THE PEPTAIBIOME AND PEPTAIBOMICS. STRUCTURAL CHARACTERIZATION OF THE ENTIRETY OF PEPTAIBIOTICS EXPRESSED BY FUNGI

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Introduction

In analogy to the terms "proteome" and "proteomics" we define the "peptaibiome" as the entirety of fungal peptides containing the non-protein α-aminoisobutyric acid (Aib). Accordingly, "peptaibiomics" is the analytical methodology for the structural characterization of the entirety of peptaibiotics expressed in filamentous fungi [1]. A peptaibiotic is defined as fungal peptide containing Aib and exhibiting antibiotic or other bioactivities. Peptaibiotics consists of mixtures containing up to twenty residues peptides that have, in part, unusual N- and C-termini. We present a rapid and sensitive method for the detection and structural characterization of the peptaibiome expressed by fungal multienzyme complexes [2].

Results and Discussion

For peptaibomics the fungal cultures were grown for seven days on malt-extract agar in Petri dishes. Extraction was performed with three 5 ml portions of MeOH/dichloromethane (1/1, v/v). Extracts were cleaned up on a Sep-Pak C-18 cartridge (1.5 cm x 1.0 cm) [1]. Aliquots of 10 µl were analyzed by HPLC-ESI-MS using gradient elution with MeOH/MeCN/0.1% TFA mixtures (for HPLC and ESI conditions see [1]).

Use of a Kromasil KR100 column together with the gradient system provided best separations for medium-chain (11 - 17 residues) and long-chain (18 - 20 residues) peptaibiotics of varying lipophilicity. For scanning of molecular masses and fragments resulting from cleavage of the extremely labile Aib-Pro bond no collision induced dissociation (CID) energy was used, whereas a CID energy of 45% generated series of characteristic fragment ions [1]. From the mass differences (Δm) of fragment ions the presence of the marker amino acid Aib, characterized by Δm = 85.1 Da, as well as other constituents could be deduced.

Using peptaibomics, characteristic fragment ions were detected and the resulting partial structures were compared with structures in data bases [3]. Suitability of the method is demonstrated with the analysis of different *Trichoderma* strains. As an example, Fig. 1 shows of partial sequences of the mold *Trichoderma asperellum* (CBS 433.97). Comparison of these sequences with peptaibiotics already described in [3] shows that sequence no. 7 from *T. asperellum* might be identical with trichotoxin A 50E and sequence no. 9 might be trichotoxin A 50I from *Trichoderma* viride, strain NRRL 5242. For the partial sequences of peptides from some other Trichoderma strains [1] no correspondence with known structures were found. Thus they are assumed to represent new micro heterogeneous peptaibiotics.

The peptaibomic technique applying HPLC-ESI-MS to extracts of molds cultured on agar plates, as described above, provides reliable diagnostic information on the totality of peptaibiotic production. Differentiation among new and already known structures is possible by comparison with sequences stored in relevant databases.

Trichoderma asperellum (CBS 433.97)

	MW
1 [227]–Aib–Lxx–Aib–Aib–Ala–Aib–763–Aib-Valol	1702
2 [156]–Ala–Aib–Vxx–Aib–Aib–Ala–Aib–[909]	1646
3 [227]–Aib–Lxx–Aib–Aib–Ala–Aib–[923]	1674
4 [270]–Lxx–Aib–Gln–Aib–Aib–Aib–158–Aib–210–Aib–Aib–Gln–Valol	1705
5 [270]–Lxx–Aib–Gln–Aib–Aib–Aib–172–Aib–210–Aib–Aib–Gln–Valol	1719
6 [270]–Lxx–Aib–Gln–Aib–Aib–Vxx–128–Aib–210–Aib–Aib–Gln–Valol	1689
7 [270]–Lxx–Aib–Gln–Aib–Aib–Aib–Ala–Ala–Aib–210–Aib–Aib–Gln–Valol	1689
8 [270]–Lxx–Aib–Gln–Aib–Aib–Aib–Ala–Aib–Aib–210–Aib–Aib–Gln–Valol	1703
9 [270]–Lxx–Aib–Gln–Aib–Aib–Aib–156–Aib–[625]	1717

Fig. 1 Examples of partial sequences from *Trichoderma asperellum* (CBS 433.97) analyzed for peptaibiotics; sequences containing less than 5 AA are not shown; abbreviations according to the standard three-letter nomenclature; Aib = α-aminoisobutyric acid; Lxx = Leu or Ile; Vxx = Val or Iva (isovaline); Valol = valinol; MW, molecular weight. Numbers in square brackets refer to not identified fragment ions.

References

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