

SUMMARY OF PH.D DISSERTATION

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

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INTRODUCTION

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 plant pathogens throughout their biocontrol activity, or during infections of mushrooms, or even humans.

Trichoderma species are the main producers of peptaibols, that are short peptides, usually 5–20 amino acid residues long, containing α -aminoisobutyric acid (Aib) and isovaline, as well as acetylated N-terminus and 1,2-amino alcohols at the C-terminus. The name ‘peptaibol’ is formed from the word **peptide** and the two most characteristic residues, **Aib** and the C-terminal amino alcohol. They 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.

Species of the *Trichoderma* 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*.

In this study the peptaibol profiles and related bioactivities of *T. koningiopsis*, *T. gamsii*, *T. aggressivum* f. *europaeum*, *T. pleuroti*, as well as 17 species from clade Longibrachiatum were examined, in order to broaden our knowledge in the field of *Trichoderma* peptaibiomics.

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 plant pathogens 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 high performance liquid chromatography (HPLC) - mass spectrometry (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

METHODS

Culturing methods:

- Solid phase cultivation in Petri-dishes
- Solid phase cultivation in large plates (40 × 40 cm)

Separation techniques:

- Extraction of peptaibols
- Analysis of peptaibols using HPLC-MS
- Purification of peptaibols using semi-preparative HPLC

Quantitative and qualitative investigation of peptaibols:

- Primary structure determination using mass spectrometry
- Peptaibol profile determination using mass spectrometry
- Peptaibol profile analysis using cluster analysis in the ClustVis web tool

Bioassays:

- Testing the effect of peptaibol extracts on bacteria and yeasts
- Testing the effect of peptaibol extracts on filamentous fungi

- *In vitro* confrontation assays
- Bioassays for peptaibol toxicity on mushroom mycelia
- Bioassays for peptaibol toxicity on filamentous fungi
- Bioassays for peptaibol effects on plants (*Arabidopsis thaliana*)
- Peptaibol bioactivity assays against mammalian cells (porcine kidney tubular epithelial cell line (PK-15) and boar sperm)

RESULTS

1. Optimization of HPLC-MS to identify peptaibol compounds

Based on using alamethicin (ALM) as standard, the HPLC-MS Agilent 1100 system and the HPLC-Orbitrap-MS, Dionex UltiMate 3000 instrument were optimized to identify peptaibol compounds.

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

The study of the peptaibiome composition of *T. koningiopsis* and *T. gamsii* from clade Viride of the genus *Trichoderma* revealed a total of 30 peptaibol sequences (26 new and 4 known). The novel peptaibol group named koningiopsins was described with 11 new 19-residue-long sequences, which were produced by *T. koningiopsis*.

3. Peptaibol production of *Trichoderma* species causing mushroom green mould diseases

During the study of the peptaibol production of *Trichoderma* species causing green mould disease, 20 new and 5 already known hypomurocin-like compounds were detected from *T. aggressivum* f. *europaeum*. In the case of *T. pleuroti*, 24 novel, 18-residue-long peptaibol compounds were identified and named as tripleurins.

4. Peptaibol production of *Trichoderma* species from clade Longibrachiatum

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 (*T. aethiopicum*, *T. andinense*, *T. capillare*, *T. citrinoviride*, *T. effusum*, *T. flagellatum*, *T. ghanense*, *T. konilangbra*, *T. longibrachiatum*, *T. novae-zelandiae*, *T. pinnatum*, *T. parareesei*, *T. pseudokoningii*, *T. reesei*, *T. saturnisporum*, *T. sinensis* and *T. orientale*), including 53 new and 75

recurrent compounds. Furthermore, 8 novel 19-residue-long sequences were identified from the extracts of *T. flagellatum*, *T. sinensis* and *T. parareesei* and named as brevicelsins. The peptaibols from clade Longibrachiatum could be categorized into groups A, B and C based on their primary structure, where groups A and B consist of only 20-residue-long peptaibols, while group C comprises exclusively of the new, 19-residue-long brevicelsins.

5. Phylogenetic relations based on peptaibol production

Although, we found that the phylogenetic relationships between the producer species within clade Longibrachiatum are less reflected by the clustering based on peptaibol profiles than based on gene sequences, the peptaibol profiles of the species from this clade may still provide insights into their evolution. 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.

6. Bioactivity of peptaibols produced by *T. gamsii* and *T. koningiopsis*

The examination of the antibiotic activity of *T. gamsii* and *T. koningiopsis* 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 on yeasts, while filamentous fungi showed considerable sensitivity, suggesting that the type of the host cell wall may play a role in the sensitivity to peptaibols.

