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Characterization of a heavy metal-tolerant endomycorrhizal fungus from the surroundings of a zinc refinery

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Abstract. An arbuscular mycorrhizal fungus was isolated from the rhizosphere of *Agrostis capillaris* growing in the contaminated surroundings of a zinc refinery in The Netherlands. After examination of the infection pattern and the spores, it was characterized as *Scutellospora dipurpurescens,* which was first isolated from a reclaimed coal mine area in West Virginia.

Key words: *Agrostis* – Arbuscular mycorrhiza – Heavy metal contamination – *Scutellospora dipurpurescens* – Zinc

Introduction

High concentrations of heavy metals in soils are strong selective agents which result in highly metal-resistant ecosystems with low species diversity at each ecosystem level (Ernst 1990). Mycorrhizal fungi of various groups have evolved resistance to heavy metals, depending on the degree of soil pollution and the occurrence of host plants, i.e. ectomycorrhizae (Colpaert and Van Assche 1987), ericoid mycorrhizae (Bradley et al. 1981) and vesicular-arbuscular (VA) mycorrhizae.

With the latter type of mycorrhizae, most interest in ecological research has been attached to the host species, which is usually well characterized, or to the mycorrhizal association, although the various fungi within this group do not all function in the same way and may differ significantly in their effectiveness (e.g. Abbott and Robson 1978; Allen and Boosalis 1983; Hayman and Tavares 1985). Less attention has been paid to the fungal species itself.

To be informative about the ecological implications of (V)A-mycorrhizal associations, records on and experiments with this group of fungi should describe the specific species found or worked with. In this present study, dealing with the adaptation and functioning of VA-mycorrhizal associations in heavy metal-enriched or contaminated areas, spores of VA-mycorrhizal fungi were isolated and the species was determined. Because species of all (V)A-mycorrhizal genera have been isolated from heavy metal-contaminated soils (as summarized in Table 1), no particular individual species was expected at the contaminated site examined.

Materials and methods

Soil was collected from the rhizosphere of a field population of *Agrostis capillaris* L. (=*A. tenuis* Sibth.) at a zinc refinery in Budel (The Netherlands; 5°36′E, 51°14′N). The concentration and availability of the heavy metals involved was reported previously (Griffioen et al. 1994). The soil was stirred in tap water, allowed to settle for 20 s, and then washed through a 500-μm sieve. The water fraction was stirred and allowed to settle for a further 20 s before being decanted onto a 45-μm sieve. The material on the sieve was retained.

Spores of (V)A-mycorrhizal fungi were isolated from soil using a centrifugation-sugar flotation method. After 4 min at maximum speed (3500 rpm) in a bench-top centrifuge, the supernatant was poured off carefully. The pellet was resuspended in a 2 M sucrose solution and centrifuged for 30 s at 3500 rpm. After decanting the sucrose supernatant onto a 45-μm sieve, the sucrose in the residue was washed out with water and back-washed onto a 0.45-μm membrane filter (Schleicher and Schüll). Spores were examined and isolated using a stereomicroscope. Spores were mounted in polyvinyl alcohol-lacto-glycerol (PVLG) (Koske and Tessier 1983) and PVLG/Melzer's solution, and at least some of them were flattened. Characterization was carried out using Schenck and Pérez (1990) spore wall terminology and characteristics suggested by Walker (1983, 1986) and Morton (1986).

Root infection was recorded using a line-intersect method (Giovannetti and Mosse 1980) after staining with chlorazol black E (Brundrett et al. 1984) according to Koske and Gemma (1989).

The infection of the Budel population was compared with that of a population from an uncontaminated dune area (Schiermonnikoog, The Netherlands; 6°12′E, 53°29′N) and from a copper-enriched area (Imsbach, Germany; 7°54′E, 49°35′N).

Results

The morphology of the VA-mycorrhizal infection at the Budel location was examined closely. Besides arbuscules (Fig. 1), the infection pattern included auxilia-

Table 1. Fungal species having arbuscular mycorrhizal associations with higher plants on heavy metal-enriched or -contaminated areas. Scientific names are according to Walker and Trappe (1993). Heavy metals polluting the soil to a lesser extent are given in parentheses

