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Technical note: *Piriformospora indica* hyphae and chlamydospores by scanning electron microscopy

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Piriformospora indica **is an endophytic fungus with a wide host range. The hyphae of the fungus can penetrate into the root cortex and form chlamydospores. After culturing of *P. indica* on synthetic media we performed scanning electron microscopy to analyze the form and structure of the hyphae and chlamydospores. The hyphae are straight and hyaline, and the surface of the hyphal walls is smooth. The chlamydospores are pear-shaped and have smooth walls. We provide a protocol for the visualization of fungal hyphae and spores by Scanning Electron Microscopy.**

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Introduction

The endophytic fungus *Piriformospora indica* interacts with the roots of many different species and promotes growth and performance of the hosts. *Oryza sativa, Triticum sativum, Hordeum vulgare*, *Zea mays, Setaria italica, Sorghum vulgare* (Varma et al. 1999, 2000); *Spinacia oleracea* (Kumari et al. 2003), *Glycine max*, *Cicer arietinum*, *Solanum melongena*, *Nicotiana tabacum*, *Petroselinum crispum*, *Artemisia annua*, *Bacopa monniera*, *Cymbidium spp.* (Varma et al. 2000), *Spilanthes calva, Withania somnifera, Adhatoda vasica Nees* (Varma et al. 1999, 2000; Kumari et al. 2003), *Arabidopsis thaliana* (Shahollari et al. 2005) and *Brassica chinensis* (Sun et al. 2010) have been investigated so far. *P. indica* colonizes the roots, grows inter- and intracellularly, forms pear-shaped spores within the cortex, but does not invade the endodermis and aerial parts of the plants. The endophytic fungus also promotes nutrient uptake, allows plants to survive under water, temperature and salt stress, and confers tolerance to toxin, heavy metal ions and pathogenic organisms (Oelmüller et al. 2009). For beneficial interactions, the degree of root colonization is very critical. Over- and under-colonization impairs the benefits for both interaction partners (Sherameti et al. 2008). Here, we describe a protocol for the visualisation of the ultrastructure of the fungus, and present first results.

Until now, the morphology of *P. indica* hyphae and spores in colonized roots is mainly analysed by staining methods. Others studies used epifluorescense (Verma et al. 1998) or confocal microscopy (Stein et al. 2008, Waller et al. 2005). Those methods allow the analysis of the morphology of the hyphae and spores only in two dimensions. We used scanning electron microscopy (SEM) to analyse *P. indica* spores and hyphae since SEM is a useful technique to study morphology and surface microstructure of various biological specimens in three dimensions (Bricelj 2006).

The Method

SEM (Figure 1) is a type of electron microscopy that images the sample surface by scanning it with a high-energy beam of electrons in a raster scan pattern. The electrons interact with the atoms and produce signals about the sample's external morphology, texture, crystalline structure and orientation of surface-bound structures (Vernon-Parry 2000). The method is outlined in the Method box –buffers and solution- and box –protocol-.

Results

The *P. indica* hyphae are straight. The surface of the hyphal walls is smooth and almost hyaline, but in older cultures the hyphae become irregularly inflated. After 15 days of cultivation of the fungus on PDA medium (Figure 2), the pear-shaped chlamydospores are formed from vesicles at the tips of the hyphae. They appear singly or in clusters. The walls of chlamydospores are smooth.

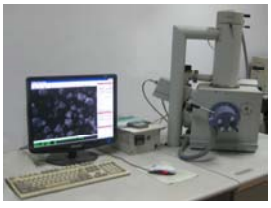

Figure 1: Scanning electron microscope XL30E, Philips (NL)

Figure 2: *P. indica* grown for 15 days on plates with PDA medium

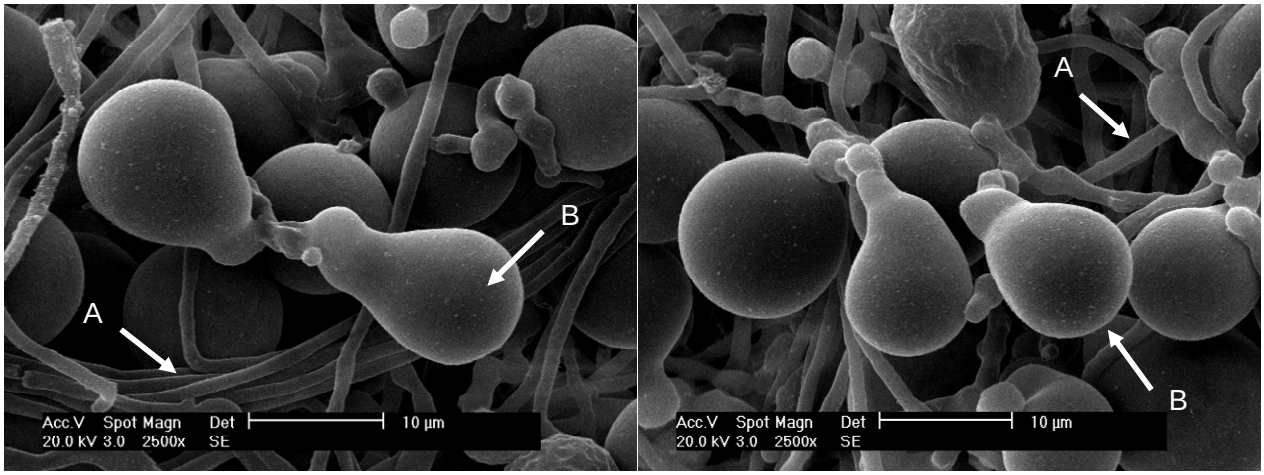

Figure 3: The hyphae and chlamydospores of *P. indica* under SEM (magnification times 2500). The fungus grown for 15 days on PDA medium. (A) Hyphae, (B) chlamydospore.

