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# Interspecies virus transfer via protoplast fusions between Fusarium poae and black Aspergillus strains

A D. van Diepeningen
*Wageningen University and Research*

A J M Debets
*Wageningen University and Research*

S M. Slakhorst
*Wageningen University and Research*

*See next page for additional authors*



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## Abstract

Similarities between the genome organisation of dsRNA mycoviruses and dsRNA patterns in different fungal species suggest a relatedness between these viruses, which could be the result of co-evolved infections or of interspecies transfer. Such interspecies transfer between species is suggested by our observation of transfer and maintenance of mycoviral dsRNAs between *Fusarium* and *Aspergillus* via protoplast fusion.

## Authors

A D. van Diepeningen, A J M Debets, S M. Slakhorst, C Fekete, L Hornok, and R F. Hoekstra

## Interspecies virus transfer via protoplast fusions between *Fusarium poae* and black *Aspergillus* strains

Anne D. van Diepeningen[1], Alfons J.M. Debets[1], S. Marijke Slakhorst[1], Csaba Fekete[2], László Hornok[2] and Rolf F. Hoekstra[1] - [1]Laboratory of Genetics, Wageningen University and Research, Dreijenlaan 2, 6703 HA Wageningen, The Netherlands; [2]Agricultural Biotechnology Centre, 2101 Gödöllö, P.O. Box 411, Hungary

Similarities between the genome organisation of dsRNA mycoviruses and dsRNA patterns in different fungal species suggest a relatedness between these viruses, which could be the result of co-evolved infections or of interspecies transfer. Such interspecies transfer between species is suggested by our observation of transfer and maintenance of mycoviral dsRNAs between *Fusarium* and *Aspergillus* via protoplast fusion.

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Viruses are commonly found in animals, plants, bacteria and fungi. Most of the fungal viruses or 'mycoviruses' have genomes of double-stranded (ds) RNA and are transmitted only via intracellular routes (Buck 1998, In: Molecular variability of Fungal pathogens, Bridge *et al.*:53-72). Similarities between the genome organisation of dsRNA mycoviruses and dsRNA patterns in different fungal species, suggest a relatedness between these viruses, which could be the result of coevolved infections or of interspecies transfer.

In the black *Aspergilli*, ten percent of the world-wide population is infected with a great variety of dsRNA mycoviruses, with similar infections in distantly related strains. The wide-spread heterokaryon incompatibility between different *Aspergillus niger* strains in nature blocks the intraspecies transfer of these viruses via direct hyphal contact in laboratory experiments (Van Diepeningen *et al.* 1997 Curr. Genet 32:209-217). Even in protoplast fusion experiments, artificially bypassing of part of the heterokaryon incompatibility reactions, the black *Aspergillus* intraspecies transfer is limited (Van Diepeningen *et al.* 1998 Fungal Genetics and Biology 25: 171-180). From a population dynamic point of view these viruses with (small) deleterious effects on fitness are expected to decline in the *A. niger* population without (intraspecies) means of transfer, but the natural infection frequency seems stable world-wide. In *Fusarium poae*, another ascomycete, all natural isolates were found to contain dsRNA viruses (Fekete *et al.* 1995 FEMS Microbiol. Lett. 131:295-299). We have tested the qualitative possibility of virus transfer and maintenance of several mycoviruses from *F. poae* in black *Aspergillus* strains.

As means of transfer polyethylene glycol (PEG) mediated protoplast fusion was used. The *F. poae* strains, isolated from wheat kernels, contained different dsRNA infection patterns and were used as donors in these experiments. These strains could best be protoplasted from an overnight culture on Czapek-Dox medium enriched with yeast extract, casamino acids and neopepton (3 g $l^{-1}$ each). Harvested mycelium was protoplasted for 2-2½ h at 30°C with either 2 mg $ml^{-1}$ Novozym 234 or with Novozym in combination with an lytic enzyme mixture from *Oerscovia* in 0.7M NaCl, 0.2M $CaCl_2$ (±1800 mOsm). The black *Aspergillus* strains used were isolated from soil on 20% tannin medium and all contained an introduced chlorate resistance marker. They are naturally virusfree but earlier experiments showed that the used *A. niger* and *A. tubingensis* strains can maintain viruses originating from other black *Aspergillus* strains. The *Aspergilli* were protoplasted as described before (Van Diepeningen *et al.* 1998 Fungal Genetics and Biology 25: 171-180). Equal amounts of donor and acceptor protoplasts ($10^6$-$0.5x10^7$) were fused in a 30% w/v PEG6000, 50 mM $CaCl_2$ solution for 45min at 30°C. The fusion mixture was gradually diluted to 1:1 with STC and plated on osmotically stabilised medium. In two replicate experiments, acceptor strains were selected after two rounds of selection on their chlorate resistance and these were tested with gel electrophoresis on infection with viral dsRNA fragments (see Figure 1).

