Closing report of the NKFI project „Discovering the potential inside of bioactive peptaibol compounds: a step towards practical applications"

During the project period, the *Trichoderma* strain repository of the Szeged Microbiological Collection (SZMC) was extended with several new *Trichoderma* strains isolated from vegetable rhizosphere, mushroom cultivation substrate and forest soil samples, as well as Iranian soil isolates and representatives of the *Longibrachiatum* clade of the genus procured from culture collections. Isolations were carried out on selective dichlorane-Rose Bengal medium. Followed by an initial, morphology-based identification, the isolates were subjected to species-level, DNA barcode-based molecular identification by the sequence analysis of the internal transcribed spacer (ITS) region of the ribosomal RNA gene cluster and where necessary, a fragment of the translation elongation factor 1 alpha (*tef1*) gene. Genomic DNA was isolated from the strains and subjected to PCR-amplification, amplicon sequencing and sequence analysis by the barcoding software *TrichO*key 2.0 available online at http://www.isth.info as well as by nucleotide-nucleotide BLAST analyses performed at http://blast.ncbi.nlm.nih.gov. The isolates from vegetable rhizosphere represented 12 species: *T. harzianum, T. pleuroticola, T. koningiopsis, T. virens, T. atroviride, T. longibrachiatum, T. citrinoviride, T. hamatum, T. gamsii, T. asperellum, T. brevicompactum* and *T. koningii*. From mushroom cultivation substrates, *T. pleuroti* and *T. pleuroticola* (oyster mushroom) as well as *T. aggressivum* (white button mushroom) and *T. decipiens* strains were isolated, among them, the *T. aggressium* f. *aggressivum* was firstly detected in Europe, while *T. decipiens* was firstly detected from *Agaricus* production. Iranian isolates were belonging to *T. viride, T. viridescens, T. asperellum, T. longibrachiatum* and *T. citrinoviride*, while the Hungarian forest isolates were identified as *T. virens* and *T. harzianum*. Culture-collection derived *Trichoderma* strains from species of section *Longibrachiatum* were *T. orientale, T. aethiopicum, T. andinense, T. capillare, T. effusum, T. flagellatum, T. ghanense, T. konilangbra, T. novae-zelandiae, T. pinnatum, T. pseudokoningii, T. reesei* and *T. sinensis*.

For initial screening of *Trichoderma* isolates, 15 previously isolated and identified *Trichoderma* strains were selected from the Szeged Microbiological Collection. The selected strains were from different habitats (wheat rhizosphere, mushroom compost, natural substrates and fruiting bodies of wild-growing mushrooms). The 15 strains represented *T. aggressivum* f. *aggressivum, T. atroviride, T. brevicompactum, T. cerinum, T. citrinoviride, T. gamsii, T. ghanense, T. hamatum, T. harzianum, T. koningiopsis, T. rossicum, T. spirale* and *T. virens*.

A rapid and sensitive biological test system was established for the detection of bioactive peptaibols from *Trichoderma* isolates. Plates were inoculated with 4 different species of bacteria *(Bacillus subtilis, Escherichia coli, Micrococcus luteus, Serratia marcescens*). Mycelia and ferment broths of *Trichoderma* strains grown in shaken or standing cultures in yeast extract-glucose medium were liophilized and dissolved in methanol. Extracts were filled into holes bored in the surface of agar plates. Bacteria were mixed into a covering agar layer poured onto the surface of the medium. Based on size of the inhibition zones and sensitivity to alamethicin as a peptaibol reference compound, *M. luteus* was selected as test organism for the development of an easy-to-perform, plate assay. Pre-cultivation time and inoculum concentration of *M. luteus* were optimized and standardized: a pre-cultivation of 2 days and an inoculum concentration of 200000 bacteria per ml revealed the largest inhibition zones. The developed method was able to detect alamethicin at a relatively low concentration (detection limit at 62.5 µg/ml), suggesting its suitability for peptaibol screening. After the establishment and optimization of the biotest, the screening was extended to isolates from further habitats (wheat straw-based cultivation substrate of oyster mushroom, compost used for the cultivation of white button mushroom, samples from clinical patients, forest-derived isolates).

