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MICROBIOLOGICAL IMAGE

Mycoparasitic interaction between *Trichoderma afroharzianum* strain Th2RI99 and *Rhizoctonia solani*

Interacción micoparasítica entre *Trichoderma afroharzianum* cepa Th2RI99 y *Rhizoctonia solani*

Eliana Melignani[a,*], Mara E. Martin[b], Vanesa Y. Mema[c], Catalina B. Taibo[c], Julia V. Sabio y García[c], Viviana A. Barrera[c]

[a] *Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Av. Int. Güiraldes 2160, C.A.B.A., C.P. C1428EGA, Argentina*
[b] *Instituto de Agrobiotecnología y Biología Molecular (IABIMO), INTA-CONICET, Las Cabañas y De los Reseros s/n, Hurlingham, C.P. 1686, Castelar, Buenos Aires, Argentina*
[c] *Instituto de Microbiología y Zoología Agrícola (IMYZA), INTA, Las Cabañas y De los Reseros s/n, Hurlingham, C.C. 25 (1712) Castelar, Buenos Aires, Argentina*

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The behavior of *Trichoderma afroharzianum* strain Th2RI99 confronted to *Rhizoctonia solani* AG4-HG II in dual culture was observed under light (LM) and scanning electron microscope (SEM). There is evidence of mycoparasitism in the *Trichoderma harzianum* complex, but no report is available regarding *T. afroharzianum*, since it has been just recently described as a new species, previously identified with phylogenetic analysis with 3 genes[1].

Two mycelial plugs (5 mm diam.), from Th2RI99 and the pathogens *Bipolaris sorokiniana*, *Fusarium graminearum*, *Sclerotium minor* and *R. solani* cultures (grown in potato dextrose agar for 72 h at 25 °C in darkness), were placed 3 cm apart from each other on 1.2% water agar plates in independent assays. Three replicates were incubated and observed at 24, 46, 47, 48 and 72 h. Rectangular sections from the confrontation area were mounted on slides in 3% KOH, stained with 10% lactophenol blue or 5 mM white calcofluor (Fluka, Sigma-Aldrich) for 5 min and washed with distilled water[2,4,5]. The observation of samples under LM (Olympus BX51) coupled to a digital camera (Cool Snap-Pro) was carried out using bright field, differential contrast interference and epifluorescence (360–370 nm). Digital images were taken using Image-Pro Solution software (Media Cybernetics).

Samples for SEM observation were prepared following[3]. Only samples against *R. solani* showed signs of mycoparasitism. At 46 and 47 h, multiple coilings from Th2RI99 hyphae around *R. solani* mycelium were evident (Figs. 1A and B). At 48 h, the cell wall of *R. solani* presented the formation of a pore, caused by the penetration tube of Th2RI99 (Fig. 2A).

* Corresponding author.
E-mail address: elianameli@bg.fcen.uba.ar (E. Melignani).

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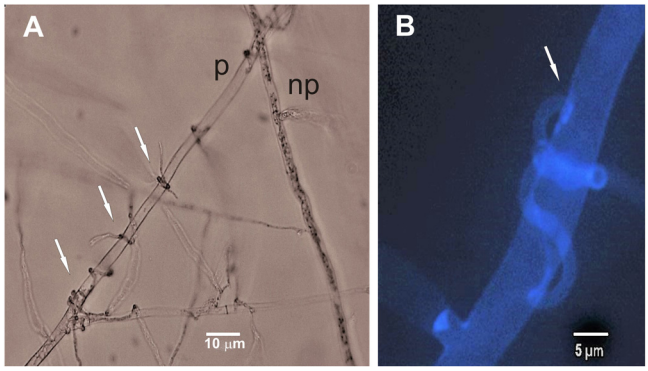

Figure 1 Light microscope observation of the interaction between hyphae of *Trichoderma afroharzianum* Th2RI99 (T) and *Rhizoctonia solani* AG4-HG II (R) in dual culture (WA 1.2%) after 47 h. (A) Arrows: coilings of Th2RI99 on *R. solani*; p: plasmolized hyphae; np: not plasmolized hyphae. (B) Calcofluor dye. Arrow: detail of coiling of Th2RI99 on *R. solani*.

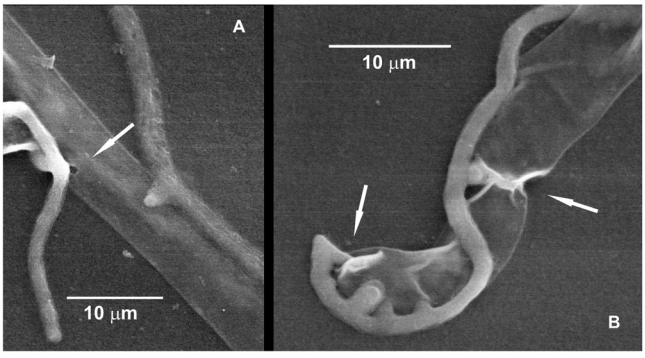

Figure 2 Scanning electron microscope observation of the interaction between hyphae of *Trichoderma afroharzianum* Th2RI99 (T) and *Rhizoctonia solani* AG4-HG II (R) in dual culture (WA 1.2%) after 48 h. (A) Arrow: formation of penetration pore in hypha of *R. solani*. (B) Arrows: Th2RI99 penetration tubes around the apical hypha of *R. solani*.

In one of the mycoparasitism events, the apical zone of a *R. solani* hypha had up to 5 penetration tubes from the antagonist (Fig. 2B).

This is the first report of mycoparasitic interaction between *T. afroharzianum* and *R. solani*.

Conflicts of interest

This work has been carried out as a Technological Association Agreement with Rizobacter S.A.

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