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Exposure of polysaccharides in lignocellulosics with peroxy acids produced by enzymatic perhydrolysis

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In enzymatic saccharification of lignocellulosics, access of enzymes to exposed cellulose surfaces is an initial key step in triggering the hydrolysis. Peroxy acids are potential agents to degrade plant cell wall components to expose cellulose. In this study the author focused on enzymatic production of peroxy acids, and evaluated their reactivity to expose cellulose surfaces in lignocellulosic biomass. For the production of peroxy acids, perhydrolysis of fatty acids by lipase was applied. Exposure of the polysaccharide by peroxy acids was analyzed using green fluorescent protein (GFP)-labeled carbohydrate-binding modules (CBMs) from *Clostridium josui*. As a specific markers for crystalline and non-crystalline cellulose, *Cj*CBM3 and *Cj*CBM28 were used, respectively. Distribution of the exposed cellulose surfaces after reaction was analyzed using confocal laser scanning fluorescence microscope.

Japanese cedar wood was partially delignified by glycerolysis at 200°C for 6 min using microwave irradiator. Insoluble pulp fraction was separated, washed successively with acetone and water, and used for the analysis of the exposed cellulose. Peroxy acids were prepared by the reactions of lipase from *Penicillium camemberti* with octanoic acid and H_2O_2 in chloroform. *m*-Chloroperbenzoic acid (*m*CPBA) was used as a standard compound of peroxyacid.

The partially delignified pulp was treated with peroxyoctanoic acid, and adsorption of *Cj*CBM28-GFP and *Cj*CBM3-GFP on the substrate was analyzed before and after reaction. Adsorption of these probe molecule to crystalline and non-crystalline cellulose increased depending on the conditions for the pretreatment. Increase in the adsorption of fluorescent CBMs were also found in the treatments with *m*CPBA. Analysis with confocal laser fluorescence microscope showed that the lignocellulosics treated with peroxy acid was extensively fibered (Figure 1).

Peroxy acids formed by enzymatic perhydrolysis are the potential agent to expose cellulose surfaces in lignocellulosics. This expands roles of lipase and other lipid-related (per)hydrolytic enzymes from the degradation of lipids to the exposure of cellulose surfaces, thereby accelerating microbial and enzymatic degradation of lignified plant cell walls.

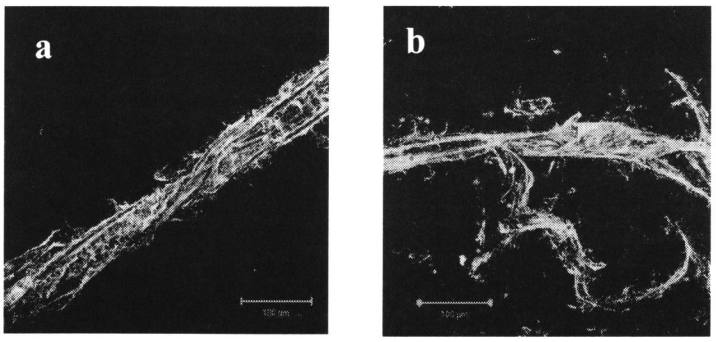

Figure 1 Confocal laser fluorescence microscopic photographs of partially delignified pulp from Japanese cedar wood after adsorption of *Cj*CBM28-GFP.
(a) untreated, (b) treated with peroxyoctanoic acid