7. Bioactivity of peptaibols produced by *T. aggressivum* f. *europaeum* and *T. pleuroti*

The peptaibols produced by *T. aggressivum* f. *europaeum* and *T. pleuroti* were found to be potential growth inhibitors of mushroom mycelia, therefore their contribution to the antagonistic arsenal of mushroom green mould species can be suggested. 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.

8. Bioactivity of *T. reesei* peptaibols

Although, peptaibols are toxic to bacteria and fungi, no negative effects could be detected on *A. thaliana* plants at concentrations of *T. reesei* peptaibols below 0.1 mg ml⁻¹. The 0.1 mg ml⁻¹ concentration of the peptaibol extract is still toxic to plant pathogenic filamentous fungi. This extract is also toxic to PK-15 and boar sperm cells, but it needs direct contact with cell membranes, like in the case of common amphiphilic detergents. These findings may be useful in tropical agricultural environments, as peptaibol solutions with biocontrol capabilities may replace the use of thermotolerant *Trichoderma* strains with the potential of causing opportunistic infections in humans.

Altogether, this work highlights the peptaibiome diversity within the genus *Trichoderma*, the biological activity of peptaibols towards various organisms and opens an avenue to their potential exploitation in plant disease management.

SUMMARY

- The HPLC-MS instruments were optimized to identify peptaibols from *Trichoderma* extracts.
- We identified 26 new and 4 known peptaibol compounds from *T. gamsii* and *T. koningiopsis*, including the 11 new 19-residue-long sequences which were named as koningiopsins.
- We detected 20 new and 5 known hypomurocin-like compounds in *T. aggressivum* f. *europaeum* extracts, and 24 novel, 18-residue-long peptaibol compounds were identified and named as tripleurins from *T. pleuroti* extracts.
- A total of 128 20-residue-long peptaibols from the 17 examined species belonging to clade Longibrachiatum were identified, including 53 new and 75 recurrent compounds, as well as 8 novel 19-residue-long sequences, which were named as brevicelsins.
- The peptaibol extracts of *T. gamsii* and *T. koningiopsis* strongly inhibited the growth of Gram-positive bacteria and filamentous fungi, while Gram-

- negative bacteria seemed to be less sensitive and no inhibitory effects of the studied peptaibol extracts could be detected on yeasts.
- The peptaibols produced by *T. aggressivum* f. *europaeum* and *T. pleuroti* are potential growth inhibitors of mushroom mycelia. We detected changes in the quantity of peptaibol compounds during *in vitro* confrontation of *Trichoderma* species with *Agaricus* and *Pleurotus*.
 - No negative effects could be detected on *A. thaliana* plants at concentrations of *T. reesei* peptaibols below 0.1 mg ml⁻¹. The 0.1 mg ml⁻¹ concentration of peptaibol extract is still toxic to plant pathogenic filamentous fungi.
 - The 0.1 mg ml⁻¹ concentration of *T. reesei* peptaibol extract is toxic to PK-15 and boar sperm cells.

LIST OF PUBLICATIONS RELATED TO THIS THESIS

ARTICLES SUMMARIZING THE RESULTS OF THIS THESIS

Marik, T., Szekeres, A., Várszegi, C., Czifra, D., Vágvölgyi, C., and Kredics, L. (2013). Rapid bioactivity-based pre-screening method for the detection of peptaibiotic-producing *Trichoderma* strains. *Acta Biol. Szeged.* 57, 1-7. (IF₂₀₁₃: 0)

Marik, T., Várszegi, C., Kredics, L., Vágvölgyi, C., and Szekeres, A. (2013). Mass spectrometric investigation of alamethicin. *Acta Biol. Szeged.* 57, 109–112. (IF₂₀₁₃: 0)

Marik, T., Urbán, P., Tyagi, C., Szekeres, A., Leitgeb, B., Vágvölgyi, M., et al. (2017) Diversity profile and dynamics of peptaibols produced by green mould *Trichoderma* species in interactions with their hosts *Agaricus bisporus* and *Pleurotus ostreatus*. *Chem. Biodivers.* 14, 33. doi: 10.1002/cbdv.201700033. (IF₂₀₁₇: 1.449)

Marik, T., Tyagi, C., Racić, G., Rakk, D., Szekeres, A., Vágvölgyi, C., et al. (2018). New 19-residue peptaibols from *Trichoderma* clade Viride. *Microorganisms* 6, 85. doi: 10.3390/microorganisms6030085 (IF₂₀₁₈: 4.167)

