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Isolation, culture and host colonization of *Entrophospora schenckii* (Glomales), an arbuscular mycorrhizal fungus

by

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With 2 figures

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Abstract: *Entrophospora schenckii* was found for the first time in Argentina. Single-spore cultures were established to observe root colonization and developmental stages of spore production. The fungus produced vesicular-arbuscular mycorrhizae.

Key words: arbuscular mycorrhiza, Argentina, colonization.

Introduction

Entrophospora schenckii Sieverding & S. Toro (Sieverding & Toro 1987) is recognizable by its hyaline and thin spore walls and small spore diameter. Spores are usually devoid of their collapsed sporiferous saccule and the remnants of its subtending hypha, making the generic determination difficult. The spore wall structure was examined following the method of Walker (1983), as modified in Walker & Vestberg (1998). The spore wall consists of three components (1-3) in two groups (A and B): group A with two components (1 and 2) and group B with only one (3).

E. schenckii has so far been reported only in two sites from Colombia (Sieverding & Toro 1987), but it has recently been isolated for the first time from an uncultivated soil in the province of Salta, in the Northwest of Argentina. Single-spore cultures were established in order to observe the root colonization process as well as spore production.

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Materials and methods

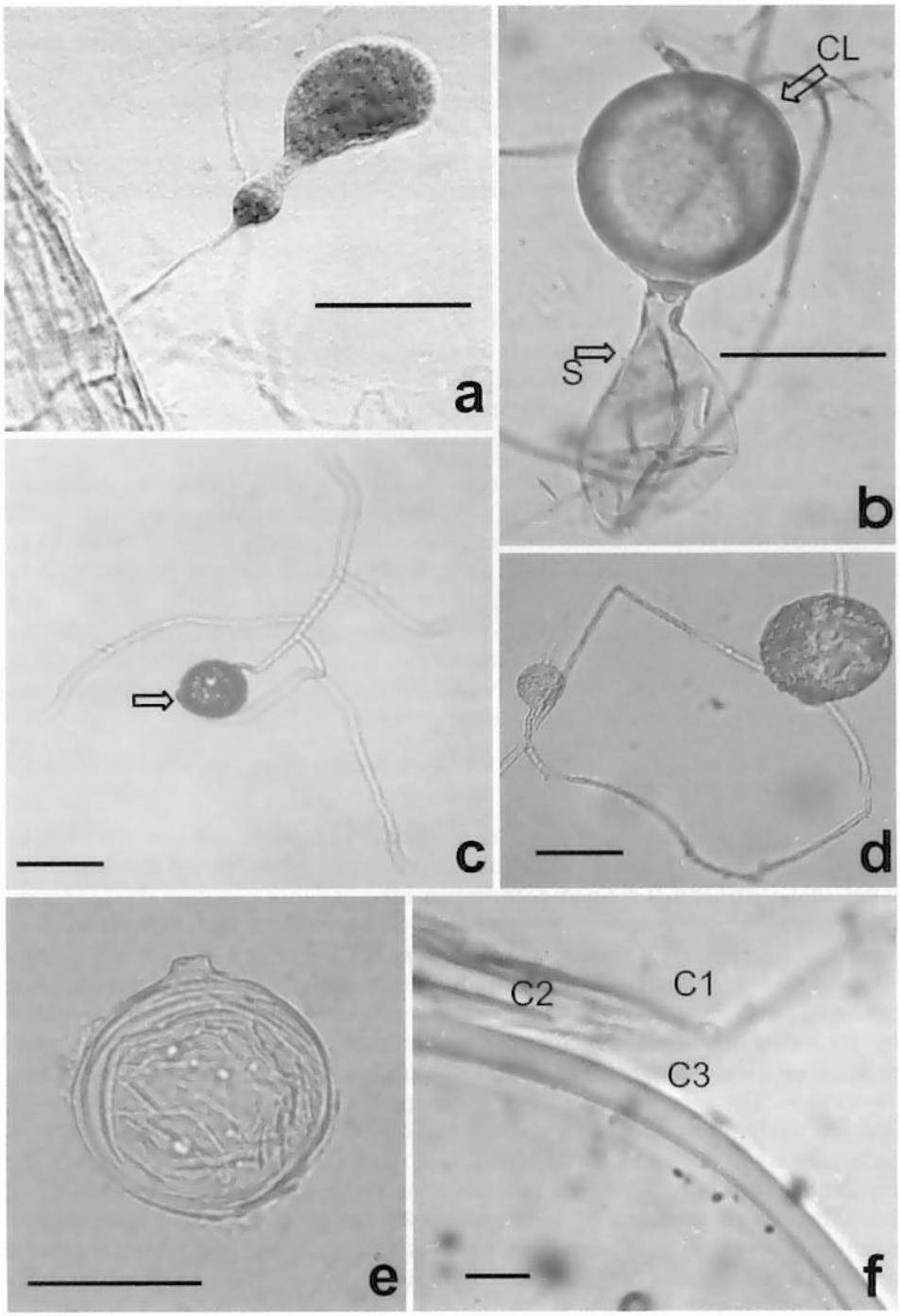
Rhizosphere soil was collected from a grass sample on a sandy loam soil, pH 7, at 3000 m alt. in the Puna region of the Salta province (Argentina), kept in a refrigerator until processing. Spores were extracted by wet sieving and decanting (Gerdemann & Nicolson 1963). Single-spore and multi-spore cultures were set up (Fracchia et al. 2001), using tomato (*Lycopersicon esculentum*), white clover (*Trifolium repens*) and *Vigna* sp. as hosts. Plants were cultured in 5-cm diam Petri dishes with autoclaved (120°C, 20 min) vermiculite-perlite mixture (1/1, V/V). Seeds were surface-sterilized with 10% hypochlorite solution in water for 2 min, sown on the vermiculite:perlite, incubated in a greenhouse at 20-25°C and intermittently watered with deionized water and Hewitt solution (Hewitt 1952) until fungal sporulation occurred. Surface-sterilized spores were transferred to a 5-cm diam. Petri dish containing 0.4% gellann gum (Gel-Gro™ ICN catalog No. 150180) in 10 mM 2-(N-morpholin) ethane sulphonic acid (MES) buffer (pH 7), and incubated at 25°C. Spore germination and hyphal development were periodically observed under a dissecting microscope. Vermiculite-perlite dishes with 2-3 week-old seedlings were transferred over the Gel-Gro™ medium with the germinated spore. Hyphal development and root colonization were observed daily through the bottom of the Petri dish under a dissecting microscope. Root fragments were excised, stained with Trypan blue (Phillips & Hayman 1970), and assayed for succinate dehydrogenase activity (SDH) (MacDonald & Lewis 1978).

