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DISSERTATION SUMMARY

Examination of biochemical and molecular genetic background of ochratoxin production in *Aspergillus* species

Krisztina Rigó

Department of Microbiology, University of Szeged, Szeged, Hungary

Ochratoxins (especially ochratoxin A, OA) are economically important mycotoxins produced by *Aspergillus* and *Penicillium* species. The main aim of our project is to examine the spread of OA production among *Aspergillus* species, and to clarify the biochemical and genetic background of ochratoxin biosynthesis in order to develop a molecular detection method for ochratoxin producing fungi. Phylogenetic analysis of OA producing isolates was also carried out using ITS sequences and other features. The examined taxa involve *Aspergillus* sections *Circumdati*, *Flavi* and *Fumigati*. Phenotypic features and sequences of the intergenic transcribed spacer regions and the 5.8 S rRNA gene of type or neotype strains and other isolates of the 17 species currently assigned to *Aspergillus* section *Circumdati* and some potentially related species indicated that *Aspergillus* section *Circumdati* is paraphyletic. *Aspergillus campestris*, *A. lanosus*, and *A. dimorphicus* with *A. sepultus* were found to be more closely related to *Aspergillus* sections *Candidi*, *Flavi* and *Cremei*, respectively. *A. robustus* and *A. ochraceoroseus* were found not to be related to any of the species examined. Species of the proposed revised *Aspergillus* section *Circumdati* formed two main clades, which could also be distinguished based on phenotypic methods. OA producing isolates were scattered on the dendrogram. A similar study of species of *Aspergillus* section *Flavi* indicated that these species form distinct clades. The three main clades identified based on sequence data could also be distinguished based on colony colour, and their ubiquinone systems (*"A. flavus", "A. tamarii"* and *"A. alliaceus"* clades). The synnematous species *A. coremiiformis* was closely related to species in the *"A. tamarii"* clade. Three species, *A. nomius, A. avenaceus* and *A. leporis* were found to form separate lineages not closely related to any of the main clades identified. The intraspecific variability of the *Aspergillus viridinutans* species and its relatives was examined using various techniques including morphological examinations, carbon source utilization tests, restriction enzyme analysis of the mitochondrial and nuclear DNA, and sequence analysis of part of the β-tubulin gene. The ochratoxin A producing *A. viridinutans* strain IMI 306135 was most closely related to an asexual isolate. These two latter strains were more closely related to *A. fumigatus* and *N. fischeri* than to any *A. viridinutans* strains, and possibly represent a new species in *Aspergillus* section *Fumigati*.

Examination of the mycoflora of agricultural products led to the conclusion that *A. ochraceus* is not the only source of OA contamination in these products. Kinetics of ochratoxin A production was also examined in a number of ochratoxin producing isolates representing different sections of the *Aspergillus* genus. Both weak and high ochratoxin producers were tested using immunochemical or HPLC methods. All isolates were found to produce the highest amounts of ochratoxin A after 7-10 days of incubation. The *A. albertensis* and *A. melleus* isolates examined were found to produce ochratoxin A constitutively. Ergosterol content and ochratoxin production of *A. albertensis* cultures were in good correlation.

OA degrading activities of large numbers of Aspergilli were also tested. An *A. niger* isolate was selected for further studies, which could degrade OA and ochratoxin A. Further studies are in progress to apply this isolate or its enzymes for OA decontamination.

OA non-producing mutants of *A. albertensis* were also isolated and characterized. In the future, OA producing and non-producing variants of *A. albertensis* are planned to be screened by RAPD using large numbers of random primers, with degenerate primer pairs based on sequences of polyketide synthase domains, and by the differential display technique. The differentiating DNA fragment would be cloned, characterized and used as a probe against a gene bank of an OA producing *A. albertensis* isolate. The hybridizing clones would be further characterized. Finally, a DNA probe would be developed for fast molecular detection of OA producing fungi in foods and feeds.

Supervisor: János Varga
E-mail: christa@bibl.szef.u-szeged.hu