Monitoring of Ochratoxin A biosynthesis related genes in *Penicillium verrucosum* by differential-display reverse transcription-PCR (DDRT-PCR) experiments.

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Ochratoxin A (OTA), is an isocumarin derivative of the secondary metabolism of different filamentous fungi. Main producers are filamentous fungi belonging to the genera *Aspergillus* and *Penicillium*. OTA is a genotoxic carcinogen for animals and humans. In addition OTA can cause severe effects on fetal development and the immune system. Sources for this mycotoxin can be as well plant materials, e.g. cereals, coffee, beer, wine and fruit juices as food products based on animal tissues except for ruminants. Despite the importance of this mycotoxin for human and animal health nearly nothing is known about the genetic basis for ochratoxin biosynthesis. A presumable biosynthetic pathway for OTA is proposed and most of the metabolic intermediates are isolated and described. But structural as well as regulatory genes remain unknown, they still have to be identified. Besides others one possible approach in this concern is the analysis of differentially expressed genes by DDRT-PCR experiments. A stringent prerequisite for the performance of this type of experiments is the knowledge about permissive/restrictive growth conditions for OTA biosynthesis and/or the existence of ochratoxin A negative mutant strains besides the ochratoxin A producing wild type strain respectively. A well defined minimal medium supplemented by glycerol/NH_4^+ was used for permissive growth condtions and the same minmal medium supplemented by glucose/NO_3^- was used for restrictive growth conditions respectively. Differentially expressed RNA populations as well from permissive/restrictive growth conditions as from OTA^- mutants strains/OTA^+ wild type strains respectively have been labeled by [α 33P] dATP and run on a polyacrylamide sequencing gel. The detection of differentially expressed genes was performed by autoradiography on X-ray films. Differentially expressed DNA bands were selected, eluted out of the polyacrylamide gel and used as a template in succeeding PCR reactions for amplification. These differentially expressed DNA bands will be sequenced and the results will be compared to different data bases.