Genetics and Molecular Biology, 23, 1, 61-63 (2000)

Short Communication

Effects of double-stranded RNA on virulence of *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) against the silverleaf whitefly, *Bemisia tabaci* strain B (Homoptera: Aleyrodidae)

Andréia Cristiane Souza Azevedo[1], Daniel Ricardo Sosa-Gómez[2], Marcos Rodrigues Faria[3] and Maria Helena Pelegrinelli Fungaro[1]

Abstract

Bands of double-stranded RNA (dsRNA) were detected in three out of twelve isolates of *Paecilomyces fumosoroseus*. Identity of these bands was confirmed by RNAse, DNAse and S1 nuclease treatments. The cure of dsRNA for one isolate (P92) was successfully carried out for a single conidium subculture. Isogenic strains, with or without dsRNA, were submitted to virulence tests against the whitefly *Bemisia tabaci* strain B. In contrast to findings for some phytopathogenic fungi, these dsRNA fragments did not cause hypovirulence in *P. fumosoroseus.*

INTRODUCTION

Whiteflies are found worldwide and cause considerable damage to a variety of high-value crops (Gerling and Mayer, 1996). The entomopathogenic fungus *Paecilomyces fumosoroseus* (Wise) Brown & Smith is an important natural control agent of homopteran insects, and has become a commercial product in Mexico and the United States. This fungus is associated with *Bemisia tabaci* in different global regions (Lacey *et al.*, 1996), including Brazil, where strain B has been observed on vegetables and soybean (Sosa-Gomez, D.R. and Faria, M.R. de, unpublished results). Also in Brazil, recent whitefly outbreaks have been recorded (França *et al*, 1996) and, since chemical control has proven inefficient, use of entomopathogenic fungi as mycoinsecticides has been considered. Double-stranded RNAs (dsRNA) are commonly found in fungi, and some are associated with virus-like particles (Michelmore and Hulbert, 1987). Effects of dsRNA on fungal pathogenicity have been studied mainly in plant pathogenic species, and their hypovirulence to hosts is well documented (Castanho *et al*., 1978; Anagnostakis and Day, 1979; Pusey and Wilson, 1982; Boland, 1992). The only study done so far on entomopathogenic fungi effects employed *Metarhizium flavoviride* (= *M. anisopliae* var. *acridum*) and the grasshopper *Rhammatocerus schistocercoides* (Martins *et al*., 1999). In the present communication we report the occurrence of dsRNA elements in *P. fumosoroseus* isolates, and whether the dsRNA affected their virulence with respect to the silverleaf whitefly, *B. tabaci* strain B.

MATERIAL AND METHODS

The geographic origin, insect hosts and isolation data of twelve *P. fumosoroseus* strains used in this study are presented in Table I. Cultures were maintained on complete medium agar plates as described by Pontecorvo *et al*. (1953). Incubation was performed at 28°C. Mycelia were ground with liquid N_2 using a mortar and pestle; genomic DNA was extracted according to Bogo *et al*. (1996). For RNAse A digestion, the enzyme (Gibco Life Technologies) was added to 3 µg total nucleic acids for a final concentration of 8 µg/ml in 100 mM Tris-HCl, 10 mM EDTA, pH 7.5 and incubated at 37°C for 30 min. For S1 nuclease digestion 35 U of enzyme (Gibco Life Technologies) was added per µg of total nucleic acids; the reaction was performed according to supplier's instructions. For DNAse digestion, the samples were treated with enzyme (Pharmacia) at 20 µg/ml in 30 mM $MgCl_2$ at 37°C for 20 min. To test for dsRNA transmission through conidia, ten single-conidial isolates were obtained from P92. A conidial suspension was spread over a water-agar plate and incubated overnight. Agar blocks bearing single conidia with short germ tubes were aseptically cut and transferred to CM tubes.

For virulence test, melon leaves, hybrid AF682, were removed from plants and put in glass vials (7 x 3.5 cm), with petiole immersed in tap water. Leaves were kept individually inside plastic cups (14 x 9 cm) with a screened top, in an incubator regulated to 27 ± 1°C, 70 ± 5% RH and at a 12-h photophase. Each leaf was infested by 18 adult couples of *B. tabaci* biotype B (Hemiptera: Aleyrodidae) for 26 h. Six days after adult removal, 1- and 2-instar nymphs

[1]*Centro de Ciências Biológicas, Universidade Estadual de Londrina,Caixa Postal 6001, 86051-970 Londrina, PR, Brasil. Send correspondence to M.H.P.F. Fax: +55-43-371-4207. E-mail: hfungaro@sercomtel.com.br*
[2]*CNPSo/EMBRAPA, Londrina, PR, Brasil.*
[3]*Cenargen/Embrapa, Brasília, DF, Brasil.*

Table I - Origin of *P. fumosoroseus* isolates.