Marik, T., Tyagi, C., Balázs, D., Urbán, P., Szepesi, Á., Bakacsy, L., et al. (2019). Structural diversity and bioactivities of peptaibol compounds from the Longibrachiatum clade of the filamentous fungal genus *Trichoderma*. *Front. Microbiol.* 10, 1434. doi: 10.3389/fmicb.2019.01434 (IF₂₀₁₉: 4.259)

BOOK CHAPTER:

Marik, T., Szekeres, A., Andersson, M. A., Salkinoja-Salonen, M., Tyagi, C., Leitgeb, B., et al. (2017). Bioactive peptaibols of forest-derived *Trichoderma* isolates from section Longibrachiatum, In: *Soil Biological Communities and Ecosystem Resilience*, eds: Lukac, M., Grenni, P., and Gamboni, M. *Springer International Publishing*, Cham, Switzerland, 277–290. doi: 10.1007/978-3-319-63336-7_17

OTHER ARTICLES

- Hatvani, L., Manczinger, L., **Marik, T.**, Bajkán, S., Vidács, L., Bencsik, O., et al. (2013). The complete degradation of acetanilide by a consortium of microbes isolated from River Maros. *Acta Biol. Szeged*, 57, 117-120 (IF₂₀₁₃: 0)
- Körmöczy, P., **Marik, T.**, Manczinger, L., Sajben-Nagy, E. I., Vágvölgyi, C., and Kredics, L. (2014). *Trichoderma* isolates from vegetable rhizosphere samples: potential for the biological control of *Botrytis* species. *Rev. Agric. Rural Devel.* 3, 324-330 (IF₂₀₁₄: 0)
- Tamandegani, P. R., Zafari, D., **Marik, T.**, Szekeres, A., Vágvölgyi, C., and Kredics, L. (2016). Peptaibol profiles of Iranian *Trichoderma* isolates. *Acta Biol. Hung.* 67, 431-441. doi: 10.1556/018.67.2016.4.9 (IF₂₀₁₆: 0.679)
- Castagnoli, E., **Marik, T.**, Mikkola, R., Kredics, L., Andersson, M. A., Salonen, H., et al. (2018). Indoor *Trichoderma* strains emitting peptaibols in guttation droplets. *J. Appl. Microbiol.* 125, 1408-1422. doi:10.1111/jam.13920 (IF₂₀₁₆: 2.683)
- Castagnoli, E., Salo, J., Toivonen, M. S., **Marik, T.**, Mikkola, R., Kredics, L., et al. (2018). An evaluation of boar spermatozoa as a biosensor for the detection of sublethal and lethal toxicity. *Toxins*, 10, 463. doi:10.3390/toxins10110463 (IF₂₀₁₈: 3.895)
- Vornanen-Winqvist, C., Järvi, K., Toomla, S., Ahmed, K., Andersson, M. A., Mikkola, R., **Marik, T.**, et al. (2018). Ventilation positive pressure intervention effect on indoor air quality in a school building with moisture problems. *Int. J. Environ. Res. Pub.* 15, 230. doi:10.3390/ijerph15020230 (IF₂₀₁₈: 2.468)
- Vornanen-Winqvist, C., Salonen, H., Järvi, K., Andersson, M. A., Mikkola, R., **Marik, T.**, et al. (2018). Effects of ventilation improvement on measured and perceived indoor air quality in a school building with a hybrid ventilation system. *Int. J. Environ. Res. Pub.* 15, 1414. doi:10.3390/ijerph15071414 (IF₂₀₁₈: 2.468)
- Salo, M. J., **Marik, T.**, Bencsik, O., Mikkola, R., Kredics, L., Szekeres, A., et al. (2019). Screening mold colonies by using two toxicity assays revealed indoor strains of *Aspergillus calidoustus* producing ophiobolins G and K. *Toxins*, 11, 683. doi:10.3390/toxins11120683 (IF₂₀₁₉: 3.531)
- Salo, M. J., **Marik, T.**, Mikkola, R., Andersson, M. A., Kredics, L., Salonen, H., et al. (2019). *Penicillium expansum* strain isolated from indoor building material was able to grow on gypsum board and emitted guttation droplets containing chaetoglobosins and communesins A, B and D. *J. Appl. Microbiol.* 127, 1135-1147. doi:10.1111/jam.14369 (IF₂₀₁₉: 3.066)
- Tischner, Z. B., Kredics, L., **Marik, T.**, Vörös, K., Kriszt, B., Péter, B., et al. (2019). Environmental characteristics and taxonomy of microscopic fungi isolated from washing machines. *Fungal Biol-UK*, 123, 650-659. doi:10.1016/j.funbio.2019.05.010 (IF₂₀₁₉: 2.699)
- Tischner, Z. B., Kredics, L., **Marik, T.**, Vörös, K., and Magyar, D. (2019). Hazai háztartásokban üzemelő mosógépek gombaszennyezettsége a használati szokások

tükreben = Fungal contamination of washing machines in domestic households in the light of usage habits. *Egészségtudomány*, 63, 45-65. doi:10.29179/EgTud.2019.1-2/45-65 (IF₂₀₁₉: 0)

