STUDY OF CHEMICAL TREATMENT COMBINED WITH RADIATION TO PREPARE BIOTIC ELICITOR FOR UTILIZATION IN AGRICULTURE

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Abstract

Chitosan was prepared from shrimp shell (alpha chitosan) and from squid pen (beta chitosan) with degree of deacetylation of about 70%. Degradation of chitosan in flake form by combined treatment with H_2O_2 and gamma Co-60 radiation was carried out. Results showed that combined treatment was highly effective for degradation of chitosan to obtain low molecular weight of 1-2 $\times$ 10^5. Oligochitosan was prepared by irradiation of chitosan solution of 50g/l (5%, w/v). The dose required for oligochitosan with water soluble content of more than 70% was of 32kGy and 48kGy for beta and alpha chitosan, respectively. Synergic effect of degradation of chitosan in solution with H_2O_2 and gamma Co-60 radiation was also investigated. The dose to obtain oligochitosan was reduced from 32kGy to 4kGy for beta chitosan and from 48kGy to 8kGy for alpha chitosan.
The elicitation and growth promotion effect of oligochiotsan for sugarcane and rice were investigated. Results showed that oligochitosan with water soluble content of 70-80% (Mw~5,000-10,000) exhibited the most effective elicitation and growth promotion for plant. The optimum oligochitosan concentration by spraying was of 30 and 15ppm for sugarcane and rice, respectively. The disease index of *Ustilgo scitaminea* and *Collectotrichum falcatum* on sugarcane were reduced to 44.5 and 72.3% compared to control (100%). The productivity of sugarcane was increased about 13% (8tons/ha). The disease index of *Pyricularia grisea* on rice was reduced to 53.0% for leaf and 34.1% for neck of bloom compared to control (100%). The productivity of rice was increased for 11-26% (0.6-1.4 tons/ha). The obtained results indicated that oligochitosan is promising to use as a biotic elicitor for plant particularly for sugarcane and rice. The procedure for production of oligochitosan elicitor by γ- irradiation method was described.

1. INTRODUCTION

Radiation technology based on the use of gamma Co-60 sources and electron beam has been increasingly applied in many countries. More than 30 years in the past, industrial application of radiation processing technology has been mainly focused to 1) crosslinking of wire and cable, heat shrinkable tube and sheet, vulcanization of rubber for tire production, 2) sterilization of medical supplies, cosmetics and pharmaceuticals, 3) food irradiation and more recently treatment of flue gas and waste water [1, 2]. Radiation technology has been considered as a useful tool for the production of new and value-added products. In addition, radiation technology is also considered as an environmentally friendly and energy saving technology. More recently, radiation processing of natural polysaccharides such as alginate, chitin/chitosan, carraginnane, modification of cellulose, starch and their derivatives is promissing to apply on large scale [3].

The effect of oligosaccharides on plants was studied since 1980 [4]. In 1985, the elicitation effect of oligosaccharides was for the first time reported [5] and then a series of research on this field was worked out [6-13]. However, up to now the product oligosaccharides as elicitor has been produced limitedly in some countries for instance “Enerplant” in Mexico and “Iodus” in France. In Vietnam, since 1996 research on radiation degradation of natural polysaccharides such as alginate used as plant growth promoter and chitosan used as fungicide for plants. In addition oligochitosan, an active exogenous elicitor, also exhibited growth promotion effect for plants.

In order to set up large scale production of biotic elicitor oligochitosan for application in agriculture, the research project “Study of chemical treatment combined with radiation for production of biotic elicitor for utilization in agriculture for rice and sugarcane” has been carried out.

2. EXPERIMENTAL

2.1. Chemicals

Alpha-chitin, beta-chitin and absolute ethanol were products of Vietnam. Sodium hydroxide, lactic acid and hydroperoxide were purchased from Shanghai Chemical Agent Co., China. The others reagents were analytical grade and used as received.

2.2. Methods

2.2.1. Preparation of chitosan: Chitins from shrimp shells and squid pens were deacetylated with sodium hydroxide 50 and 30% respectively at room temperature. The obtained chitosan was washed by water and dried in force air oven at 60^0C.

2.2.2. Degradation of chitosan by hydroperoxide: Chitosan flake was degraded in aqueous solution of H_2O_2 with concentration of 1% for beta chitosan and 1.5% for alpha chitosan, pH9 at room temperature.

2.2.3. Degradation of chitosan by gamma Co-60 radiation: Chitosan flake was irradiated by gamma Co-60 radiation at the VINAGAMMA Center up to 50 kGy.