Newly-formed spores were selected with tweezers from the culture and mounted in polyvinyl alcohol-lactic acid-glycerol (PVLG) (Omar et al. 1979, Koske & Tesier 1983) or Melzer's reagent. Spore wall characteristics and terminology are those suggested by Walker and Vestberg (1998). Spore colour was determined according to Munsell (1954). Voucher specimens were preserved in PVLG and deposited in the herbarium of the Department of Biological Sciences, School of Exact and Natural Sciences, University of Buenos Aires (BAFC N° 50914)

Results and discussion

Spore germination in Petri dishes was observed 15 days after the culture was set up (Fig. 1 c-d). Nearly all of the plated spores germinated, producing one germination tube. Sometimes the germ tube emerged beneath the spore, and then branched immediately giving the appearance of multiple germ tubes. Three weeks after transferring the seedlings on the gellann gum medium with the germinated spore, extraradical mycelium was observed growing abundantly in the substrate. Trypan blue staining showed mycorrhiza development in *Vicia* sp. but not in tomato or clover. However, SDH activity could be observed in all the three plant species. Pieces of roots stained with Trypan blue and SDH showed the presence of extraradical, sinuous, 1-5 µm wide hyphae, and typical arbuscular mycorrhizal structures such as hyphal invasion points with appressoria, intraradical vesicles, arbuscules and coiled hyphae (Fig. 2 a-d). The two last structures stained only lightly in trypan blue, but were easily visible with the SDH reaction. These observations show that *E. schenckii* forms functional vesicular-arbuscular mycorrhizas (Becard & Piché 1989).

