Molecular characteristics of fumonisin-producing Fusarium spp. from asparagus

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Introduction

Fusarium proliferatum (teleomorph: *Gibberella intermedia*) is one **causal agent of crown rot of *Asparagus officinalis*** and is one potential **fumonisin producing** species within the genus *Fusarium*. It colonises roots and crowns of asparagus plants, but could also be isolated from symptomless asparagus spears.

F. proliferatum is a well known producer of polyketide derived B type fumonisins. Accumulation of high levels of these secondary metabolites has been described for several *F. proliferatum* strains.

Aims

F. proliferatum strains isolated from asparagus spears from different locations in Austria and Germany, which exhibit DNA polymorphisms, were selected and the presence of two essential genes ***FUM1*** (polyketidesynthase) and ***FUM8*** (aminoacyltransferase) from the fumonisin biosynthetic pathway of *F. proliferatum* were probed by PCR. The **sequence variability** of these gene fragments were analyzed and related to the whole-genome genetic variability determined by fingerprint methods.

Results

Genetic fingerprinting of 45 isolates by RAPD- and DAF-PCR revealed genetic heterogenicity of *F. proliferatum* by establishment of 14 different fingerprint groups (not shown). Most isolates differentiated into three major clusters, but no association was found between fingerprint group and origin of isolates.

In all isolates tested, both initial genes (*FUM1*, *FUM8*) were detectable. Amplified FUM gene fragments exhibited nucleotide polymorphisms, as was shown by sequencing, but were usually not discriminated by PCR-RFLP (Fig. 1 and Fig. 2). Sequence variability of *FUM1* and *FUM8* gene fragments of the *F. proliferatum* strains was below 1%. Interspecific divergence was considerably higher. Gene fragments from *F. verticillioides* shared only 84% (*FUM1*) and 77% (*FUM8*) sequence identity with *F. proliferatum* sequences (Tab. 1). Phylogenetic analysis based upon *FUM1* and *FUM8* sequence fragments was in accordance with genetic relationships within the *Fusarium* genus determined by housekeeping genes (Fig. 3).

PCR-RFLP analysis of *FUM* gene fragments

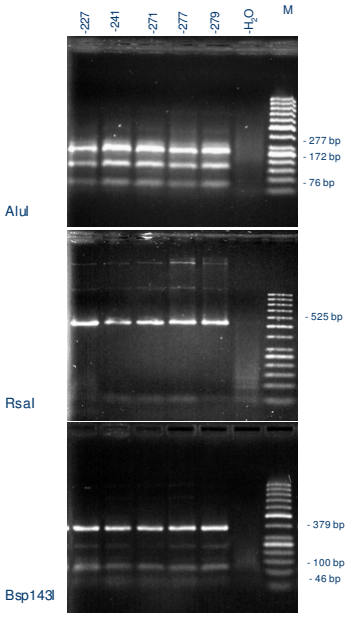

Fig. 1: PCR-RFLP pattern of *FUM1* fragments, amplified from *F. proliferatum* isolates from asparagus, with AluI, RsaI and Bsp143I. M = Marker 50 bp Ladder, Fermentas

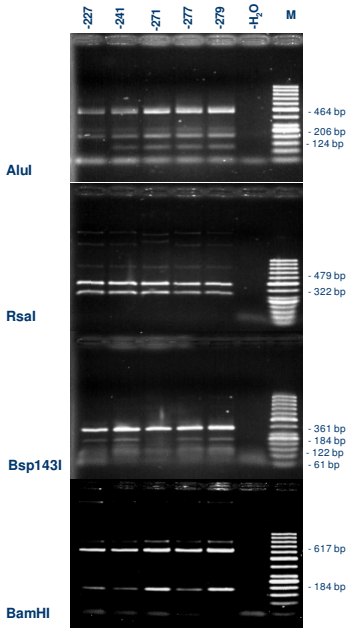

Fig. 2. PCR-RFLP pattern of *FUM8* fragments, amplified from *F. proliferatum* isolates from asparagus, with AluI, RsaI, Bsp143I and BamHI. M = Marker 50 bp Ladder, Fermentas

Sequence comparisons of *FUM1* and *FUM8* gene fragments

Tab. 1: Intraspecific and interspecific sequence variability of *FUM1* and *FUM8* gene fragments from various *Fusarium* species. Pairwise comparisons of nucleotide sequence identities are given in percent. Fragment sizes excluding primer sequences are given.

