PEPTAIBIOMICS: SURVEY OF THE FUNGAL GENUS HYPOCREA

Corina Krause, Sabine Kempff, Jochen Kirschbaum and Hans Brückner

Department of Food Sciences, Interdisciplinary Research Center, University of Giessen, Giessen, Germany

Introduction

Peptaibiotics are defined as a family of fungal peptides containing a high proportion of the nonproteinogenic amino acid Aib (α-aminoisobutyric acid) and showing biological activities. *N*-acetylated members of this group containing a C-terminal 1,2-amino alcohol are defined as peptaibols. Lipopeptaibols are acylated with a fatty acid at the N-terminus, and aminolipopeptides contain unusual heterocyclic residues at N- and/or C-termini. We apply peptaibiomics to fungal cultures grown on single agar plates. Here we present a survey of the peptaibiome of *Hypocrea* species. Peptaibiomics is the analytical methodology for the structural characterization of the totality of peptaibiotics expressed by filamentous fungi [1]. By comparing partial sequences deduced from analytical data with those compiled e.g. in the "Peptaibol Database" [2] the judgment is possible whether or not structures are novel, or related or identical to structures described.

Results and Discussion

Peptaibiomics comprises growth of species of the genus *Hypocre*a on potato-glucose-agar Petri dishes followed by treatment of mycelia with MeOH/DCM (1/1, v/v) and solid-phase extraction with Sep-Pak C-18 cartridges (1.5 cm x 1 cm). The purified peptides were analyzed by online coupling of HPLC with ESI-MS (for HPLC and ESI conditions see [1]). 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 application of a CID energy of 45% generated series of characteristic fragment ions [1]. The resulting partial structures were compared with structures in data bases [2]. Fig. 1 presents examples of partial sequences of *Hypocrea* species screened for peptaibiotics. In extracts of *Hypocrea semiorbis, H. vinosa, H. dichromospora, H. gelatinosa, H. nigricans, H. muroiana* and *H. lactea* a multitude of short-, middle- and long-chain Aib-containing peptides were characterized. Comparison of these sequences with peptaibiotics stored in [2] shows that *H. vinosa* and *H. lactea* produce peptides which are new analogs of the peptaibiotics trichogin and trichokingin from species of *Trichoderma.* For the other fragments and partial sequences no similarity could be validated. The data establish *Hypocrea* as a rich source of peptaibiotics.

***Hypocrea muroiana* MUCL 28442**		**MW**
1	[291]-Phe-Aib-Lxx-Aib-Lxx-[186]	1020
2	[199]-Ala-Aib-Aib-213-Aib-Aib-Ser-Aib-Lxx-[842]	1950
3	[199]-Ala-Aib-Aib-213-Aib-Aib-Ser-Vxx-Vxx-[816]	1924
4	[199]-Ala-Aib-Aib-213-Aib-Aib-Ser-Aib-Lxx-196-Aib-Lxx-Gln-Gln-Pheol	1909

***Hypocrea nigricans* MUCL 28439**		**MW**
1	[157]-Vxx-Vxx-Vxx-Aib-[623]	1162
2	[270]-Vxx-Aib-Lxx-[623]	1190

***Hypocrea gelatinosa* CBS 724.87**		**MW**
1	[142]-Gln-Lxx-Lxx-Aib-[n.i.]	n.i.
2	[157]-Vxx-Lxx-Lxx-Aib-Vxx-[510]	1176
3	[142]-Gln-Lxx-Lxx-Aib-[623]	1204

***Hypocrea dichromospora* CBS 337.69**		**MW**
1	[184]-Ala-Vxx-Aib-Aib-Aib-Leuol	726
2	[184]-Ala-Lxx-Aib-Gly-Lxx-Leuol	740
3	[184]-Ala-Lxx-Aib-Ala-Lxx-Leuol	754
4	[255]-Lxx-Aib-Gly-Lxx-Vxx-[247]	969

***Hypocrea vinosa* CBS 247.63**		**MW**
1	[212]-Gly-Vxx-Aib-Gly-Gly-Vxx-Aib-Gly-Lxx-Leuol	1038
2	[212]-Gly-Lxx-Aib-Gly-Gly-Vxx-Aib-Gly-Lxx-Leuol	1052
3	[212]-Gly-Vxx-Aib-Gly-Gly-Lxx-Aib-Gly-Lxx-Leuol	1052
4	[212]-Gly-Lxx-Aib-Gly-Gly-Lxx-Aib-Gly-Lxx-Leuol	1066

***Hypocrea semiorbis* CBS 244.63**		**MW**
1	[284]-Ala-Aib-Ala-213-Aib-Aib-Leu-Gly-Aib-[788]	1937
2	[284]-Ala-Aib-Ala-213-Vxx-Aib-Aib-Gly-Leu-[774]	1937
3	[284]-Ala-Aib-Aib-213-Vxx-Aib-Aib-Aib-Aib-[774]	1951
4	[284]-Ala-Aib-Aib-213-Ala-Leu-Aib-Gly-Leu-[788]	1965

***Hypocrea lactea* CBS 853.70**		**MW**
1	[212]-Gly-Lxx-Aib-Gly-Gly-Vxx-Aib-Gly-Vxx-Leuol	1038
2	[212]-Gly-Vxx-Aib-Gly-Gly-Lxx-Aib-Gly-Vxx-Leuol	1038
3	[212]-Gly-Vxx-Aib-Gly-Gly-Lxx-Aib-Gly-Lxx-Leuol	1052
4	[212]-Gly-Lxx-Aib-Gly-Gly-Lxx-Aib-Gly-Lxx-Leuol	1066

Fig. 1 Examples of partial sequences from species of Hypocrea analyzed for peptaibiotics; sequences containing less than 3 residues are not shown; abbreviations according to the standard three-letter nomenclature; Aib = α-aminoisobutyric acid; Lxx = Leu or Ile; Vxx = Val or Iva (isovaline); Leuol = leucinol; Pheol = Phenylalaninol; MW, molecular weight; n.i., not identified. Numbers in square brackets refer to not identified fragment ions.

References

1. Krause C., Kirschbaum J. and H. Brückner, Amino Acids, 30 (2006) 435.
2. Whitmore L., Chugh J.K., Snook C. F. and B.A. Wallace, J. Peptide Sci., 9 (2003) 663.