Fig. 1. Light photomicrographs of *Entrophospora schenckii*. a. Spore and sporiferous saccule stained dark with SDH during early stage of spore formation, b. mature spore with sporiferous saccule (arrow). CL: chlamydospore-like structure. S: sporiferous saccule stained with trypan blue; c. and d. germinated spores showing germ tubes (arrow shows scar), e. mature spore mounted in Melzer's reagent, f. wall components (numbering explained in the text). Bars: a. 100 µm; b, c, e. 50 µm; d. 25 µm, f. 5 µm.


a
CL
S
b
c
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e
C2
C1
C3
f

Extraradical terminal and intercalary chlamydospore-like structures staining pink in Melzer's reagent were observed (Fig. 1d). These globose to subglobose, thin-walled structures, 19-29 µm diam., correspond with those mentioned by Sieverding & Toro (1987) in the protologue, although they were described as appearing terminally and bigger in size. After 5 months, spores were formed terminally in the mycelium (Fig. 1 a and b). Sporiferous saccule development and migration of the sporiferous saccule content into the spore were completed in 7 and 2 days, respectively. We were able to cultivate three generations of *E. schenckii* by re-inoculating newly formed spores in vitro, showing conclusively that the fungus completes its life cycle in the plant.

The specimen of *E. schenckii* was collected in Salta in soil with neutral pH similar to that in Colombia. This contrasts with other species like e.g. *E. colombiana* Spain & N.C. Schenck, which appear to occur only in acidic soils. Our strain of *E. schenckii* corresponds well the original description with regard to the spore morphology and wall structure.

Spores hyaline at maturity, globose to subglobose (49-)50-60(-72) µm diam. sometimes showing one scar (Fig. 1e), formed singly in the soil or in cortical cells of the root. Sporiferous saccule hyaline, one-layered, globose to subglobose, 50-60 µm diam., with a 8-10 µm wide neck. Spore walls of three components (1, 2 and 3), 0.8-2.1 µm thick. The outermost hyaline component, continuous with, and thus part of, the wall of the saccule neck (0.5-1.0 µm), usually sloughed when saccules are detached. Components 1 and 2 are similar in width (0.4-1 µm) and 3 is thicker than the other two (1.5-2.0 µm, Fig. 1 f). Numerous folds were observed in components 1 and 2. No component reacted in Melzer's reagent.

At germination, mounted spores did not show a germ-shield in water or Melzer's reagent (Spain 1992).

In the original publication, Sieverding & Toro (1987) could not confirm whether this species formed vesicular-arbuscular mycorrhizae since, except for spores, no other fungal structures stained with trypan blue or other common dyes. Morton (1990) considered this species together with *G. leptotichum* N.C. Schenck & G.S. Smith, *G. occultum* C. Walker, *G. tortuosum* N.C. Schenck & G.S. Smith, and *Acaulospora myriocarpa* Spain, N.C. Schenck & G.S. Smith, as hypothetical ancestors, with incomplete development of a mutualistic association characterized by: (i) compatible hyphal development in plant roots, (ii) poorly developed or non-existent arbuscules, and (iii) abundant sporulation to offset a tenuous symbiotic association. The arbuscular status of these species was assumed by analogy with species having similarities in spore structure, and because of the absence of pathogenicity in pot-culture with plants. Our work shows that, on the contrary, the mycorrhizas are fully developed and functional, with well-formed arbuscules (Fig. 2d). There is no evidence of incompatibily in the roots (i.e., no hypersensitive reaction). Sporulation is abundant, but this feature is not uncommon amongst arbuscular mycorrhizal fungi and may have no easily determined relationship with colonization density or efficacy.

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