DIVERSIFICATION AMONG MUSHROOMS: ANALYZING RATES OF EVOLUTION IN THE AGARICALES

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The order Agaricales is the most species-rich group of the Basidiomycota, numbering ca. 480 genera and 14,000 described species. Extremely diverse fungi belong to this order, bearing highly distinct morphologies and displaying life history traits which show considerable diversity, yet the driving forces of evolutionary diversification in these mushrooms are poorly known. Understanding why and how certain lineages became extremely species-rich, while others are only represented by a few species, determining the timing of major lineage expansions, or the impact of fruiting body morphologies on speciation rates may have an effect on fungal conservation and taxonomy, however, theoretical and experimental evidence so far remained spurious. A recently launched 4-year project, ADiv, aims to investigate patterns of speciation, extinction and variations in the rate of evolution in the Agaricales. To address these questions, we will use statistical models of lineage diversification in a phylogenetic framework. Modeling of diversification will rely on a new two-gene dataset (referred to as diversity dataset) for ca. 3,000 species accepted in the Agaricales. For molecular investigations we have selected the nLSU and RPB2 loci, which are known to provide sufficient phylogenetic information for relationships at the intrageneric level. In addition to the diversity dataset, a phylogenomic dataset is also being produced, which will provide robust support for the backbone of the Agaricales to reinforce phylogenetic inference from the diversity dataset. These two datasets will allow us to examine general patterns of speciation and extinction, to identify shifts in diversification rates and whether transitions between different fruiting body types have an effect on rates of speciation and extinction in the Agaricales. In this contribution, we report an experimental workflow designed specifically for the Agaricales, progress in generating sequence data and preliminary results about adaptive radiations in the Agaricales.

REVIEW OF PEPTAIBOLS PRODUCED BY *TRICHODERMA* STRAINS RELATED TO THEIR PURIFICATIONS, STRUCTURAL ELUCIDATIONS AND BIOLOGICAL ACTIVITIES

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The efficiency of antimicrobial chemotherapy is increasingly challenged by the emergence of pathogenic strains exhibiting high levels of antibiotic resistance. Therefore, it is very important to search for novel compounds produced by living organisms, which are undergoing intensive investigations. The filamentous fungi are valuable sources of new biologically active compounds and their application in different medicines has a long history. The soil borne *Trichoderma* species (Ascomycota) are known producers of numerous secondary metabolites such as different antibiotics and peptaibols as well as several plant promoting compounds, which could enable their use in the green-agriculture as biocontrol agents. Peptaibols are linear, amphipathic polypeptides forming a family of peptide antibiotics of fungal origin, whose number is constantly growing since the first member, alamethicin was reported in 1966. A considerable part of these compounds was isolated

from the soilborne filamentous fungal genus *Trichoderma*. These peptaibols are composed of several amino acids (7-20) containing characteristic nonproteinogenic amino acid residues. Their purification from the ferment broth and mycelia contained usually comparable isolation and separation steps regarding their similar structures and chemical properties. Furthermore, the elucidations of the peptaibol structures were generally undertaken using mass spectrometric methods using ESI ionization techniques, where the peptide fragmentation pattern enables the de novo peptide sequencing. These types of antibiotics show interesting physico-chemical and biological properties, such as the formation of pores in bilayer lipid membranes, as well as antibacterial, antifungal, and occasionally antiviral activities, and may elicit plant resistance. The aim of this review is to summarize the significant amount of information about the purifications, structural elucidations and biological activities of peptaibols.

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SCREENING METHOD FOR THE RAPID DETECTION OF PEPTAIBOLS PRODUCED BY *TRICHODERMA* STRAINS

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Species of the imperfect filamentous fungal genus *Trichoderma* with teleomorphs belonging to the Hypocreales order of Ascomycota are of great economic importance as sources of antibiotics, enzymes, as plant growth promoters, decomposers of xenobiotics, and as commercial biofungicides. The peptaibols and related peptaibiotics (PrPs) are secondary metabolites constituting a family of fungal peptide antibiotics, which is constantly growing since alamethicin was isolated from cultures of strain NRRL 3199 of *Trichoderma arundinaceum* (initially identified as *T. viride*). These compounds are linear, amphipathic oligopeptides composed of 5-20 amino acids which usually contain several nonproteinogenic amino acid residues representing characteristic building blocks of the structure. The major part of the already described peptaibols is also produced by members of the genus *Trichoderma* including the species *T. aggressivum*, *T. asperellum*, *T. atroviride*, *T. aureoviride*, *T. brevicompactum*, *T. citrinoviride*, *T. harzianum, T. inhamatum, T. koningii, T. longibrachiatum, T. parareesei, T. parceramosum, T. polysporum, T. pseudokoningii, T. pubescens, T. reesei, T. saturnisporum, T. strigosum, T. stromaticum, T. virens* and *T. viride*. In our present work, a rapid bacterium-based screening method was developed and optimized for the detection of peptaibol production using the commercially available alamethicin as a peptaibol reference compound. Initially, a sensitive bacterium strain able to indicate the presence of alamethicin (*Micrococcus luteus*) was selected from the Szeged Microbiological Collection using plate assays. In the final method, the agar overlay technique was applied, which resulted in a homogeneous lawn of bacteria within a thin layer of agar across the surface of the plate. The inhibitory effects of solved compounds were investigated in standard-sized holes bored in the plates. During the experiments the pre-cultivation time and the concentration of the inoculated suspension of the selected *M. luteus* strain were optimized and standardized. The limit of detection and reproducibility of the assay were also determined. The developed method is able to detect the selected peptaibol molecule at relatively low concentration. Thus, it seems to be suitable for our peptaibol screening programme.