Note

Effects of Chitosan, Pectic Acid, Lysozyme, and Chitinase on the Growth of Several Phytopathogens

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Chitosan [(1→4)-linked 2-amino-2-deoxy-β-D-glucan] and chitin (*N*-acetylchitosan) are present in the cell walls of phytopathogens. Chitinase (EC 3.2.1.14) activity has been found in almost all plant tissues,[1] and the enzyme actually degrades fungal cell walls[2] to inhibit fungal growth.[3] Such a phenomenon seems to be very important in the interaction between plants and phytopathogens, and several reports are available concerning the inhibition of phytopathogen growth, though only in a few species, by chitinase.[3] Chitosan itself has also been shown to inhibit phytopathogen growth.[4~7] In this study, we have studied the relationship between the degree of polymerization of chitosan and the inhibition grade, and compared the inhibitory effects of chitosan, chitinase, and lysozyme.

Potato dextrose agar (PDA, Difco Lab., Detroit) was used as the basal culture medium (pH 5.6). Each of 18 phytopathogens was grown on the PDA medium containing a test material in a concentration of 0.01, 0.1, or 1.0 mg/ml in a Petri dish (diameter 8.5 cm). A control was run with the PDA medium containing no test materials. The test materials used are the lactate salt of high-molecular-weight (HMW)-chitosan [crab shell, MW 400,000, the degree of substitution 0.95 for NAc],

Table I. GROWTH INHIBITION OF SEVERAL PHYTOPATHOGENS BY INOCULATING THEIR MYCELIA AND SPORES ON PDA MEDIUM[a]

Pathogen	Radial growth (% of control)[b]			
	HMW-chitosan	LMW-chitosan	Chitosan oligosaccharides	Pectic acid
Rhizopus nigricans	84±4	62±6	80±5	n.d.
Valsa mali	79±5	54±4	n.i.	n.i.
Botrytis cinerea	81±7	n.i.	n.i.	n.i.
Rhizoctonia solani	73±8	87±4	n.i.	109±5
Ceratobasidum fragariae	79±6	66±6	n.i.	n.i.
Helminthosporium oryzae	49±9	n.i.	88±5	84±4
Phomopsis fukushi	83±9	83±5	n.i.	77±10
Fusarium oxysporum f. sp. *melonis*	56±9	86±3	82±4	84±2
	(65±8)[c]	(81±5)[c]	(89±3)[c]	
Fusarium oxysporum F. sp. *lycopersici*	51±9	69±2	85±5	86±6
	(63±10)[d]	(75±2)[d]	(87±1)[d]	
Alternaria alternata	69±7	86±2	94±3	85±3
Alternaria alternata apple pathotype	74±10	n.i.	n.i.	n.i.
Alternaria alternata Japanese pear pathotype	66±3	n.i.	n.i.	n.i.
Aspergillus niger (IAM 3001)	n.d.	107±7	116±8	n.d.
Cladosporium cucumerinum	72±11	n.i.	114±10	76±5
Glomerella cingulata	n.i.	n.i.	n.i.	n.i.
Pyricularia oryzae	n.i.	n.i.	n.i.	68±3
Penicillium citrinum (ATCC 9849)	n.d.	107±9	n.i.	n.d.
Venturia inaequalis	n.d.	23±1	35±1	n.d.

[a] A circular portion (diameter 5 mm) of the pathogen mycelia was inoculated in the center of the PDA medium containing a test material (1.0 mg/ml) in a Petri dish. For the inoculation of spores, the spore suspension (20 μl each) was put on the center of the PDA medium. The plates were incubated at 25°C for 3~7 days until the colony diameter was in the range from 5 to 6 cm. The longest and shortest diameters for each of the five colonies were measured, and the average value was expressed with the standard deviation.

[b] The growth on the PDA was defined as 100 (control): inhibition, <100; growth stimulation, >100; n.i., not inhibited; n.d., not determined.

[c] By inoculating the spores, 1.14×10^4/20 μl.

[d] By inoculating the spores, 2.96×10^4/20 μl.

Table II. GROWTH INHIBITION OF SEVERAL PHYTOPATHOGENS BY INOCULATING THEIR MYCELIA ON PDA MEDIUM[a]

Pathogen	Growth inhibition				
	HMW-chitosan	LMW-chitosan	Chitosan oligosaccharides	Chitinase[b]	Lysozyme[c]
Rhizopus nigricans	−	−	+	−	−
Valsa mali	++	+++	++	+	++
Rhizoctonia solani	−	++	+	−	−
Phomopsis fukushi	+	+++	++	−	+
Fusarium oxysporum f. sp. *melonis*	−	+++	++	−	−
Fusarium oxysporum F. sp. *lycopersici*	++	+++	+++	−	++
Alternaria alternata	+	++	++	−	−

[a] For the experimental procedure, see ref. 3. All the tests were done at pH 5.6 at the concentration of 1.0 mg/ml. Key: −, no inhibition; +, weak inhibition; ++, medium inhibition; +++, strong inhibition.
[b] From *Streptomyces* sp. (5∼15 units/mg, WAK-83, Wakunaga).
[c] From hen egg-white (Grade III, Sigma).

low-molecular-weight (LMW)-chitosan[8] and chitosan-oligosaccharides (d.p. 2∼8, Katakurachikkarin Co., Ltd.),[9] and the sodium salt of pectic acid (MW 30,000) from orange peels.

Increases in the concentrations of these compounds resulted in increases in the grades of the inhibition of phytopathogen growth. As shown in Table I, the growth of 13 fungi was inhibited more than 10% by HMW-chitosan, 9 fungi by LMW-chitosan, 7 by pectic acid, and 6 by chitosan oligosaccharides. More than 30% inhibition was observed with *V. inaequalis*, *H. oryzae*, *F. oxysporum* f. sp. *melonis*, *F. oxysporum* f. sp. *lycopersici*, *A. alternata*, and *A. alternata* (Japanese pear pathotype) by HMW-chitosan, *R. nigricans*, *V. mali*, and *C. fragariae* by LMW-chitosan, and *P. oryzae* by pectic acid. The growth of *Pyricularia oryzae* was inhibited about 30% by pectic acid but not by chitosan. The inhibition was also confirmed by inoculating their spores. The formation of a growth inhibition zone around a well containing each of test materials was examined by the method of Boller *et al.*[3] for 7 phytopathogens (Table II). Strong growth inhibition was oserved with LMW-chitosan and chitosan oligosaccharides, but only weak inhibition with HMW-chitosan, chitinase, and lysozyme. The HMW-chitosan was highly viscous and may not diffuse into the agar gel medium around the well. These enzyme proteins had no inhibitory effects when denatured.

The growth of *Fusarium* genus was inhibited by chitosan in agreement with earlier data.[4,5] Decreases in the degree of polymerization of chitosan resulted in decreases in the number of inhibited fungus species. These data, together with the inhibition of phytopathogen growth by chitosan but not by *N*-acetylchitosan,[4] indicate that the functional groups for the growth inhibition are the cationized amino group (at pH 5.6) of chitosan and the carboxyl group of pectic acid. Chitosan may make polyelectrolyte complexes with the acidic and basic groups of the cell surface to disorder it.[10] The inhibitory activities by lsozyme and chitinase[7] were lower than those by LMW-chitosan and chitosan oligosaccharides. This strongly suggests that the depolymerized products of chitosan are effective for growth inhibition. Chitosan acts in the growth inhibition of several fungi by inducing plant chitinase activity.[11] Therefore, the mechanism of the growth inhibition by chitosan differs distinctly from that of the currently used synthetic fungicides.

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