Control experiments showed that colonies obtained from single protoplasts of the *F. poae* donor strains never lost their specific dsRNA banding patterns. Likewise, in controls with PEG-treated *Aspergillus* strains no spontaneous infections appeared. Figure 1 shows an ethidium bromide-stained electrophoresis gel showing nucleic acids extracted from fusion products and parental strains. Both partial and total transfer of dsRNA banding patterns could be detected. The dsRNA nature could be confirmed by digestions with DNase and RNase under different salt regimes (Fekete *et al.* 1995 FEMS Microbiol. Lett. 131:295-299). The viral fragments are maintained stable in their new host species, also after multiple rounds of serial subculturing. Some of the fragments are able to reproduce on their own without the accompanying fragments present in their original host strain. The fact that in some cases not all dsRNA bands are recovered in the new host may reflect multiple infections and/or hitchhiking by defective particles in the original host.

The similarity between dsRNA virus patterns as found in all natural isolates of *F.poae* and in approximately 10% of the wild black *Aspergilli*, combined with the observation that dsRNAs from *F. poae* can be transferred to and maintained in black *Aspergillus* strains, supports the hypothesis of (rare) interspecies transfer of mycoviral dsRNAs in nature.

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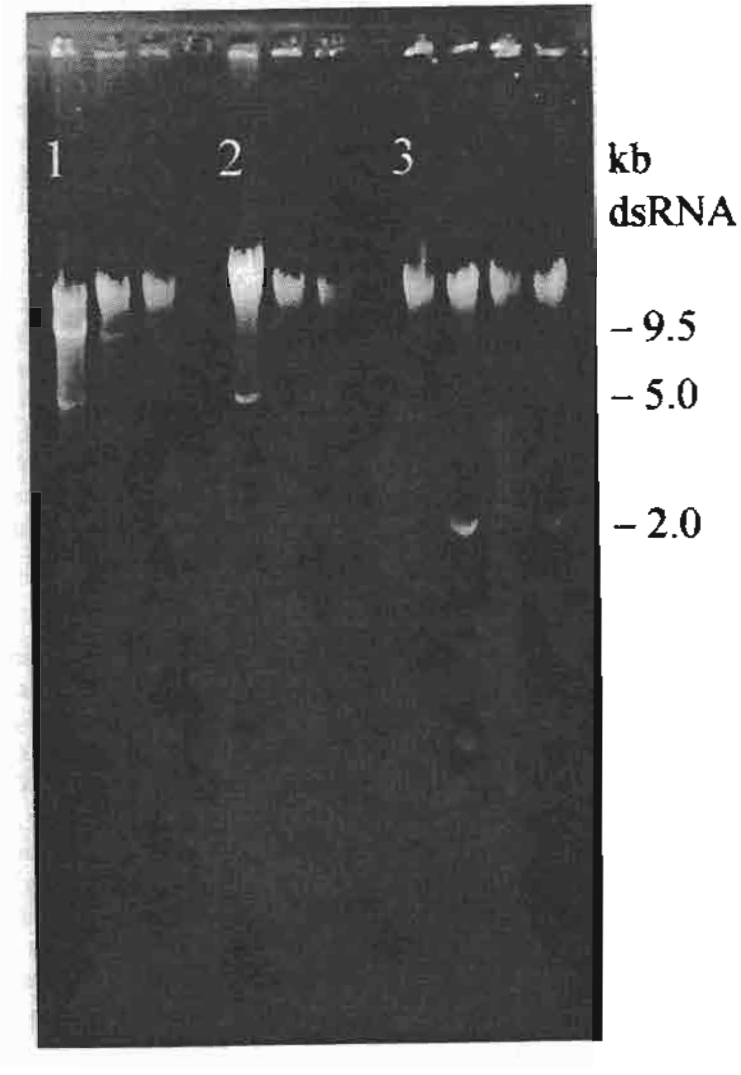


Figure 1. Gel electrophoresis patterns of phenol/chloroform extracted nucleic acid preparations of three sets of fusion products and parental strains. *A. niger* Ind 1.8.9 is the acceptor in all three combinations, a nucleic acid preparation of it is shown in the left lane of combination three, where only DNA is visible. *F. poae* TAPO-1, TAPO-30 and A-11 are the donors in combination 1, 2 and 3 respectively. The left lanes of combinations 1 and 2 show TAPO-1 and TAPO-30, with a bright DNA-dot and the smaller dsRNA fragments lower in the gel. The second lane of combination three similarly shows *F. poae* A-11. The *A. niger* acceptors of each combination, done in duplicate, are shown in the two right lanes of the three sets shown. Combination 1 shows transfer of the total parental dsRNA pattern, combination 3 shows partial transfer.