The diversity of the peptaibols produced by *Trichoderma* isolates was compared on different solid media (yeast extract glucose agar, malt extract agar, champignon powder agar, oyster mushroom powder agar). The spectrum and amount of the produced peptaibols proved to be dependent from the media: the highest amounts and widest spectra of the peptaibols were observed in the extracts of strains cultivated on malt extract agar medium, which was identified as the optimal culture condition for the isolation of peptaibols. Liquid malt extract medium was also tested, however, solid media proved to be more efficient for the production and extraction of peptaibols.

Purification was started with the bioactive components produced by a *T. citrinoviride* strain showing the largest inhibition zone in the bio-assays on *M. luteus*. After the large volume fermentation process, liquid extraction of filtered ferment broth aliquots was optimized with different organic solvents (hexane, pentane, acetone, ethyl-acetate, dichlorometane, chloroform). The latter two extracts produced the highest inhibition zones and the chloroform-ferment broth biphasic system showed lower amount of phase disturbation effects at the phase surfaces. Chloroform extract of the whole ferment broth was concentrated with rotary evaporator, subjected to an own-filled medium pressure normal phase (silica, 40-60 μm) glass column and separated using chloroform-methanol gradient elution. Neighbouring bioactive fractions were pooled and evaporated to dryness. The dry material was dissolved in the mixture of methanol and water and applied on octadecyl-silica solid phase extraction column eluted with stepwise methanol gradient. The separated material was used for the development of a TLC run to separate the bioactive component. At the fine-tuned TLC-mobile phase combination, observed spots were scrapped and tested for bioactivity. Purity of the active fraction was analyzed on an analytical HPLC-UV system at 205 nm. This optimized purification protocol was applied for the purification of peptaibols from all other *Trichoderma* isolates.

For the mass spectrometric (MS) measurement of peptaibol molecules, single quadrupole MS measurement conditions were developed using alamethicin as standard in ESI positive mode. A number of series flow injection analyses was carried out without any chromatographic column at a mobile phase flow rate of 0.2 ml/min, while product ions were monitored in full scan mode in the range of m/z 500-2000. The Curved Desolvatation Line, Q-array and interface voltages were tuned and nebulizing gas pressure as well as temperatures of capillary and block heaters optimized. On the acquired mass spectra, the molecular ions ($[M+H]^+$) of alamethicins (ALM-F30 and ALM-F50) showed very low abundance, but sodium adducts ($[M+Na]^+$) of the molecules appeared with higher intensities. The multiple charged ions were the base peaks on the spectra possessing a sodium and a hydrogen ion ($[M+H+Na]^{2+}$). Detection of these characteristic ions substantially aided the identification of the molecular mass and rapid detection of the purified unknown peptaibols. Based on the MS data, a gradient HPLC-MS method was also established.

The mass spectrometric structure analysis of peptaibols produced by *Trichoderma* strains was then performed by on-line reverse-phase HPLC coupled to electrospray ionization ion trap (IT) MS. Initially, the parameters of the IT-MS were optimized by the continuous infusion of an ALM standard solution directly into the ESI source. Then, the proper HPLC method was developed for the analysis of the alamethicin components, which was capable to separate the peaks of F50-5, F50-6a, F50-7 and F50-8b from each other, which could be identified based on their mass spectra. In the standard solution, the highest amount among the detected components of ALM was ALM F50-7 (57%). The amounts of ALM F50-5, ALM F50-6b and ALM F50-8b were 26%, 12% and 11%, respectively. In the different peaks, the following typical fragments of the peptides were detected and analyzed: $[M+Na]^+$, $[M+2Na]^{2+}$, the major mass with Na^+ ion or two Na^+ ions; b_{13} and y_7p fragments, fragments of the major peptides fragmentized in the very labile Aib-Pro bounds, and some other b-fragments like b_{12}, b_{10}, b_9, b_8, b_6, b_5 and b_4. Then, the compounds of the peptaibols produced by the examined

Trichoderma strains were examined, where the results achieved during the optimization process had contributed to the identification of yet undescribed peptaibol molecules isolated from different *Trichoderma* strains based on the gathered data about the MS fragmentation properties. The novelty of the determined sequences was validated by using the 'Peptaibiotics Database' (https://peptaibiotics-database.boku.ac.at/django).