Isolates		Host	Location
CNPSo-Pf77 (ARSEF 5154)	*P. fumosoroseus*	*Bemisia argentifolii*	Brazil
CNPSo-Pf78 (ARSEF 5155)	*P. fumosoroseus*	*B. argentifolii*	Brazil
CNPSo-Pf80 (ARSEF 5157)	*P. fumosoroseus*	*B. argentifolii*	Brazil
CNPSo-Pf81 (ARSEF 5158)	*P. fumosoroseus*	*B. argentifolii*	Brazil
CNPSo-Pf85	*P. fumosoroseus*	*B. argentifolii*	Brazil
CNPSo-Pf92 (ARSEF 3638)	*P. fumosoroseus*	Soil	Brazil
CNPSo-Pf93 (ARSEF 2956)	*P. fumosoroseus*	*Spaethiella* sp.	Brazil
CNPSo-Pf125 (ARSEF 2658)	*P. fumosoroseus*	*Trialeurodes vaporariorum*	United States
CNPSo-Pf126 (ARSEF 3083)	*P. fumosoroseus*	*B. tabaci*	United States
CNPSo-Pf121 (ARSEF 3303)	*P. fumosoroseus*	*Bemisia* sp.	Mexico
CNPSo-Pf124 (ARSEF 3660)	*P. fumosoroseus*	*B. tabaci*	United States
CNPSo-Pf127 (ARSEF 3699)	*P. fumosoroseus*	*B. tabaci*	India

were selected through observation under a dissecting microscope and ink-marked with a spot near their position. For spraying conidial suspension, a Potter Spray Tower (Burkard Manufacturing, Hertfordshire, England) operating at 12.5 psi and applying 2 ml conidial suspension (1.0 x 10^7/ml) per leaf was used. In the control treatment, a 0.1% Tween 80 solution was applied. Incubation conditions were the same as mentioned above. Assessments were performed 12 days post-spray, when numbers of live and dead nymphs and adults were determined.

RESULTS AND DISCUSSION

The CNPso-Pf80, CNPso-Pf81 and CNPso-Pf92 *P. fumosoroseus* isolates showed extra-bands after electrophoretic separation of undigested nucleic acids (Figure 1). These bands proved to be dsRNA, as evidenced by sensitivity to RNAse in buffer with low salt concentration, and insensitivity to DNAse and S1 nuclease, which had no effect on these bands but eliminated the single-stranded RNA (ssRNA). Based on number and molecular size, two distinct banding patterns were observed. Isolates CNPso-Pf80 and CNPso-Pf81 were identical in pattern bands, showing three bands of 1.7, 1.3 and 0.5 kb. The isolate CNPso-Pf92 showed three bands of 4.5, 3.5 and 3.2 kb. This polymorphism type has also been observed in other fungi species, including entomopathogenic fungi, e.g., *Metarhizium anisopliae* (Bogo *et al.*, 1996) and *Metarhizium flavoviride* (= *M. anisopliae* var. *acridium*) (Martins *et al.*, 1999). Band profile similarity of P80 and P81 isolates is probably because they are from the same geographic region and, consequently, infected by the same virus. Isolates CNPso-Pf77, CNPso-Pf78, CNPso-Pf85, CNPso-Pf93, ARSEF 2658, ARSEF 3303, ARSEF 3083, ARSEF 3660 and ARSEF 3589 did not contain any detectable extra-bands.

Inability to transmit dsRNA by simple infection or transduction is the major obstacle to directly analyzing the role of these elements in regulating virulence and related traits. So, the relationship of these genetic elements to virulence variability has mostly been inferred from correlative evidence, supported in some cases by curing and transmission experiments (Nuss and Koltin, 1990). In attempts to eliminate dsRNA from various fungal species, several treatments have been used, e.g., chlorate selection of *nit* mutants, incubation at high temperature, hot water exposure, antibiotics, acridine dye, single conidium subculture, hyphal tip transfer, UV-irradiation, and cycloheximide treatment (Pusey and Wilson, 1982; Fulbright, 1984; Hunst *et al.*, 1986; Kousik *et al.*, 1994; Elias and Cotty, 1996). Although these methods would fail in some

