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Spatial distribution and catalytic mechanisms of β-glucosidase activity at the root-soil interface

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Abstract

© 2016 Springer-Verlag Berlin Heidelberg We compared modifications of soil zymography, a new in situ technique to visualize enzyme activities, based on contact of fluorgenic substrate-saturated membranes with soil either through the gel layer (gel zymography) or without gel application (direct zymography). We coupled zymography with quantitative measurements of enzyme kinetics to characterize catalytic mechanisms of β-glucosidase activity at the plant-soil interface including root surface (rhizoplane), rhizosphere, and bulk soil. Direct zymography refined and focused image resolution. The area of hotspots (i.e., spots with most intensive enzyme activity) as well as color intensity ratios estimated using direct zymography exceeded by a factor of 2 the corresponding values obtained with gel zymography. As determined by direct zymography, the percentage of hotspots associated to root surfaces was 58–68 % of total hotspot area. Hotspot area comprised only 6.8 ± 0.1 % of the total area of an image and 9.0 ± 3 % of the root surface area. The intensity of β-glucosidase activity, however, was up to 20 times higher in the hotspots versus bulk soil. The contribution of rhizosphere to β-glucosidase activity of the whole image (77–82 %) was four times higher than the contribution of the root surface. Enzyme kinetic parameters indicated different enzyme systems in bulk and rhizosphere soil. Higher substrate affinity and catalytic efficiency in bulk than in rhizosphere soil suggested relative domination of microorganisms with more efficient enzyme systems in the former. Coupling direct zymography and kinetic assays enabled mapping the two-dimensional (2D) distribution of enzyme activity at the root-soil interface and estimating the catalytic properties of root-associated and soil-associated enzymes.

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Keywords

Enzyme kinetics, Enzyme mapping, Rhizosphere hotspots, Root exudates, Root-soil interface, Zymography, β-glucosidase