During the project period, the total number of *Trichoderma* strains examined for peptaibol production reached 51, out of which 46 strains were found to produce peptaibols in high, while 5 strains in low quantities. Using a 3D IT-MS instrument, the sodium adducts ($[M+Na]^+$) of various peptaibol molecules were detected with prominent intensities on the full scan ESI-MS spectra in all cases. Furthermore, the appearance of the characteristic *m/*z values due to the protonated $[M+H]^+$ and double charged $[M+2H]^{2+}$, $[M+H+Na]^{2+}$ and $[M+2Na]^{2+}$ pseudomolecular ions on the mass spectra were generally observed, which confirmed the molecular mass of each detected compound. Moreover, the acquired full scan MS spectra contained also the series of the fragment ions (b ions) related to the N-terminal parts of the peptaibol sequences and a C-terminal fragment ion (y) arising from the cleavage of the Aib-Pro bond via in-source collision-induced dissociation (CID). Therefore, the first 12 N-terminal residues of the detected peptaibols could be determined directly from the MS spectra, but to interpret the sequences of C-termini, MS^2 investigations were needed. In that cases, the identified acylium ions (y_6) in the full scan measurements were applied as precursor ions resulting the b-ion series of the C-terminal residues.

From the crude extracts of white button mushroom pathogenic *T. aggressivum* f. *europaeum*, peptaibols positionally isomeric with hypomurocins B1, B3a, B3b and B4 as well as several new, yet unreported compounds with sequences very similar to hypomurocins B were identified based on the MS and MS^2 spectra. In *T. aggressivum* f. *aggressivum*, 19 hypomurocin B-like compounds were detected, which, however, proved to differ from the hypomurocin B-like compounds of *T. aggressivum* f. *europaeum* by containing an Lxxol residue in the R18 position instead of Vxxol. In the oyster mushroom pathogenic *T. pleuroti* a new group of compounds was detected, which did not match any of the known groups of peptaibols. A relatively close group according to the b_{12} ion is the group of trichokindins, which, however, have a Leu in the R8 position and a completely different y6 ion part with m/z 625. According to the MS^2 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*, 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, trichorzianin PAU was also reported with a further Iva-Aib exchange in position R5. For this new group of peptaibols detected in our study we introduced the name “tripleurin”. Putative NRPS genes enabling the biosynthesis of the detected peptaibols could be found in the available, raw genome sequences of both *T. aggressivum* f. *europaeum* and *T. pleuroti* by bioinformatic analysis. From the peptaibol extract of *T. pleuroticola* (the other oyster mushroom pathogenic species), 35 new peptaibol compounds were identified. These compounds proved to be entirely different from the tripleurins produces by the genetically closely related *T. pleuroti*, and proved to be closely related to the trichovirin II group with a single amino acid difference in position R16. This peptaibol group is also similar to hypomurocins with one further amino acid difference in position R2, thus it represents another new group of *Trichoderma* peptaibols. *In vitro* experiments revealed that peptaibols produced by the *Trichoderma* green mould strains are potential growth inhibitors of mushroom mycelia, and that the host mushrooms may have an influence on the peptaibol profiles of green mould agents.

