Note

Solid State $^{13}$C-NMR Analysis of Cell Wall Components of Fusarium oxysporum

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It is known that many fungal species have chitin as a main component of the cell wall. In higher plants, chitinases have been implicated in the defense reaction against pathogens, because they directly attack the cell wall and inhibit fungal growth in vitro. However, since the fungal cell wall contains chitosan to some extent, it is uncertain whether chitinase indeed functions in the degradation of the cell walls of pathogens. In order to efficiently control the fungal plant-pathogen, therefore, it is important to confirm the structural details of the chitinous component in the intact cell wall by a nondestructive method.

Solid state cross-polarization magic-angle spinning (CP/MAS) NMR spectroscopy is one of the most useful methods for structural elucidation under nondestructive conditions, and has been applied to the structural analyses of the bacterial cell wall, insect cuticle, and other insoluble materials. In this paper, we report the CP/MAS $^{13}$C-NMR spectrum of the cell wall of Fusarium oxysporum f. sp. lycopersici race 1, and discuss the structure of the cell wall and its decomposability by the chitinases.

Dried F. oxysporum cells were suspended in distilled water and completely ground in a mortar with a pestle. After confirming cell destruction under a microscope, the homogenate was centrifuged at 3500 g for 20 min. The precipitate was washed with distilled water and then lyophilized. Chitin (0% deacetylated) and chitosan 70M (65-75% deacetylated) were purchased from Wako Pure Chemical Industries Ltd., and were used as references. CP/MAS $^{13}$C-NMR spectra were obtained at 67.8 MHz by using a JNM-GSX 270 spectrometer with 5 ms cross-polarization transfers from protons, a 27 kHz radiofrequency field, and with decoupling performed at 111 kHz. The dried sample was spun at 3.4 kHz in a Kel-F rotor.

Fig. 1. Solid State $^{13}$C-CP/MAS NMR Spectra of Fusarium oxysporum Dried Cells (A), Its Cell Wall Fraction (B), Chitosan 70M (C), and Chitin (D).
Figures 1A and 1B show the spectra for the dried cells and the cell wall fraction of *F. oxysporum*. The spectra for chitin and chitosan 70M are also shown as references (Figs. 1D and 1C). Each resonance in the spectrum for chitin has already been assigned by Saito et al.6) as indicated in the figure. The small peaks near 120 and 230 ppm are the spinning sidebands. The resonances in the spectrum for chitosan 70M could be assigned on the basis of the $^{13}$C-NMR chemical shift of the chitosan oligomer7) as indicated. Peak widths of the resonances for the chitosan are larger than those for the chitin, indicating the overlap of the resonances for the acetylated and deacetylated residues. In the spectrum for dried cells of *F. oxysporum* (Fig. 1A), minor resonances for the carbonyl and methyl carbons were detected in addition to main three peaks (50–110 ppm). In the spectrum for the cell wall fraction of the fungus (Fig. 1B), however, the carbonyl and the methyl carbon resonances are more explicit, indicating that the cell wall fraction had been satisfactorily purified by the procedure mentioned above.

The resonances for carbonyl, methyl, C1 and C4 of the chitosan chain in the cell wall fraction could be assigned by referring to the spectrum for chitosan 70M, and the relative intensities of the resonances for the cell wall fraction were quite similar to those for chitosan 70M. In the region of C2, C6, C5 and C3 (50–80 ppm), however, extra resonances were superimposed on the resonances derived from chitosan. These are most likely due to the other components of the cell wall and/or to the insoluble membrane fraction. Despite these minor contaminants, it is concluded that the cell wall of *F. oxysporum* contains a chitosan chain in which the degree of acetylation is comparable to that in chitosan 70M.

Mitsutomi et al.8) have reported that 66% deacetylated chitosan is successfully hydrolyzed by *Aeromonas hydrophila* chitinase, suggesting that chitinase can also attack the chitosan chain with a low degree of acetylation and, hence, most probably hydrolyzes the *F. oxysporum* cell wall. On the other hand, the decomposability of the chitosan chain by the chitinase apparently depends not only on the degree of acetylation but also on the distribution of the acetylated sugar residue in the chitosan chain. Further investigation of the chitosan chain in the *F. oxysporum* cell wall is in progress through an analysis of the enzymatic reaction products of the cell wall.

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References