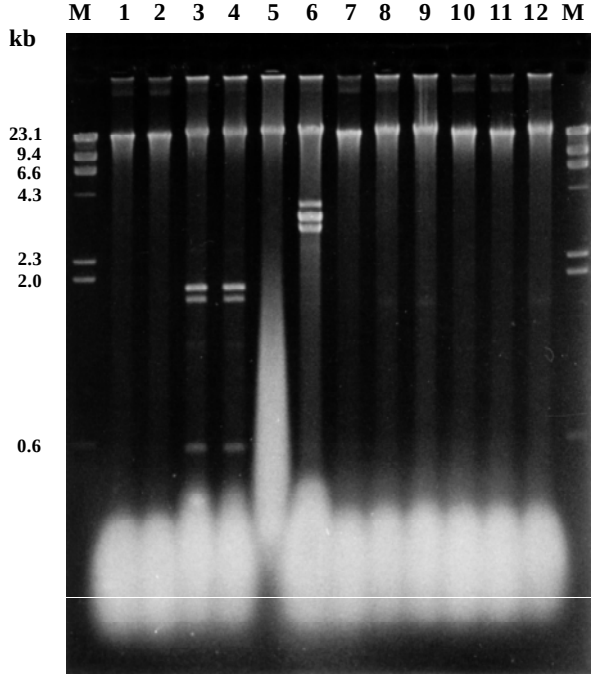

Figure 1 - Agarose gel electrophoresis of total nucleic acid extracted from *Paecilomyces fumosoroseus*. Lane M, Molecular markers (*Hin*dIII cut λ DNA); Lane 1, CNPso-Pf77; Lane 2, CNPso-Pf78; Lane 3, CNPso-Pf80; Lane 4, CNPso-Pf81; Lane 5, CNPso-Pf85; Lane 6, CNPso-Pf92; Lane 7, CNPso-Pf93; Lane 8, ARSEF 2658; Lane 9, ARSEF 3083; Lane 10, ARSEF 3303; Lane 11, ARSEF 3660; Lane 12, ARSEF 3589.

Table II - Virulence tests of P92 strain with dsRNA and P92 strain without dsRNA. Data represent percentage of mortality 12 days post-spray conidial suspension.

Strain	P92 strain with dsRNA	P92 strain without dsRNA
RI	73.9	71.7
RII	92.1	77.3
RIII	75.9	87.8
M ± SD	80.6[a] ± 10.0	78.9[a] ± 8.1

[a] Means followed by the same letter are not different according to Tukey's test ($P < 0.05$); RI, RII and RIII are replicates.

entomopathogenic species (Martins *et al*., 1999), we did obtain cured strains of the P92 isolate by single conidium subculture. Four out of ten single-conidial P92 isolates showed no dsRNA banding profiles, indicating that dsRNA components were not perfectly transferred during asexual subculture. The absence of dsRNA in the four cured strains after five successive subcultures assured cure.

Bioassays using CNPSo-Pf92 with and without dsRNA fragments against the whitefly were performed, in order to investigate if the dsRNA in *P. fumosoroseus* induces hypovirulence. Results of the bioassays are presented in Table II. Although this isolate was obtained from soil, its virulence is as high as that observed by Vidal *et al.* (1997) for isolates obtained from different whitefly species. Comparison of mean mortality by the Tukey test showed no statistical differences among isolates, indicating that these dsRNA fragments did not cause hypovirulence in *P. fumosoroseus*, in contrast to those found in some phytopathogenic fungi (Castanho *et al*., 1978; Ghabrial, 1980; Brasier, 1983; Naiki and Cook, 1983; Fulbright, 1984), but in accordance with findings for the entomopathogenic fungus *M. flavoviride* (Martins *et al*., 1999). Absence of hypovirulence effects of dsRNA for *P. fumosoroseus* has been shown for the first time in the present work.

ACKNOWLEDGMENTS

This investigation was supported by PRONEX and CNPq. A.C.S.A. was the recipient of a CNPq fellowship.

RESUMO

Bandas de dsRNA foram detectadas em três dos doze isolados de *Paecilomyces fumosoroseus*. A identidade destas bandas foi provada através de tratamentos com RNAse, DNAse e S1 nuclease. A cura do dsRNA para um dos isolados (P92) foi obtida através do isolamento de colônias monospóricas. Linhagens isogênicas, com e sem dsRNA, foram submetidas ao teste de virulência contra a mosca branca *Bemisia tabaci* biotipo B. Ao contrário do que ocorre para vários fungos fitopatogênicos, os fragmentos de dsRNA não causaram hipovirulência em *P. fumosoroseus*.