Fungal species	Soil enriched with	Reference
Acaulospora bireticulata Rothwell & Trappe	Zn (Cu, Pb, Ni, Cd)	Sambandan et al. 1992
Acaulospora delicata Walker, Pfeiffer & Bloss	Zn (Cu, Pb, Ni, Cd)	Sambandan et al. 1992
Acaulospora nicolsonii Walker, Reed & Sanders	Pb	Walker et al. 1984
Gigaspora gigantea (Nicol. & Gerd.) Gerd. & Trappe	Zn (Cu, Pb, Ni, Cd)	Sambandan et al. 1992
Glomus aggregatum Schenck & Smith emend. Koske	Zn (Cu, Pb, Ni, Cd)	Sambandan et al. 1992
Glomus albidum Walker & Rhodes	Zn (Cu, Pb, Ni, Cd)	Sambandan et al. 1992
Glomus deserticola Trappe, Bloss & Menge	Mn, Zn	Arines and Vilarino 1991
Glomus deserticola	Zn (Cu, Pb, Ni, Cd)	Sambandan et al. 1992
Glomus fasciculatum (Thaxter) Gerd. & Trappe emend. Walker & Koske	Fe, Mn, Zn	Ernst et al. 1984
Glomus fasciculatum	Fe, Mn, Zn	Dueck et al. 1986
Glomus fasciculatum	Cd, Zn	Ietswaart et al. 1992
Glomus fasciculatum	Pb, Zn	Ietswaart et al. 1992
Glomus geosporum (Nicol. & Gerd.) Walker	Zn (Cu, Pb, Ni, Cd)	Sambandan et al. 1992
Glomus intraradix Schenck & Smith	Zn (Cu, Pb, Ni, Cd)	Sambandan et al. 1992
Glomus macrocarpum Tul. & Tul.	Zn (Cu, Pb, Ni, Cd)	Sambandan et al. 1992
Glomus microcarpum Tul. & Tul.	Zn (Cu, Pb, Ni, Cd)	Sambandan et al. 1992
Glomus mosseae (Nicol. & Gerd.) Gerd. & Trappe	Cd (Zn)	Gildon and Tinker 1981
Glomus mosseae	Cd (Zn)	Gildon and Tinker 1983
Glomus mosseae	Mn	Bethlenfalvay and Franson 1989
Glomus occultum Walker	Zn (Cu, Pb, Ni, Cd)	Sambandan et al. 1992
Glomus pubescens (Sacc. & Ellis) Trappe & Gerd.	Zn (Cu, Pb, Ni, Cd)	Sambandan et al. 1992
Glomus tenue (Greenall) Hall	Zn, Cu	Christie and Kilpatrick 1992
Glomus tortuosum Schenck & Smith	Zn (Cu, Pb, Ni, Cd)	Sambandan et al. 1992
Sclerocystis rubiformis Gerd. & Trappe	Zn (Cu, Pb, Ni, Cd)	Sambandan et al. 1992
Sclerocystis heterogama (Nicol. & Gerd.) Walker & Sanders	Zn (Cu, Pb, Ni, Cd)	Sambandan et al. 1992
Sclerocystis weresubiae Koske & Walker	Cu	Koske and Walker 1986

ry cells (Fig. 2). Vesicles, however, were absent and indications for the genus *Scutellospora* were found from the presence of a sporogenous cell and a germination shield (Fig. 3). The purple staining of the two inner walls in PVLG/Melzer's solution was specific for *Scutellospora dipurpurescens* Morton & Koske (Fig. 4). The VA-mycorrhizal infections found at the uncontaminated Schiermonnikoog site and the copper mine of Imsbach contained many vesicles but no auxiliary cells, and the spores were typical of a *Glomus* species.

Discussion

Zinc has been emitted by the Budel refinery for about 100 years. During this period the grass *Agrostis capillaris* has evolved a zinc- and cadmium tolerance (Dueck et al. 1984). The occurrence of an arbuscular mycorrhizal fungus in this contaminated site indicates the potential of such species to develop metal tolerance. The infection pattern, the staining reaction of the isolated spores in Melzer's solution and the spore wall structure indicate the presence of *S. dipurpurescens*,

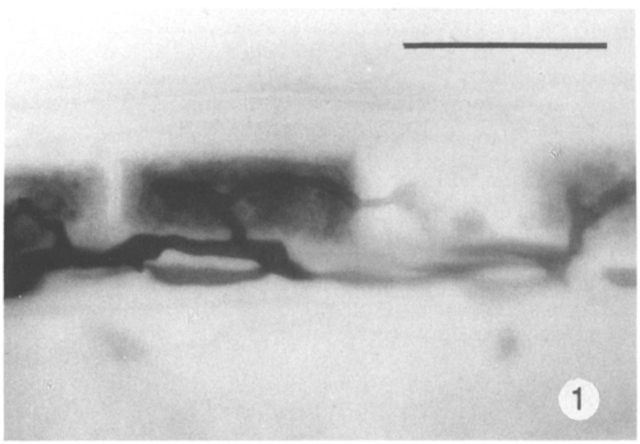

Fig. 1. Arbuscules in *Agrostis capillaris* isolated from the surroundings of a zinc refinery at Budel, stained with chlorazol black E. *Bar* = 50 μm