Tyagi, C., **Marik, T.**, Szekeres, A., Vágvölgyi, C., Kredics, L., and Ötvös, F. (2019). Tripleurin XIIc: Peptide folding dynamics in aqueous and hydrophobic environment mimic using accelerated molecular dynamics. *Molecules* 24, 358. doi: 10.3390/molecules24020358 (IF₂₀₁₉: 3.060)

Tyagi, C., **Marik, T.**, Vágvölgyi, C., Kredics, L., and Ötvös, F. (2019). Accelerated molecular dynamics applied to the peptaibol folding problem. *Int. J. Mol. Sci.* 20, 4268. doi: 10.3390/ijms20174268 (IF₂₀₁₉: 4.183)

Tamandegani, P. R., **Marik, T.**, Zafari, D., Balázs, D., Vágvölgyi, C., Szekeres, A., and Kredics, L. (2020). Changes in peptaibol production of *Trichoderma* species during *in vitro* antagonistic interactions with fungal plant pathogens. *Biomolecules* 10, 730. doi: 10.3390/biom10050730 (IF₂₀₁₉: 4.082)

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OTHER PUBLICATIONS

Kredics, L., **Marik, T.**, Antal, Z., Nagy, L., and Vágvölgyi, C. (2011). *In silico* analysis of fungal PDR-type ABC transporters. *Acta Microbiol. Immunol. Hung.* 58, p. 174.

Kredics, L., **Marik, T.**, Oláh, S., Terhes, D., Danilovic, G., Pankovic, D., et al. (2012). *Trichoderma* species occurring in the rhizosphere of vegetables in different regions of Hungary. In: 14th DKMT Euroregional Conference on Environment and Health. CD-ROM.

Kredics, L., **Marik, T.**, Oláh, S., Terhes, D., Danilovic, G., Pankovic, D., et al. (2012). Species composition of *Trichoderma* communities in Hungarian soils used for vegetable cultivation. *Rev. Agric. Rural Devel.* 1, p. 483.

Körmöczi, P., Oláh, S., **Marik, T.**, Terhes, D., Danilovic, G., Pankovic, D., et al. (2012). A *Trichoderma* nemzetség biodiverzitása magyarországi zöldséghizoszféra mintákban = Biodiversity of the genus *Trichoderma* in Hungarian vegetable rhizosphere samples. *Mikol. Közl. Clusiana*, 51, pp. 140-141.

Körmöczi, P., **Marik, T.**, Manczinger, L., Sajben-Nagy, E. I., Vágvölgyi, C., Danilovic, G., et al. (2013). Laccase production of *Trichoderma* strains from vegetable rhizosphere. In: 15th Danube-Kris-Mures-Tisa (DKMT) Euroregion Conference on Environment and Health with satellite event LACREMED Conference "Sustainable agricultural production: restoration of agricultural soil quality by remediation": Book of Abstracts, ed: B. Škrbić, University of Novi Sad, Novi Sad, Serbia, p. 56.

