LIVE 3D IMAGING OF ROOTS AND MICROORGANISMS IN NOVEL TRANSPARENT SOIL

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1. BACKGROUND

Roots and their interactions with microorganisms are important in nutrient uptake and disease transmission [1], but techniques for observing roots and associated microorganisms in situ are lacking. To date, the primary limitation has been the opacity of sufficiently heterogeneous soils. We present the development of a transparent, heterogeneous substrate for the culture and live imaging of soil biological organisms which allows imaging using OPT, CLSM, and SPIM. We show physical and chemical characterisation of the substrate and demonstrate applications in studying root system architecture [2] and microbial activity.

2. METHODS

Our novel transparent soil consists of particles of the transparent, low refractive index (RI), ion exchange polymer, nafion. The material was freezer milled to produce a range of particle sizes (250-1600 µm), and hydrolyzed to reduce hydrophobicity. The particle surface was modified, allowing adsorption of mineral ions and fluorescent dyes such that roots and particles can be simultaneously imaged. A RI matched nutrient solution was used to saturate the medium before imaging such that no loss of image quality resulted from scattering by the soil particles. This allowed us to use standard CLSM and OPT plus a novel form of SPIM we term Lightsheet Tomography.

3. RESULTS AND CONCLUSIONS

The ability to image plant roots in situ allowed us to observe the development of micro-colonies of the human pathogen *Escherichia coli* O157:H7 on lettuce roots with CLSM (figure 1) and quantify 3D root system architecture using lightsheet tomography. Transparent soil combines complex soil-like environment and the capabilities of imaging biological processes at different scales, resolutions and throughput rates using modern microscopy techniques. Applications include root biology, crop genetics and soil microbiology.


Figure 1. Lettuce root in transparent soil. Bar = 200 µm