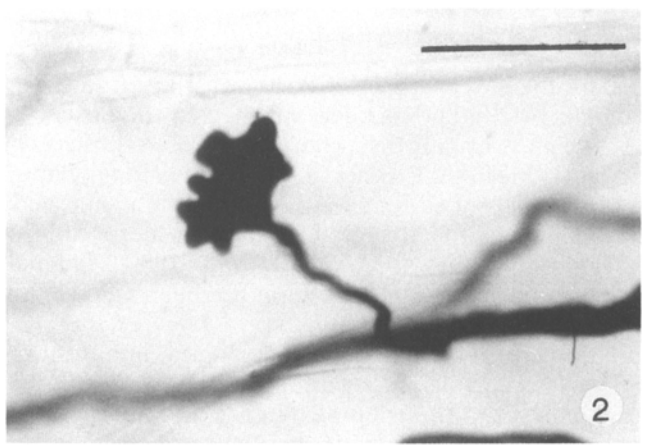

Fig. 2. Auxiliary cell near infected roots of *A. capillaris* from the surroundings of a zinc refinery at Budel, stained with chlorazol black E. *Bar* = 50 μm

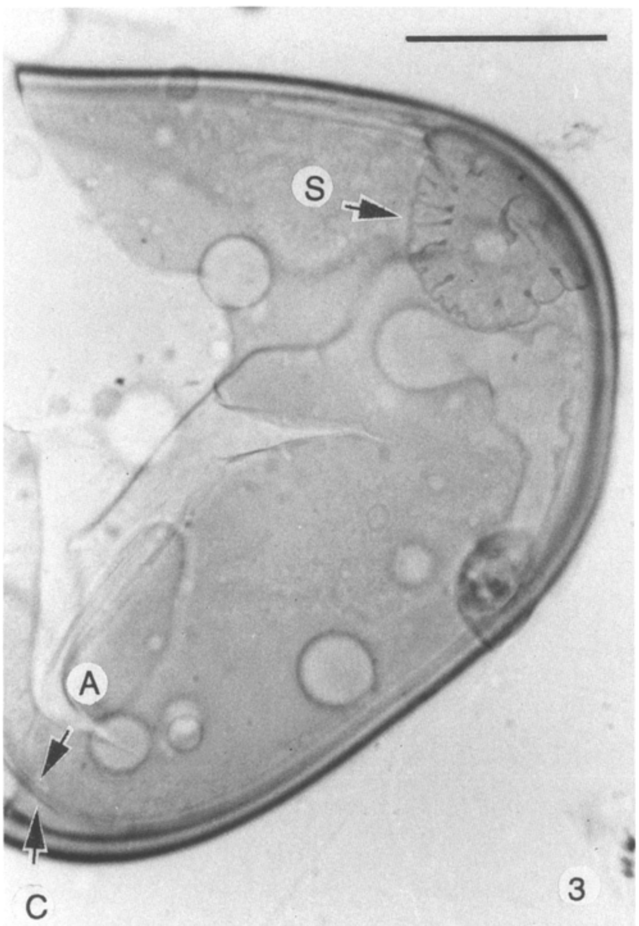

Fig. 3. Part of a crushed isolated spore showing the germination shield (*S*) and the most inner (*A*) and outer-inner (*C*) wall. Spore embedded in PVLG without stain. *Bar*=50 μm

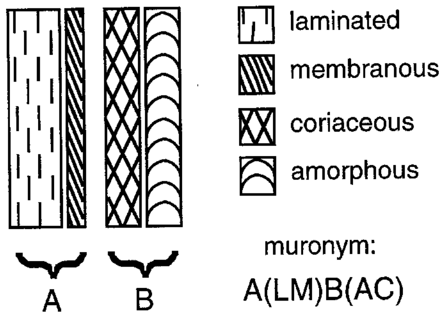

Fig. 4. Murograph and muronym of the spores isolated from soil near zinc refinery at Budel

first isolated and described from a reclaimed coal mine area in West Virginia (Morton and Koske 1988) and perhaps also present in Poland (Blaszkowski 1991). Examination of soil samples from the rhizosphere of the same host (*A. capillaris*) on the island of Schiermonnikoog and in the surroundings of a copper mine in Germany did not reveal spores of this mycorrhizal species. Although present in both America and Europe, this arbuscular mycorrhizal fungus does not seem to be a common species. The distribution of the fungal species may depend upon the importation of zinc ore from natural ore outcrops and was thus imported into The Netherlands or Europe, as is the case for the occurrence of the copper moss *Scopelophila cataractae* (Mitt.) Broth. in South Wales (Corley and Perry 1985) and in Central Europe (Sotiaux et al. 1987). Further investigations should be made of the presence of *S. dipurpurescens* at other heavy metal-contaminated sites.

The role of *S. dipurpurescens* and that of other (V)A-mycorrhizal fungi in the heavy metal tolerance of their hosts is still under discussion. They may function by a decrease in the translocation of heavy metals, as found for the ericoid mycorrhiza of the heath (Bradley et al. 1981). However, the role may only involve the supply of some major nutrients and/or water, as no impact was found of (V)A-mycorrhizae on the metal concentrations in *A. capillaris* at the Budel site or at the ore outcrop area near Breinig (Ietswaart et al. 1992).

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