In addition to *Trichoderma* species causing green mould disease in mushroom cultivation, the non-aggressive mushroom compost colonizers *T. atroviride* (clade *Viride*) and *T. harzianum* (clade *Harzianum*) were also included in the screening for peptaibol production with the optimized biotest method, which was followed by chromatographic separation and the determination of their peptaibol components. Several known peptides belonging to the group of trichorzianins were identified from the examined *T. atroviride* strains, including trichorzianins TA IIIb/IIIc, TA/TB IIa, TB IVb, TA II a, TA/TB Vb, TAP-14a, TA VII and TA/TB VI, as well as new, previously unknown trichorzianins. The four examined *T. harzianum* strains proved to produce 14 to 24 hypomurocin B-like compounds, which are more similar to those of the green mould biotype *T. aggressivum* f. *europaeum* than to the ones of *T. aggressivum* f. *aggressivum.*

In the case of *T. viride*, *T. viridescens* (clade *Viride*) and *T. asperellum* (clade *Hamatum*), trichotoxins were the most prominent group of peptaibols detected. Some compounds proved to be similar to trichotoxin A-50 E (T5E), trichotoxin A-50 F (T5F), trichotoxin A-50 I (T5I) and trichotoxin A-50 J (T5J) as well as trichotoxin T5D2 and trichotoxin sequence 05. We also found 21 yet undescribed trichotoxin sequences obtained as microheterogenous mixtures produced by the members of the *Viride* clade.

T. cerinum (clade *Harzianum*), *T. rossicum* (clade *Stromaticum*) and *T. virens* (clade *Virens*) were also found to produce hypomurocin B-like compounds (21, 21 and 8 compounds, respectively), suggesting that the ability to produce this group of peptaibols is widespread across different clades of the genus. In *T. brevicompactum* (clade *Lutea*) we detected 7 compounds from the group of trichobrachins. *T. hamatum* (clade *Hamatum*), *T. gamsii*, *T. koningiopsis* (clade *Viride*) and *T. spirale* (*Lone lineages*) produced 7, 8, 6 and 3 compounds from the group trichostrigocins. The 3 compounds from *T. spirale* were also found in *T. gamsii*. *T. koningiopsis* sequences contain a different amino acid in position R5 compared to the compounds of the other three species. *T. hamatum* produced peptaibols which are one amino acid longer than those of the other 3 species: the sequences proved to be the same with one additional amino acid in position R4 and an exchange in position R6, thus representing a new group of peptaibols related with trichostrigocins.

Twenty-residue, trichobrachin-like peptaibols were detected in the case of the isolates belonging to the two clinically most relevant species of the genus *T. longibraciatum* and *T. citrinoviride* (clade *Longibrachiatum*). Some of the detected compounds were similar to previously described sequences including Longibrachin AI (Trilongin BI), Longibrachin BII (Trilongin CI), Longibrachin AIII, Longibrachin B III (Trilongin CIII) and Longibrachin A II (Trilongin BII). The production of yet unknown, 20-residue peptaibols related with trichobrachins and suzukacillin could also be detected in the examined *T. longibrachiatum* isolates. The optimized detection and purification protocol has been applied to a series of further species belonging to the clinically and biotechnologically relevant clade *Longibrachiatum* of the genus *Trichoderma*: *T. orientale, T. aethiopicum, T. andinense, T. capillare, T. effusum, T. flagellatum, T. ghanense, T. konilangbra, T. novae-zelandiae, T. pinnatum, T. pseudokoningii, T. reesei* and *T. sinensis*. Production of peptaibols in large amounts could be detected in most of these species with the exception of *T. andinense, T. effusum* and *T. konilangbra*, which produced lower amounts of peptaibols. Forest-derived strains of *T. aethiopicum, T. novae-zelandiae* and *T. pseudokoningii* produced 20-21-residue trichobrachin-like compounds, the sequences of which are different from any peptaibols reported in the literature so far. The spectra of the peptaibols produced by these isolates were entirely different from each other. The largest amount of peptaibols consisting of four yet unknown compounds was produced by *T. aethiopicum*, while seven and six new, trichobrachin-like compounds were detected from *T. pseudokoningii* and *T. novae-zelandiae*, respectively. Feline fetal lung cell proliferation inhibition tests and membrane damage bio-assay with boar sperm cells revealed that although

T. novae-zelandiae produced the least amount of peptaibols, its compounds were the most inhibitory to mammalian cells. Similar trichobrachin-like compounds were also identified from *T. orientale*, *T. andinense*, *T. capillare*, *T. effusum*, *T. flagellatum* (11), *T. ghanense*, *T. konilangbra*, *T. pinnatum*, *T. reesei* and *T. sinensis*, suggesting that the production of trichobrachin-like peptaibols is a general characteristic of species from *Trichoderma* section Longibrachiatum.