	FUM1					*FUM8*				
	n[a]	[bp][b]	Mean (S.D.)	Min	Max.	n[a]	[bp][b]	Mean (S.D.)	Min	Max.
Intraspecific sequence identity										
F. proliferatum	11	484	99.59 (± 0.25)	99.1	ID[c]	10	758	99.54 (± 0.21)	99.0	ID
F. verticillioides	10	488	99.70 (± 0.27)	99.1	ID	2	775	ID	ID	ID
Interspecific sequence identity of *F. proliferatum* isolates										
***Gibberella fujikuroi* species complex**										
F. fujikuroi	1	484	96.91 (± 0.22)	96.6	97.1	1	758	98.63 (± 0.18)	98.4	99.0
F. globosum	1	484	96.24 (± 0.17)	96.0	96.4	1	758	98.50 (± 0.18)	98.2	98.9
F. antophilum	1	484	92.94 (± 0.17)	92.7	93.1	1	759	90.53 (± 0.19)	90.3	90.9
F. nygamai	1	489	84.84 (± 0.17)	84.6	85.0	1	783	76.92 (± 0.21)	76.7	77.4
F. verticillioides	10	488	84.32 (± 0.25)	83.6	84.6	2	775	77.17 (± 0.17)	77.0	77.6
***Fusarium oxysporum* species complex**										
F. oxysporum	1	484	86.24 (± 0.17)	86.0	86.4	1	757	81.19 (± 0.20)	81.0	81.6

(a) sequences per *Fusarium* species included in the pairwise comparison,
(b) compared fragment length without primer sequences, (ID) identical sequences

Phylogenetic analysis based on *FUM1* and *FUM8* gene fragments

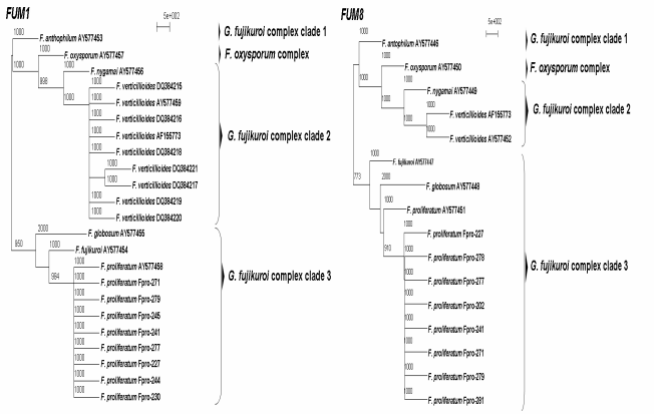

Fig. 3: Maximum-parsimony phylograms of *FUM1* and *FUM8* nucleotide fragments obtained from *F. proliferatum* strains in comparison with corresponding *Fusarium* spp. sequences from the database (indicated by accession numbers behind the *Fusarium* species). Species complexes according to O´Donnell et al. (1998) are indicated in the middle. Bootstrap values (n = 1000 repetitions) over 700 are indicated on branch nodes.

Conclusions

FUM genes were detectable in all investigated _F. proliferatum_ strains obtained from asparagus spears.

Verification of _FUM_ genes suggests that these fungal strains are able to produce fumonisins and responsible of contamination of asparagus spears with this mycotoxin.

Genome wide variability of _F. proliferatum_ occurring in asparagus obtained by RAPD- and DAF markers is mirrored by _FUM1_ and _FUM8_ gene diversity, but obviously not in the presence or absence of the fumonisin gene cluster within this species.

FUM gene based sequence comparisons confirmed phylogenetic relationships of _Fusarium_ spp..

Literature

O´Donnell K, Cigelnik E, Nirenberg, 1998. Molecular systematics and phylogeography of Gibberella fujikuroi species complex. Mycologia 90: 465–493