

INDIRECT IMMUNO-FLUORESCENCE ASSAY

(Wright, 2002)

Introduction

This procedure is used to identify the location of glomalin on fungal structures, roots, soil particles, mesh, etc. The anti-glomalin monoclonal antibody (MAb32B11) binds to antigenic sites on glomalin. [The anti-glomalin antibody is currently not available commercially, but the B cell line for production of the antibody is available for purchase from ATCC (American Type Culture Collection). The catalog number is CRL-2559. See the procedure for antibody production to learn how to grow the cell line or follow the procedures recommended by ATCC.] An IgM antibody conjugated with FITC binds to the anti-glomalin antibody and under blue light, the FITC produces a green color. The location of color is an indirect assessment of the location of glomalin. Unlike with the ELISA procedure, a secondary antibody conjugation is not needed, because the site specificity and intensity is not being used to determine concentration. Also, unlike the ELISA, glomalin is not being extracted and is therefore not exposed to the high temperatures, different pH levels, and aqueous extractants that may reduce immunoreactivity. Given the sporadic deposition of glomalin and the 3D nature of most of the structures upon which glomalin is present, this is a qualitative procedure not a quantitative procedure.

Materials

Small sieves (made from 10mm ID polyvinyl chloride or other plastic tubing with 20 to 40 um nylon mesh glued to the bottom) or 20 to 40 um mesh bags to hold the sample (Fig. 1)

2% non-fat milk (2 g powdered milk/100 ml PBS)

PBS (Phosphate buffered saline), pH 7.4

PBST (PBS with 0.2 ml/L Tween 20), pH 7.4

1% BSA (1 g BSA/100 ml PBS)*

MAb32B11 antibody (diluted with PBS, typically 1:1 or 1:2)**

FITC tagged goat anti-mouse IgM (24 ul/6 ml 1% BSA)**

Microscope slides and cover slipes (using depression slides especially when looking at aggregates will be most helpful)

VectaShield Mounting Media

Epi-fluorescence microscope with blue filter***

*Make about 500-1000 mls of stock and dispense in 6 ml aliquots and freeze at -20°C until needed.

**These are recommended concentrations. Chemicals obtained from different companies or with different lot numbers may need to be optimized for your conditions.

***Use a microscope with a band pass combination BP450-BP490 excitor filter, a dichroic chromatic beam splitter FT-510 filter, and a longwave pass LP-520 barrier filter

Methods

- 1) Place sample (colonized roots, soil aggregates, mesh, etc.) in small sieve or mesh bag and place sieves or bags in wells or containers where the sample will be immersed in the liquid, but the amount of liquid needed is rather small (Fig. 1)



Figure 1. Placing samples in sieves allows for submergence in the solutions while making it easy to replace solutions by first removing the sieves with forceps and then inverting the plate.

- 2) Submerge in 2% non-fat milk and incubate, while shaking for 30 min.
- 3) Remove milk by using a forceps to remove the small sieves or mesh bags from the solution. Then, invert the plate over a sink and blot by vigorously tapping the inverted plate on an absorbent paper towel.
- 4) Add the diluted MAb32B11 antibody, and incubate on shaker for 1 hour.
- 5) Remove antibody by using a forceps to remove the small sieves or mesh bags from the solution. Then, invert the plate over a sink followed by blotting with vigorously tapping of the inverted plate on an absorbent paper towel.
- 6) Add PBST and incubate on shaker for 5 min. Remove PBST by using a forceps to remove the small sieves or mesh bags from the solution. Then, invert the plate over a followed by blotting with vigorously tapping of the inverted plate on an absorbent paper towel. Repeat PBST incubation twice.
- 7) Add FITC tagged goat anti-mouse IgM and incubate on shaker for 1 hour.
- 8) Remove IgM by using a forceps to remove the small sieves or mesh bags from the solution. Then, invert the plate over a sink followed by blotting with vigorously tapping of the inverted plate on an absorbent paper towel.
- 9) Add PBST , and incubate on shaker for 5 min. Remove PBST by using a forceps to remove the small sieves or mesh bags from the solution. Then, invert the plate over a followed by blotting with vigorously tapping of the inverted plate on an absorbent paper towel. Repeat PBST incubation twice, followed by one 5-min incubation with PBS. Leave the sample in PBS and store in a refrigerator or cold room until mounted
- 10) Mount sample on slide with VectaShield mounting media, cover with a cover slip, and observe under epi-fluorescence using a blue filter (Fig. 2).

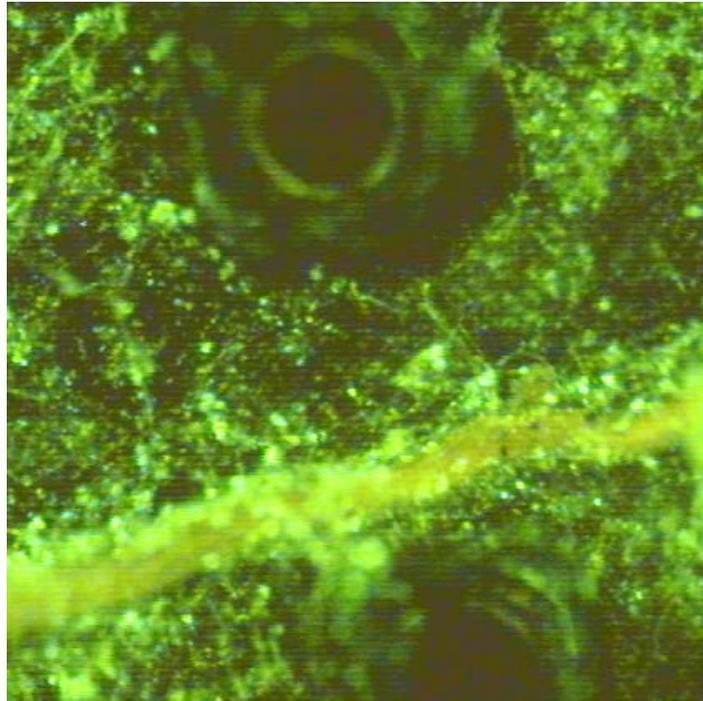


Figure 2. Bright green spots on the root, hyphae, and horticultural mesh indicate the presence of glomalin at these locations.

Note: To examine immunofluorescence of intraradical structures a pre-incubation step with boiling 10% KOH for 10-15 min and a 2-hr incubation in 1:1 diluted MAb32B11 are advised. In addition, a counterstain, such as erichrome black, may be used prior to addition of the mounting medium.

Note: Image analysis software may be used to measure intensity and approximate concentration based on pixel analysis.