REFERENCES

Anagnostakis, S.L. and **Day, P.R.** (1979). Hypovirulence conversion in *Endothia parasitica*. *Phytopathol. 69*: 1226-1229.

Bogo, M.R., Queiroz, M.V., Gimènez, D.M., Azevedo, J.L. and **Schrank, A.** (1996). Double-stranded RNA and isometric virus-like particles in the entomopathogenic fungus *Metarhizium anisopliae*. *Micol. Res. 100*: 1468-1472.

Boland, G.J. (1992). Hypovirulence and double-stranded RNA in *Sclerotinia sclerotiorum*. *Can. J. Plant Pathol*. *14*: 10-17.

Brasier, C.M. (1983). A cytoplasmatically transmitted disease of *Ceratocystis ulmi*. *Nature 305*: 220-222.

Castanho, B., Butler, E.E. and **Shepherd, R.J.** (1978). The association of double stranded RNA with Rhizoctonia decline. *Phytopathol. 68*: 1515-1519.

Elias, K.S. and **Cotty, P.J.** (1996). Incidence and stability of infection by double-stranded RNA genetic elements in *Aspergillus* section *flavi* and effects on aflatoxigenicity. *Can. J. Bot. 74*: 716-725.

França, F.H., Villas Bôas, G. and **Branco, M.C.** (1996). Ocorrência de *Bemisia argentifolii* Bellows & Perring (Homoptera: Aleyrodidae) no Distrito Federal. *An. Soc. Entomol. Bras. 25*: 369-372.

Fulbright, D.W. (1984). Effects of eliminating dsRNA in hypovirulent *Endothia parasitica*. *Phytopathol. 74*: 722-724.

Gerling, D. and **Mayer, R.** (1996). *Bemisia 1995: Taxonomy, Biology, Damage, and Management*. Intercept, Andover.

Ghabrial, S.A. (1980). Effects of fungal viruses on their hosts. *Ann. Rev. Phytopathol. 18*: 441-461.

Humber, R.A. (1992). *Collection of Entomopathogenic Cultures.* ARSEF Catalog of Strains. USDA-ARS Publication 110, Ithaca, NY. Update in http:www.ppru.cornell.edu/mycology/insect mycology.html

Hunst, P.L., Latterell, F.M. and **Rossi, A.E.** (1986). Variation in double-stranded RNA from isolates of *Pyricularia oryzae*. *Phytopathol*. *76*: 674-678.

Kousik, C.S., Snow, J.P. and **Valverde, R.A.** (1994). Comparison of double-stranded RNA components and virulence among isolates of *Rhizoctonia solani* AG-1 IA and AG-1 IB. *Phytopathol*. *84*: 44-49.

Lacey, L.A., Fransen, J.J. and **Carruthers, R.** (1996). Global distribution of naturally occurring fungi of *Bemisia*, their biologies and use as biological control agents. In: *Bemisia 1995: Taxonomy, Biology, Damage, and Management* (Gerling, D. and Mayer, R., eds.). Intercept, Andover.

Martins, M.K., Furlaneto, M.C., Sosa-Gomez, D.R., Faria, M.R. and **Fungaro, M.H.P.** (1999). Double-strand RNA in the entomopathogenic fungus *Metarhizium flavoviride*. *Curr. Genet. 36*: 94-97.

Michelmore, R.W. and **Hulbert, S.H.** (1987). Molecular markers for genetic analysis of phytopathogenic fungi. *Ann. Rev. Phytopathol*. *25*: 383-404.

Naiki, T. and **Cook, R.J.** (1983). Factors in loss of pathogenicity in *Gaeumannoomyces graminis* var. *tritici*. *Phytopathol. 73*: 1653-1656.

Nuss, D.L. and **Koltin, Y.** (1990). Significance of dsRNA genetic elements in plant pathogenic fungi. *Ann. Rev. Phytopathol. 28*: 37-58.

Pontecorvo, G., Roper, J.A., Hemmons, L.M., Macdonald, K.D. and **Bufton, A.W.J.** (1953). The genetics of *Aspergillus nidulans*. *Adv. Genet. 5*: 141-148.

Pusey, P.L. and **Wilson, C.** (1982). Detection of double stranded RNA viruses in *Ceratocystis ulmi*. *Phytopathol. 73*: 470-474.

Vidal, C., Lacey, L.A. and **Fargues, J.** (1997). Pathogenicity of *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) against *Bemisia argentifolii* (Homoptera: Aleyrodidae) with the description of a bioassay method. *J. Econ. Entom. 90*: 765-772.

(Received August 26, 1999)