- Körmöczi, P., **Marik, T.**, Sajben-Nagy, E. I., Manczinger, L., Vágvölgyi, C., and Kredics, L. (2014). Biocontrol potential of *Trichoderma* isolates from pepper and lettuce rhizosphere against plant pathogenic *Botrytis cinerea* and *B. pseudocinerea* strains. In: *16th Danube-Kris-Mures-Tisa (DKMT) Euroregion Conference on Environment and Health: Book of Abstracts*, eds: Cotoraci, C., and Ardelean, A. "Vasile Goldis" University Press, Arad, Romania, p. 40.
- Kredics, L., Szekeres, A., **Marik, T.**, Várszegi, C., Leitgeb, B., and Vágvölgyi, C. (2014). Screening of toxic fungal metabolites based on their bactericide effect. In: *SIHAM 2014: Tenth Annual Meeting of the Society for Indian Human & Animal Mycologists: Book of Abstracts*, Coimbatore, India, p. 44.
- Marik, T.**, Vágvölgyi, M., Kredics, L., Kele, Z., Vágvölgyi, C., and Szekeres, A. (2014). Trichorzianinok és rokon peptidok *Trichoderma pleuroticola* tenyészetben. In: *Elválasztástudományi Vándorgyűlés 2014: Book of Abstracts*, Pécs, Hungary, p. 116.
- Marik, T.**, Vágvölgyi, M., Kredics, L., Vágvölgyi, C., Leitgeb, B., and Szekeres, A. (2014). Trichorzianins and related compounds in *Trichoderma pleuroticola* cultures. In: *A Magyar Mikrobiológiai Társaság 2014. évi Nagygyűlése és EU FP7 PROMISE Regional Meeting: Book of Abstracts*, Keszthely, Hungary, p. 40.
- Marik, T.**, Vágvölgyi, M., Szekeres, A., Vágvölgyi, C., and Kredics, L. (2014). Trichorzianins and related compounds in *Trichoderma pleuroticola* cultures. In: *1st International Scientific Conference of young scientists and specialists: Book of Abstracts*, ed: Hüseyinov, F. National Academy of Sciences, Baku, Azerbaijan, pp. 334-335.
- Marik, T.**, Vágvölgyi, M., Szekeres, A., Vágvölgyi, C., and Kredics, L. (2014). Alamethicin F50 in ferment broth of different *Trichoderma* cultures. In: *A Magyar Mikrobiológiai Társaság 2014. évi Nagygyűlése és EU FP7 PROMISE Regional Meeting: Book of Abstracts*, Keszthely, Hungary, pp. 40-41.
- Vágvölgyi, M., **Marik, T.**, Vágvölgyi, C., Kredics, L., and Szekeres, A. (2014). Purification and structural elucidation of a bioactive compound produced by *Trichoderma harzianum* SZMC 1874. In: *A Magyar Mikrobiológiai Társaság 2014. évi Nagygyűlése és EU FP7 PROMISE Regional Meeting Keszthely: Book of Abstracts*, Hungary, pp. 78-79.
- Vágvölgyi, M., Szekeres, A., **Marik, T.**, Kele, Z., Vágvölgyi, C., and Kredics, L. (2014). Bioaktív vegyületek azonosítása egy *Trichoderma harzianum* törzsből. In: *Elválasztástudományi Vándorgyűlés 2014: Final program, Presentation- and Poster Abstracts*, Egerszalók, Hungary, p. 115.
- Kredics, L., Bóka, B., Körmöczi, P., Szabó, S., Tarnai, G., Manczinger, L., **Marik, T.**, et al. (2015). BioGO: a new soil inoculant based on the combined application of beneficial bacteria and fungi. In: *miCROPe International Symposium: Microbe-assisted crop production - opportunities, challenges and needs: Book of Abstracts*, Wien, Austria, p. 79.

Marik, T., Szekeres, A., Atanasova, L., Druzhinina, I. S., Andersson, M. A., Salkinoja-Salonen, M., et al. (2015). New bioactive trichobrachin-like peptaibols detected in natural forest habitat-derived isolates from the Longibrachiatum section of the filamentous fungal genus *Trichoderma*. In: *Soil Biological Communities and Aboveground Resilience: Proceedings of the 3rd Annual Meeting*, eds: Grenni, P., and Bevivino, A. *National Research Council*, Roma, Italy, p. 71.

Marik, T., Szekeres, A., Druzhinina, I. S., Vágvölgyi, C., and Kredics, L. (2015). Sequential diversity of peptaibol profiles of *Trichoderma* species causing green mould disease of cultivated mushrooms. *Acta Microbiol. Immunol. Hung.* 62, pp. 180-181.

Szekeres, A., Kredics, L., **Marik, T.,** Vágvölgyi, M., Bencsik, O., Keckeméti, A., et al. (2015). Purification and structural elucidation strategy of peptaibols produced by *Trichoderma* strains. In: *6th Congress of European Microbiologists (FEMS 2015)*, Maastricht, The Netherlands, Poster number: FEMS-1968.

Szekeres, A., Kredics, L., **Marik, T.,** Vágvölgyi, M., Bencsik, O., Keckeméti, A., et al. (2015). Verification of peptaibol production ability of a *Trichoderma* strain selected by a bacterium-based screening method. In: *6th Congress of European Microbiologists (FEMS 2015)*, Maastricht, The Netherlands, Poster number: FEMS-1956.

Erdenbileg, S., Volford, B., Ramasamy, A. V., Bencsik, O., **Marik, T.,** Németh, A., et al. (2016). Investigation of antimicrobial effects of endophytic fungal metabolites. In: *18th Danube-Kris-Mures-Tisa (DKMT) Euroregional Conference on Environment and Health: Book of Abstracts*, ed: Škrbić, B., *University of Novi Sad*, Novi Sad, Serbia, p. 95.

