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Towards soil micro-zymography: comparison of staining and impregnation strategies

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Enzymes are produced by microorganisms either intracellularly in cell's cytoplasm and periplasm or extracellularly either as attached to outer surface of cell membranes or released to the soil microhabitats. The distribution of microhabitats in soil is highly heterogeneous with high abundance of microorganisms in the small volume of soil hotspots, e.g., in the rhizosphere - the most important plant-soil interface with very dynamic interactions between roots and microorganisms. Soil zymography is one of the most realistic methods developed to visualize enzyme activity in undisturbed soil at the mesoscale (mm-cm) level using substrate-saturated membranes. However, visualization of enzymatic processes at the **micro**-scale level remains a challenge. We tested several impregnation strategies of soil sample (e.g., by agarose gel, silicon spray and super transparent silicon mixture) for their suitability for micro-zymography, i.e., for visualization of enzyme activity in undisturbed soil particles at the microscopic level combining fluorogenic substrates with epifluorescence microscopy. The pros- and cons- of various combinations of impregnation and staining of micro-sized soil samples will be discussed.

Keywords: enzyme activity, zymography, fluorogenic substrates