The bioactivities of crude hypomurocin, tripleurin, trichostrigocin and trichobrachin extracts were tested against a series of bacteria, yeasts and fungi known as pathogens of humans (*Staphylococcus aureus, Streptococcus faecalis, Pseudomonas aeruginosa, Candida albicans, Candida parapsilosis, Cryptococcus neoformans, Aspergillus niger*), plants (*Xanthomonas campestris, Fusarium solani, Aspergillus awamori, Fusarium oxysporum*), mushrooms (*Pseudomonas tolaasii, Mycogone perniciosa, Cladobotryum mycophilum*), as post harvest pathogens (*Rhizopus oryzae, Mucor mucedo*), and as beneficial biocontrol agents (*Bacillus subtilis, Pseudomonas fluorescens, Trichoderma virens*). *S. aureus* and *B. subtilis* proved to be especially sensitive to hypomurocins and trichostrigocins, which were also very effective against *C. albicans* and *P. tolaasii*, respectively. Except from *Rhizopus oryzae*, the 4 tested compound groups showed inhibitory effects on all tested filamentous fungi, with *A. awamori* and *M. mucedo* being the most sensitive.

A series of purified peptaibol compounds were tested for their bioactivities on boar spermatozoa and feline fetal lung cells with automatic fluorimetric methods. The toxic effects of peptaibols were evaluated by the determination of endpoints (lowest peptaibol concentrations showing toxic effect to the examined mammalian cells). Peptaibols not toxic at all to mammalian cells could not be identified, all tested compounds caused membrane damage which resulted in characteristic symptoms observable by microscopy. Boar spermatozoa proved to be more sensitive than feline fetal lung cells.

The profiles of peptaibols were examined during *in vitro* confrontation of *T. asperellum* – known as a potent biocontrol agent - against six different plant pathogenic fungi (*Fusarium moniliforme, F. culmorum, F. graminearum, F. oxysporum* species complex, *Alternaria solani* and *Rhizoctonia solani*) in order to gain information about the role of peptaibols during mycoparasitism. Analysis of the MS chromatograms of crude extracts from control and confronted cultures revealed that changes in the peptaibol profiles were depending from the confronted fungal pathogens. Certain compounds were overproduced, while some of them completely disappeared or were repressed in the extracts deriving from *T. asperellum*.

The effects of hypomurocin, tripleurin, trichostrigocin and trichobrachin extracts on the development of *Arabidopsis thaliana* roots proved to be concentration-dependent: the concentrated crude extracts inhibited root development, the inhibition effect was less expressed at 5× and 25× dilutions, while at 625× dilution all 4 peptaibol groups stimulated root growth, suggesting the possibility to develop peptaibol-based plant root growth promoting products.

Peptaibol production of 5 *Trichoderma* strains during interaction with plants infected by *Alternaria brassicae* and uninfected plants were compared with the ability of the same *Trichoderma* species to produce various peptaibols during antagonistic interaction with *A. brassicae*. For this purpose, *Arabidopsis* seedlings were inoculated with *Trichoderma* species in the roots and with *Alternaria* on the leaves. Quantification of the expression of *Trichoderma* NRPS gene *tex1* was performed from root samples in both treatments of *Trichoderma-Arabidopsis* and *Trichoderma-Arabidopsis-Alternaria*. The volumes of fold induction were obtained by division of *tex1* transcript quantification in *Trichoderma/Trichoderma-Arabidopsis* and *Trichoderma-Arabidopsis-Alternaria/Trichoderma-Arabidopsis*. Transcription of *tex1* proved to be downregulated in *Trichoderma-Arabidopsis* interactions, while a dramatic increase of *tex1* transcript levels could be detected during *Trichoderma-Arabidopsis-Alternaria* interactions, suggesting a systemic induction of this NRPS *g*ene as a response to leaf infection.