Erdenebileg, S., Volford, B., Ramasamy, A. V., Bencsik, O., **Marik, T.,** Németh, A., et al. (2016). Antimicrobial effects of fungal endophytes of *Taxus baccata*. In: *International Conference on Science and Technique Based on Applied and Fundamental Research (ICoSTAF'16): Book of Abstracts*, eds: Keszthelyi-Szabó, G., Hodúr, C., and Krisch, J., *University of Szeged Faculty of Engineering*, Szeged, Hungary, p. 50.

Marik, T., Kredics, L., Szekeres, A., Vágvölgyi, M., Büchner, R., and Vágvölgyi, C. (2016). Effect of peptaibol extracts derived from *Trichoderma* strains on mammalian cells. In: *18th Danube-Kris-Mures-Tisa (DKMT) Euroregional Conference on Environment and Health: Book of Abstracts*, ed: Škrbić, B., *University of Novi Sad*, Novi Sad, Serbia, p. 57.

Máté, V., **Marik, T.,** Tyagi, C., Kredics, L., Vágvölgyi, C., Balázs, L., et al. (2016). Peptaibol molecules in *Trichoderma harzianum*. In: *5th CESC 2016 Central European Summer Course on Mycology and 2nd Rising Stars in Mycology Workshop: Biology of pathogenic fungi: Book of Abstracts*, eds: Gácsér, A., Pfeiffer, I., and Vágvölgyi C., *JATEPress Kiadó*, Szeged, Hungary, p. 58.

Rahimi, T. P., Zafari, D., **Marik, T.,** Szekeres, A., Vahabi, K., Oelmüller, R., et al. (2016). *In vitro* assays to monitor peptaibol production during *Trichoderma* – plant,

- Trichoderma* – plant – pathogen and *Trichoderma* – pathogen interactions. In: *18th Danube-Kris-Mures-Tisa (DKMT) Euroregional Conference on Environment and Health: Book of Abstracts*, ed: Škrbić, B., *University of Novi Sad*, Novi Sad, Serbia, pp. 55-56
- Ramasamy, A. V., Bencsik, O., **Marik, T.**, Elek, G. Z., Németh, A., Kredics, L., et al. (2016). Characterisation of endophytic fungi isolated from *Hypericum perforatum*. In: *18th Danube-Kris-Mures-Tisa (DKMT) Euroregional Conference on Environment and Health: Book of Abstracts*, ed: Škrbić, B., *University of Novi Sad*, Novi Sad, Serbia, p. 97.
- Ramasamy, A. V., Bencsik, O., **Marik, T.**, Németh, A., Kredics, L., Vágvölgyi, C., et al. (2016). Isolation of endophytic fungi from medicinal plants. In: *International Conference on Science and Technique Based on Applied and Fundamental Research (ICoSTAF'16): Book of Abstracts*, eds: Keszthelyi-Szabó, G., Hodúr, C., and Krisch, J., *University of Szeged Faculty of Engineering*, Szeged, Hungary, p. 46.
- Ramasamy, A. V., Volford, B., Erdenebileg, S., Bencsik, O., **Marik, T.**, Németh, A., et al. (2016). Fungal endophytes from the common yew tree (*Taxus baccata*) produce antimicrobial metabolites. In: *Soil Biodiversity and Ecosystem Services: Meeting Programme and Abstracts COST Action FP1305 BioLink: Linking belowground biodiversity and ecosystem function in European forests*, Sofia, Bulgaria, p. 40.
- Tischner, Z. B., Kredics, L., **Marik, T.**, Vörös, K., Vágvölgyi, C., and Magyar, D. (2016). Environmental characteristics and taxonomy of microscopical fungi isolated from Hungarian washing machines. In: *18th Danube-Kris-Mures-Tisa (DKMT) Euroregional Conference on Environment and Health: Book of Abstracts*, ed: Škrbić, B., *University of Novi Sad*, Novi Sad, Serbia, p. 32.
- Tischner, Z. B., Kredics, L., **Marik, T.**, Vörös, K., Vágvölgyi, C., and Magyar, D. (2016). Human pathogenic fungi from Hungarian washing machines. In: *5th CESC 2016 Central European Summer Course on Mycology and 2nd Rising Stars in Mycology Workshop: Biology of pathogenic fungi: Book of Abstracts*, eds: Gácsér, A., Pfeiffer, I., and Vágvölgyi C., *JATEPress Kiadó*, Szeged, Hungary, p. 53.
- Tischner, Z. B., Magyar, D., Kredics, L., and **Marik, T.** (2016). Adatok a hazai mosógépekben előforduló mikroszkopikus gombák ismeretéhez. *Fiatal Higiénikusok XII. Fóruma, Összefoglalók, Egészségtudomány LX* (2), p. 38.
- Urbán, P., **Marik, T.**, Szekeres, A., Manczinger, L., Hatvani, L., Vágvölgyi, C., et al. (2016). Peptaibol production of the mushroom green mould *Trichoderma pleuroti*: from the genomic background to compound profiles. In: *Power of Microbes in Industry and Environment 2016: Programme and Abstracts*, eds: Mrša, V., Teparić, R., and Kifer D., *Croatian Microbiological Society*, Zagreb, Croatia, p. 38.
- Volford, B., Erdenebileg, S., Bencsik, O., **Marik, T.**, Németh, A., Kredics, L., et al. (2016). Taxonomical characterization of endophytic fungi isolated from Hungarian yew trees. In: *International Conference on Science and Technique Based on Applied and Fundamental Research (ICoSTAF'16): Book of Abstracts*, eds: Keszthelyi-Szabó,

