ABSTRACT OF PH.D. THESIS

USE OF MOLECULAR MARKERS FOR THE GENETIC ANALYSIS OF TOXIN-PRODUCING *FUSARIUM* SPECIES

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The genus *Fusarium* includes fungi of great economic importance. Many species of this diverse group play an important role in plant pathology, food and fodder toxicology, biodeterioration, medical and veterinary mycology and in industrial microbiology.

For many years *Fusarium* proved to be a taxonomically difficult area. There is still considerably controversy over the status of several taxa and disagreements in placing certain species in correct section. These problems may be generated by the lack of a proper knowledge on the phylogenetic relationships among these fungi.

Lesser known and interesting fungi belong to the *Fusarium* section "Arthrosporiella" and "Sporotrichiella". They are common inhabitants of small-grain cereals as weak pathogens or saprophytes. Some of them produce trichothecenes (deoxynivalenol, diacetoxyscirpenol, nivalenol, T-2 toxin, fusarenon-X), a group of sesquiterpene mycotoxins that are implicated in mycotoxicoses of domestic animals and also of humans. Very little is known about the genetics of species of these sections, since most of them are strictly asexual and great difficulties arise, too, in investigating their parasexual cycle. Our cytogenetic and molecular genetic knowledge concerning these species are extremely limited.

The purpose of our studies were to (1) determine electrophoretic karyotypes for eight species of *Fusarium*, as well as presenting data on chromosome numbers and genome sizes, (2) identify molecular karyotypes by chromosomal mappings with certain heterologous gene probes, (3) assess inter-specific karyotype

variability in the asexual fungus *F. poae*, (4) identify molecular markers suitable for estimating within-species diversity, (4) determine the nature of the extrachromosomal elements present in these fungi, (5) cloning and sequencing genes or DNA-segments that might help to understand the variability of these species.

Summary of results

Pulsed-field gel electrophoresis was used to identify karyotypes for eight species of the *Fusarium* sections *Arthrosporiella* and *Sporotrichiella*. The total number of chromosome-sized DNA molecules varied from six to nine, depending on species. The sizes of chromosomes ranged from 0.4 to ~6.5 Mb that allowed to estimate the genomes between 27.0 and 29.9 Mb.

When fractionated chromosomes of the eight species were probed with *Tox5*, a gene coding for the key-enzyme of the trichothecene biosynthesis, strong hybridization signals developed in *Fusarium poae* and *Fusarium sporotrichioides* suggesting that only these two of the eight species have the genetic potential to produce trichothecene mycotoxins.

By using heterologous probes from *Aspergillus nidulans* different *rRNA* loci have been mapped on multiple chromosomes of *Fusarium*.

Electrophoretic karyotyping provided additional information for the taxonomy of fusaria. In this respect the strong affinity of *Fusarium fusarioides, Fusarium poae* and *Fusarium sporotrichioides*

are worth mentioning. Meanwhile all these species show similar morphological characters, *Fusarium fusarioides* and *Fusarium sporotrichioides* are considered to be more closely related and hence placed into *Arthrosporiella* because of their polyblastic conidiogenous cells. However, more recent *Fusarium* systems laid greater emphasis on the presence of pyriform microconidia and placed all these species together with *Fusariun tricinctum* into the section *Sporotrichiella* irrespective of their conidiogenesis. The chromosomal DNA banding patterns identified in our study support the latter taxonomical conception. There were similarities but of lesser degree between the karyotypes of *Fusarium avenaceum* and *Fusarium pallidoroseum*, too. The karyotype of *Fusarium camptoceras* was found to be rather unique demonstrating the taxonomical distinctness of this rarely occurring species. *Fusarium tricinctum* seems to be a central member of the two sections. Its karyotype shows a definite affinity towards *Fusarium poae* and *Fusarium sporotrichioides*, but similarities between this species and the *Fusarium avenaceum-Fusarium pallidoroseum* subgroup could also be detected. The synonymy of *Fusarium chlamydosporum* and *Fusarium fusarioides* suggested by several taxonomists is unlikely if their PFGE patterns are compared.

Intra-specific karyotype polymorphism was studied in nine strains of *Fusarium poae*. All strains contained three large chromosomes (4.8, 5.2 and ~6.5 Mb), and they were rather uniform in this respect. On the other hand, great chromosome polymorphisms were found within the 1.0-3.8 Mb range.

A moderately repetitive element was isolated from the partial genomic library of *Fusarium poae* strain *K21*. This clone, 1.2 kb in size was species-specific, and selectively hybridized to the variable chromosome region of several *Fusarium poae* strains. Sequence analysis of the clone revealed no open reading frame, but comparing the sequence to computerized sequence data resulted in low levels of similarity (50-52%) of retrotransposons from various organisms. Although our clone, named *ZIT1* can not be classified as a transposon or retrotransposon element, it seems to be worthy of further studies, because it is present in *dsRNA* containing isolates of a strictly asexual fungus, selectively hybridize to variable chromosome regions, and contains a zinc-finger DNA-binding domain characteristic for retrotransposons.

More than 300 RAPD markers were identified in these fungi; when RAPD patterns were subjected to computer analysis, wide differences were demonstrated within species.

Sixty geographically different strains of *Fusarium poae* were assayed for the presence of extrachromosomal nucleic acid elements. All strains were found to harbor double-stranded RNA (*dsRNA*) elements as determined by gel-electrophoresis and contained encapsidated virus-like particles (*VLP*) detected by electron microscopy. There were great individual differences in *dsRNA* patterns of the various strains, but numbers and sizes characteristics for a given isolate remained unchanged after repeated subculturing of the fungi. The numbers of *dsRNA*s ranged from 1 to 12 among strains with sizes between 0.2 and 12,0 kb. Morphological alterations or signs

of degeneration were not observed in *dsRNA*-containing isolates. This was the first report on the ubiquitous occurrence of *dsRNA*s in a *hyphomycete* fungus species.

A trichodiene synthase gene (*Tox5*) was amplified from *Fusarium poae* by polymerase chain reaction using synthetic primers constructed on the basis of the coding part of the same gene from *Fusarium sporotrichioides*. Sequence analysis showed high degrees of homology (89 %) with other trichodiene synthase genes.

A 378 bp *Hind*III fragment of the gene that contains the genetic information for the putative active site of the trichodiene synthase enzyme, and at the same time includes the minor deletion variations discovered at the 3'-end of the gene, was radiolabelled and used for dot blot analysis and colony hybridization. This probe was suitable to trace 1 ng DNA of those fungi that harbor an unharmed *Tox5* sequence and are therefore able to synthesize toxic trichothecene compounds, but gave no reaction with trichothecene nonproducers. When agar medium covered by *Hybond-N* membrane was inoculated with various tests fungi and colonies, developed after 48 h incubation were hybridized with the probe, strong signals developed on potential trichothecene producers, while again no reaction was observed on the trichothecene nonproducing colonies. This technique offers a rapid screening procedure for the identification of potential trichothecene producing colonies in mixed fungal populations.

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