For the different non-proteinogenic amino acids and amino-alcohol residues of peptaibols, parameterization was performed, in the course of which the partial atomic charges were derived from quantum chemical calculations. The parameterizations were carried out for the following non-proteinogenic amino acids and amino alcohols: α-aminoisobutyric acid, isovaline, beta-alanine, beta-hydroxyleucine, hydroxyproline, methylproline and pipecolic acid; as well as alaninol, isoleucinol, leucinol, phenylalaninol, prolinol, serinol, triptophanol and valinol. Wide range of testing was performed in order to determine the best applicable protocol for derivation of partial atomic charges. This included 3 sets of variables: the quantum chemical method (i.e. HF, MP2, B3LYP, CCSD(T)), the applied basis set (i.e. 6-311G, 6-311G(2df,2p), Def2-TZVPP(D)), and the relevant inner preferences of quantum chemical simulation package. In the course of the derivation of partial atomic charges, spherical surfaces were placed equidistantly over every single atomic point charge, starting from a given distance. Then values of electrostatic potential were evaluated numerically on the surfaces with a given density of tagged subintervals (on a given grid). Subsequently, based on these values, point charges were calculated with the criteria of being the best possible approximation of the electrostatic potential. The inner variables were altered, such as the number of spherical surfaces, their distance from each other and the point density of grid applied for the numeric calculation of electrostatic potential. As a conclusion of our investigation over all the studied variables, considering the needed CPU time and the achievable accuracy, the protocol with the best performance consisted of the MP2 quantum chemical method, the 6-311G basis set and the internal preferences of Gaussian overlays of IOP(6/41=4, 6/42=1, 6/43=19). Sorting of partial charge values obtained from different protocols was done with respect to the parameters of Amber ff99SB force field, which was used in further molecular dynamics (MD) simulations.

Trichobrachin (TB-A I-IV and TB-B I-IV), hypomurocin A (HM A 1-5a), trichorozin (TZ I-IV), harzianin (H B I and H K VI), trichofumin (TF A and TF B), and trichorovin (TV XIIa) peptides, composed of 11 amino acids, are the members of the group of short-sequence peptaibols. A comprehensive structural characterization was performed by different MD methods in order to identify their conformational features. Backbone conformations were investigated and various secondary structural elements (e.g. types I and III beta-turns, beta-bend ribbon spiral, helical conformations) were identified in certain segments of the molecules. Side-chain conformations were also examined and the preferred rotamers of the side-chains of amino acids were determined. Furthermore, the stabilizing intramolecular H-bonds evolved between the different groups of molecules were identified. For the peptides, the following distances were measured: end-to-end distance between the N atom of the backbone NH group of Xaa1 amino acid and the C atom of the backbone CH2 group of Xaaol11 amino alcohol; residue-residue distances between all the residue pairs of molecules. Fluctuations of the heavy atoms of backbone were studied and RMSF values were calculated. The three-dimensional structure of short-sequence peptaibols were characterized in detail and compared to one another. Results indicated that these peptides possessed characteristic structural properties, however, both conformational similarities and dissimilarities could be observed.

