXVII					1.17%		1.86%			
Pept-B-XXXVIII		1.35%	0.84%							
Pept-B-XXXIX								0.70%		
Pept-B-XL	1.90%	1.39%	0.55%	3.39%						
Pept-B-XLIa	3.09%	2.63%	0.71%		3.53%		3.75%			
Pept-B-XLIb								9.36%	5.44%	7.47%
Pept-B-XLIIa	0.78%						0.44%			
Pept-B-XLIIb		0.65%	0.82%							
Pept-B-XLIII								6.53%	14.94%	
Pept-A-VIa				1.26%						
Pept-B-XLIV										10.56%
Pept-B-XLVa	2.11%	2.83%	0.59%	0.71%	8.42%	9.78%	11.62%			
Pept-B-XLVb										6.35%
Pept-B-XLVIa	0.16%	0.34%	0.33%							
Pept-B-XLVIb							0.25%			
Pept-B-XLVII								1.92%		
Pept-A-Xa				0.65%						
Pept-A-Xb	0.92%									
Pept-B-XLVIIIa					3.17%		1.24%			
Pept-B-XLVIIIb		1.05%	1.78%							
Pept-B-XLIX								2.70%	4.60%	
Pept-B-L								1.43%		
Pept-B-LI								1.86%	11.70%	4.69%
Pept-B-LII					1.45%	0.83%	1.28%			
Pept-B-LIII	0.26%						1.03%			

Table 16 | continued

	<i>T. reesei</i>	<i>T. reesei</i>	<i>T. reesei</i> Δ	<i>T. saturnisporum</i>	<i>T. andinense</i>	<i>T. effusum</i>	<i>T. parareesei</i>	<i>T. flagellatum</i>	<i>T. sinense</i>	<i>T. konilangbra</i>
Peptaibol	22614	22616	22617	22606	22610	22611	22615	22608	22609	22607
Pept-B-LIV								10.23%	7.65%	13.09%
Pept-B-LV	1.42%	0.85%	0.40%				0.90%			
Pept-A-XVIa				3.15%						
Pept-A-XVIb	0.44%									10.85%
Pept-B-LVI	0.49%						0.84%			
Pept-B-LVII								2.28%		
Pept-B-LVIII	0.31%						0.47%			
Pept-B-LIX								4.13%	6.85%	6.20%
Pept-B-LXa	0.51%									
Pept-B-LXb							2.41%			
Pept-A-XIXa	0.12%			0.89%						
Pept-A-XIXb										2.30%
Pept-B-LXI	0.47%			0.76%			0.99%			
Brevicelsin I							0.81%	4.48%	2.09%	
Brevicelsin II									1.81%	
Brevicelsin III								0.95%		
Brevicelsin IV							2.68%	3.20%	0.87%	
Brevicelsin V									0.42%	
Brevicelsin VI								4.99%	3.31%	
Brevicelsin VII								0.42%		
Brevicelsin VIII								4.41%	1.82%	