- G., Hodúr, C., and Krisch, J., *University of Szeged Faculty of Engineering*, Szeged, Hungary, p. 54.
- Bóka, B., Sipos, G., **Marik, T.**, Jakab, J., Imre, V., Chen, L., et al. (2017). Rhizomorph-associated microbiome as a potential source of biocontrol agents against *Armillaria* root rot. In: *Woody Root 7: 7th International Symposium on Physiological Processes in Roots of Woody Plants*, eds: Kriiska, K., Rosenvald, K., Meitern, A., Ostonen, I., Tartu, Estonia, p. 86.
- Castagnoli, E., Andersson, M. A., Mikkola, R., Kredics, L., **Marik, T.**, Kurnitski, J., et al. (2017). Indoor *Chaetomium*-like isolates: resistance to chemicals, fluorescence and mycotoxin production. In: *Sisäilmastoseminaari: Book of Abstracts*, ed: Sisäilmäyhdistys, R., Helsinki, Finland, pp. 227-232.
- Magyar, D., Kredics, L., **Marik, T.**, Körmöczy, P., Tischner, Z. B., Papp, T., et al. (2017). Levegőből és beltéri környezetből izolált gombák a Szegedi Mikrobiológiai Törzsgyűjteményben - Airborne and indoor fungi in the Szeged Microbiology Collection. *Mikol. Közl. Clusiana* 56, pp. 116-117.
- Marik, T.**, Balázs, D. K., Bakacsy, L., Szepesi, Á., Szekeres, A., Vágvölgyi, C., et al. (2017). A *Trichoderma* nemzetség *Longibrachiatum* szekciójába tartozó fajok által termelt peptaibolok elsődleges szerkezete és bioaktivitása - Primary structure and bioactivity of peptaibols produced by species of *Trichoderma* section *Longibrachiatum*. *Mikol. Közl. Clusiana* 56, pp. 117-119.
- Nagy, V. D., **Marik, T.**, Bóka, B., Takó, M., Szabó, S., Tarnai, G., et al. (2017). A BioeGO talajoltó készítmény *Trichoderma* komponenseinek extracelluláris polisaccharid-bontó és foszfatáz aktivitásai - Extracellular polysaccharide-degrading and phosphatase activities of the *Trichoderma* components included in the BioeGO soil inoculant. *Mikol. Közl. Clusiana* 56, pp. 121-123.
- Rahimi, T. P., Zafari, D., **Marik, T.**, Szekeres, A., Vágvölgyi, C., and Kredics, L. (2017). Peptaibol production of *T. asperellum* during *in vitro* antagonism against plant pathogenic fungi. In: *2nd Iranian Peptide Conference and Humboldt Kolleg: Bioactive Molecules: Current Trends in Synthesis, Identification and Drug Delivery: Book of Abstracts*, Tehran, Iran, p. 26.
- Tischner, Z. B., Kredics, L., **Marik, T.**, Vörös, K., Vágvölgyi, C., Sebők, F., et al. (2017). Hazai háztartásokban üzemelő mosógépek mikológiai felmérése; diverzitásvizsgálat és patogenitás - Occurrence of microscopical fungi in washing machines kept in Hungarian households; diversity and pathogenicity research. *Mikol. Közl. Clusiana* 56, pp. 141-143.
- Tyagi, C., **Marik, T.**, Szekeres, A., Vágvölgyi, C., Kredics, L., and Ötvös, F. (2017). First report of computational structure elucidation of tripleurin XIIC produced by strain *Trichoderma pleuroti* TPhu1. *Acta Microbiol. Immunol. Hung.* 64, pp. 184-185.
- Tyagi, C., **Marik, T.**, Szekeres, A., Vágvölgyi, C., Kredics, L., and Ötvös, F. (2017). Egy, a termesztett gombák zöldpenészes megbetegedését okozó *Trichoderma*