The 11 harzianin C molecules consisting of 14 amino acids are the members of the group of short-sequence peptaibols. These peptides possess a characteristic sequence pattern: they contain three consecutive (Xaa-Yaa-Aib-Pro) motifs. MD simulations were performed for these peptides in order to identify their typical conformational patterns, focusing on the secondary structural elements and intramolecular H-bonds. In the case of molecules, taking into account the various secondary structures, the presence of types I and III beta-turns was examined along the entire sequence of peptides, and the occurrence of beta-bend ribbon spiral structure was studied for the (Xaa-Yaa-Aib-Pro) motifs. The different beta-turns are usually stabilized by characteristic intramolecular H-bonds, thus the appearance of these interactions was investigated with regard to the tetrapeptide segments containing beta-turn structures. In order

to describe the dynamic behavior of peptides, based on the MD trajectories, the alterations of conformational features were examined as a function of simulation time. In the course of this investigation, it was characterized how the various structural features (i.e. secondary structural elements and H-bonds) change during the MD simulations. Based on the results the typical conformational patterns of harzianin C molecules were determined, as well as the dynamic behavior of these short-sequence peptaibols was characterized in detail.

For the short-sequence peptaibols, i.e. ampullosporin, chalciporin and tylopeptin peptides composed of 15 amino acids, as well as for the long-sequence peptaibols, such as hypomurocin B peptides consisting of 18 amino acid residues, and trichobrachin IIb peptides composed of 20 amino acids, the folding processes were explored applying MD methods. Additionally, the folding features were also investigated in the case of the L-isovaline-containing analogs of hypomurocin B and trichobrachin IIb peptides. For these analogs, the D-isovaline amino acid was replaced by L-isovaline residue, and it was examined how the chirality inversion of this amino acid affects the folding processes of peptides. Based on the MD trajectories, the formation of various helical structures (i.e. alpha-, 310- and left-handed alpha-helix) was studied, and the alterations of helicity values were investigated considering not only the entire sequence of peptides, but also each amino acid residue. The formation of different intramolecular H-bonds evolved between the backbone NH and CO groups was also examined, which were as follows: local H-bonds (i.e. the i←i+3 and i←i+4 H-bonds); non-local H-bonds (i.e. the i←i+n H-bonds, where n > 4; and all the i→i+n H-bonds). The evolution of these H-bonds was studied with regard to their average numbers, as well as to each i←i+3 and i←i+4 H-bonds, respectively. The results revealed that the appearance of local H-bonds was in agreement with the presence of helical structures, and the non-local H-bonds did not affect significantly the evolution of helical conformations. Based on our results, however, it could be concluded that the chirality inversion of isovaline amino acid produced effects on the folding processes of peptides. On the whole, the characteristic structural and folding features of short- and long-sequence peptaibols mentioned above were identified.

To characterize the micelle/membrane-bound conformations of different short- and long-sequence peptaibol molecules, MD calculations were carried out on peptide-micelle/membrane systems. Based on the MD trajectories, the interactions between the peptaibols and micelle/membrane could be characterized by different structural features, i.e., secondary structures, as well as intra- and intermolecular interplays. For the micelle/membrane-bound conformations of peptaibols, the occurring secondary structural elements (i.e. different types of turns, multiple turn structures, alpha-helix, 310-helix and beta-bend ribbon spiral) were determined and the preferred rotamer states of the side-chains of amino acids were identified. Additionally, the presence of intramolecular H-bonds stabilizing the micelle/membrane-bound conformations of peptides was investigated, as well as the appearance of intermolecular interactions formed between the peptides and lipid molecules was studied. Based on the results, the micelle/membrane-bound conformations as well as the interactions between the peptides and micelles/membranes were characterized comprehensively for short- and long-sequence peptaibol molecules. Structure-activity relationship studies were also carried out for peptaibol molecules, in order to identify the possible relationships between the structural features and the bioactivity of peptaibols. The determination of these relationships proved to be a very difficult and time-consuming task, thus so far we have not succeeded in identifying structure-activity relationships in detail for peptaibols, however, these studies are recently in progress.

In conclusion, as a step towards practical applications, the results of this project revealed that peptaibols from the groups of hypomurocins, tripleurins, trichostrigocins and trichobrachins have the potential to be developed to products promoting plant root growth, and they may also be potentially applicable as antimicrobial substances, however, in the latter field their inhibitory effects to mammalian cells should also be taken into consideration.