Conclusion

Under SEM, the morphology of the hyphae and spores of *P. indica* can be seen in three dimensions. The hyphae are smooth, straight and closely attached to each other (Figure 3). The chlamydospores are pear-shaped. They have smooth walls. These results are in agreement with the observations by others (Verma et al. 1998, Waller et al. 2005). As an endophytic fungus, the hyphae of *P. indica* penetrate into the root cortex and form coils, branches or round bodies (Varma et al. 1999). A comparative analysis of the hyphal structure in different hosts may help to understand differences in the interactions and the benefits for both symbionts.

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References

Bricelj VM. (2006) Preparation of large bivalve specimens for scanning electron microscopy using hexamethyldisilazane (HMDS). *J Shellfish Res*. 25:639-641.

Harrison MJ, Buuren ML. (1995) A phosphate transporter from the mycorrhizal fungus *Glomus ersiforme*. *Nature,* 378:626-629.

Kumari R, Kishan H, Bhoon YK, Varma A. (2003) Colonization of cruciferous plants by *Piriformospora indica*. *Curr Sci*. 85:1672-1674.

Oelmüller R, Sherameti I, Tripathi S, Varma A. (2009) *Piriformospora indica*, a cultivable root endophyte with multiple biotechnological applications. *Symbiosis*, 49:1-17.

Shahollari B, Varma A, Oelmüller R. (2005) Expression of a receptor kinase in *Arabidopsis* root is stimulated by the basidiomycete *Piriformospora indica* and the protein accumulates in Triton X-100 insoluble plasma membrane microdomains. *J Plant Physiol*. 162:945-958.

Stein E, Molitor A, Waller F. (2008) Systemic resistance in *Arabidopsis* conferred by the mycorrhizal fungus *Piriformospora indica* requires jasmonic acid signaling and the cytoplasmic function of NPR1. *Plant Cell Physiol.* 49:1747-1751.

Sun C, Johnson MJ, Oelmüller R, Lou BG. (2010) *Pirifomospora indica* confers drought tolerance in Chinese cabbage leaves by stimulating antioxidant enzymes, the expression of drought-related genes and the plastid-localized CAS protein. *J Plant Physiol*. 167:1009-1017.

Varma A, Singh A, Rexer KH, Sharma J. (2000) Plant Productivity determinants beyond minerals, water and light: *Piriformospora indica* – a revolutionary plant growth promoting fungus. *Curr Sci*. 79:1548-1554.

Varma A, Verma S, Sudha, Sahay N, Bütehorn B, Franken P. (1999) *Piriformospora indica*, a cultivable plant – growth promoting root endophyte. *Appl Environ Microbiol.* 65:2741-2744.

Verma S, Varma A, Rexer KH, Hassel A, Kost G, Sarbhoy A, Bisen P, Bütehorn B, Franken P. (1998) *Piriformospora indica*, gen. et sp. Nov., a new root-colonizing fungus. *Mycologia*, 90:896-903.

Vernon-Parry KD. (2000) Scanning electron microscopy: an introduction. Analysis III-Vs Review, 13:40-44.

Waller F, Achatz B, Baltruschat H, Fodor J, Becker K, Fischer M, Heier T, Hückelhoven R, Neumann C, Wettstein D, Franken P, Kogel KH. (2005) The endophytic fungus *Piriformospora indica* reprograms barley to salt – stress tolerance, disease resistance and higher yield. *Proc Natl Acad Sci USA,* 102:13386-13391.

Box - Buffers and Solutions

(1) 0.1 mol/L phosphate buffer
Solution A: 0.2 mol/L disodium hydrogen phosphate solution
Na_2HPO_4 28.40 g
Solution B: 0.2 mol/L sodium dihydrogen phosphate solution
Na_2HPO_4 24.00 g
Mix 61.0 ml of solution A and 39.0 ml of solution B, add 100 ml of distilled water.

(2) 2.5% glutaraldehyde solution (for 100 ml)

25% glutaraldehyde solution	10 ml
0.2 mol/L phosphate buffer	50 ml

Add distilled water to 100 ml

(3) 1% buffered osmium tetroxide (for 10 ml)

2% osmium tetroxide aqueous solution	5 ml
0.2 mol/L phosphate buffer	5 ml

Box – Protocol

Step 1	Cultivate *P. indica* on Potato Dextrose Agar (PDA) medium for 15 days. 3mm×2mm fungal plugs from the middle of the colony are taken as samples
Step 2	Fix the sample at 4°C in 2.5% glutaraldehyde solution overnight
Step 3	Remove the fixing solution and rinse the samples 3 times (15 min each) with 0.1M phosphate buffer (pH 7.0)
Step 4	Fix the samples 1-2h in 1% buffered osmium tetroxide at the room temperature
Step 5	Remove 1% buffered osmium tetroxide and rinse the samples 3 times (15 min each) with 0.1M phosphate buffer (pH 7.0)
Step 6	Dehydrate the samples in ethanol grads (70, 80, 90, 95%) for 15 min at each concentration. Finally, wash the samples in 100% ethanol twice (20 min each)
Step 7	Put the samples in ethanol/isoamyl acetate solution (v:v = 1:1) for 30 min. Then treat the samples with isoamyl acetate alone for 1-2h
Step 8	Critical point drying is processed with a HCP-2 critical point dryer. (Hitachi, Tokyo, Japan). Carbon dioxide is used as the transitional fluid
Step 9	Coat the samples with gold
Step 10	Observe the samples under SEM and take photos.