aggressivum által termelt, újonnan leírt peptaibol molekuláris dinamikán alapuló szerkezetmeghatározása - Molecular dynamics-based structure elucidation of a newly reported peptaibol from the mushroom green mould pathogen *Trichoderma aggressivum*. *Mikol. Közl. Clusiana* 56, pp. 152-154.

Vágvölgyi, C., Bóka, B., Sipos, G., Jakab, J., Imre, V., **Marik, T.**, et al. (2017). Screening of rhizomorph-associated soil samples for potential biocontrol agents against forest-damaging *Armillaria* species. 19th *Danube-Kris-Mures-Tisa (DKMT) Euroregional Conference on Environment and Health, University of Szeged, Faculty of Medicine: Program and Abstracts*, Szeged, Hungary, p. 55.

Endre, G., Dóra, P., **Marik, T.**, Kredics, L., Varga, M., Vágvölgyi, C., et al. (2018). Determination of valine and leucine isomers in peptaibols. In: 16th *Wellmann International Scientific Conference: Book of Abstracts*, ed: Monostori, T., *Szegedi Tudományegyetem Mezőgazdasági Kar, Hódmezővásárhely, Hungary*, pp. 115-116.

Endre, G., **Marik, T.**, Kredics, L., Varga, M., Vágvölgyi, C., and Szekeres, A. (2018). Determination of valine and leucine isomers in peptaibols. In: *Proceedings of the 24th International Symposium on Analytical and Environmental Problems*, eds: Alapi, T., and Ilisz, I., *Szegedi Tudományegyetem, Szeged, Hungary*, pp. 283-285.

Marik, T., Tyagi, C., Balázs, D. K., Urbán, P., Szepesi, Á., Bakacsy, L., et al. (2018). New peptaibol compounds from the genus *Trichoderma*. In: *A Magyar Mikrobiológiai Társaság 2018. évi Nagygyűlése és a XIII. Fermentációs Kollokvium: Book of Abstracts*, Eger, Hungary, p. 39.

Marik, T., Tyagi, C., Balázs, D. K., Urbán, P., Szepesi, Á., Bakacsy, L., et al. (2018). Novel sequences of peptaibiotics from the filamentous fungal genus *Trichoderma*. 20th *Danube-Kris-Mures-Tisa (DKMT) Euroregion Conference on Environment and Health: Book of Abstracts, "Vasile Goldis" University Press, Arad, Romania*, pp. 50-51.

Tyagi, C., **Marik, T.**, Szekeres, A., Vágvölgyi, C., Kredics, L., and Ötvös, F. (2018). Understanding dynamics and structure of fungal peptides: revisiting solvent effects and sampling problems. 20th *Danube-Kris-Mures-Tisa (DKMT) Euroregion Conference on Environment and Health: Book of Abstracts, "Vasile Goldis" University Press, Arad, Romania*, pp. 38-39.

Kredics, L., Urbán, P., **Marik, T.**, Allaga, H., Valasek, A., Balázs, D. K., et al. (2019). Mushroom-pathogenic *Trichoderma* species in the genomic and metabolomic era. In: 21st *Danube-Kris-Mures-Tisa (DKMT) Euroregional Conference on Environment and Health: Book of Abstracts*, ed: Škrbić, B., *Faculty of Technology, University of Novi Sad, Novi Sad, Serbia*, p. 43.

Tyagi, C., **Marik, T.**, Vágvölgyi, C., Kredics, L., and Ferenc, Ö. (2019). Using accelerated molecular dynamics to retrieve conformational ensemble of Alamethicin. In: 33rd *Molecular Modelling Workshop (MMWS2019): Program and Abstracts*, Erlangen, Germany, p. 74.

Volford, B., Erdenebileg, S., Škrbić, B., Bencsik, O., **Marik, T.**, Németh, A., et al. (2019). Taxonomical characterisation of endophytic fungi isolated from Hungarian yew trees. In: *21st Danube-Kris-Mures-Tisza (DKMT) Euroregional Conference on Environment and Health: Book of Abstracts*, ed: Škrbić, B., Faculty of Technology, University of Novi Sad, Novi Sad, Serbia, p. 95.