2.2.4. Preparation of oligochitosan: Chitosan solution 5% in dilute lactic acid with and without H_2O_2 was irradiated up to 50 kGy.

2.2.5. Determination of the content of water soluble oligomer (WSC): The yield of WSC was gravimetrically determined by neutralization of irradiated chitosan solution by NaOH to pH7. The WSC content (%) was calculated as follows:

$$\text{WSC, \%} = 100 \times (m_0 - m)/m_0$$

Where m_0 and m is the weight of initial chitosan in solution and WSC, respectively [14].

2.2.6. Determination of degree of deacetylation (DDA): Based on infrared spectra of chitosan samples, DDA% value was determined as expression:

$$A_{1320}/A_{1420} = 0.3822 + 0.0313 \times (100 - \text{DDA\%})$$

Where A_{1320}, A_{1420} were an intensive absorbency at 1320 and 1420 cm^{-1} peaks respectively [15].

2.2.7. Determination of molecular weight by viscosimeter (Mv): Mv of chitosan was measured by capillary vicosimeter and calculated as Mark-Houwink equation:

$$[\eta] = k \times M_v^{\alpha}$$

Where $k = 1.4 \times 10^{-4}$, $\alpha = 0.83$ [16].

2.2.8. Determination of molecular weight by gel permeation chromatograph (Mw): Mw of chitosan was measured by the gel permeation chromatograph method on HP-GPC 1100, using columns of Ultrahydrogel 250, 500 and refractive index detector. The standard polysaccharide was pullulan with molecular weight in the range 780 - 380,000Da and solvent was mixture solution of 0.25M CH_3COOH/0.25M CH_3COONa [16].

2.2.9. Field test of elicition effect of oligochitosan: Field test for sugarcane (variety VN84-4137) was carried out at the Institute of Sugarcane Research, Binh duong province and for rice (variety OM1490) at the Cuu long Delta Rice Research Institute, Can tho City. Field test was designed by random block with triplicate. Biotic elicitor oligochitosan was sprayed three times on sugarcane and rice plants for a crop. Disease index, productivity were investigated and data were processed by the method of analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

3.1. Preparation of chitosan

Results in Table 1 showed that when chitin treated with concentrated NaOH solution (50% w/w for alpha chitin from shrimp shells and 30% w/w for beta chitin from squid pens) at room temperature, degree of deacetylation was increased with time up to about 70% at 48h and 96h for alpha and beta chitin respectively. Deacetylation of chitin to prepare chitosan carried out at room temperature is a cost effective process.

TABLE 1. THE DEPENDENCE OF DEGREE OF DEACETYLATION ON REACTION TIME

Reaction time, h		0	32	48	72	96	144
DDA, %	βCTS NaOH 30%	10,0	61,6	72,3	76,0	-	-
	αCTS NaOH 50%	6,0	45,0	59,0	67,5	70,3	74,6

3.2. Degradation of chitosan in flake form

3.2.1. Hydroperoxide

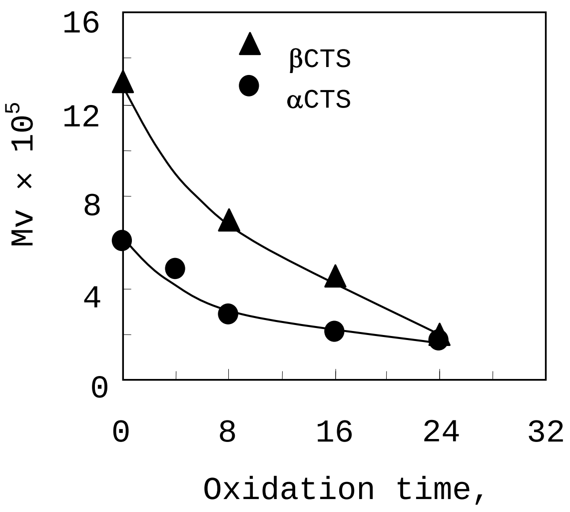

FIG. 1. The relationship of chitosan Mv with oxidation time.

Results on degradation of chitosan by H_2O_2 in flake form were shown in Fig. 1. The dimensionless relative parameter of degradation index (DI = Mvo/Mv -1) can be used to characterize quantitatively the polymer degradation process. Mv is of interest in determining the degradation extent because the dilute solution viscometry technique is reliable and has low expense. The value of DI was found out to be 0.109 and 0.189 for alpha and beta chitosan respectively. It indicated that beta chitosan is more susceptible to degradation with H_2O_2.

3.2.2. Gamma Co-60 radiation

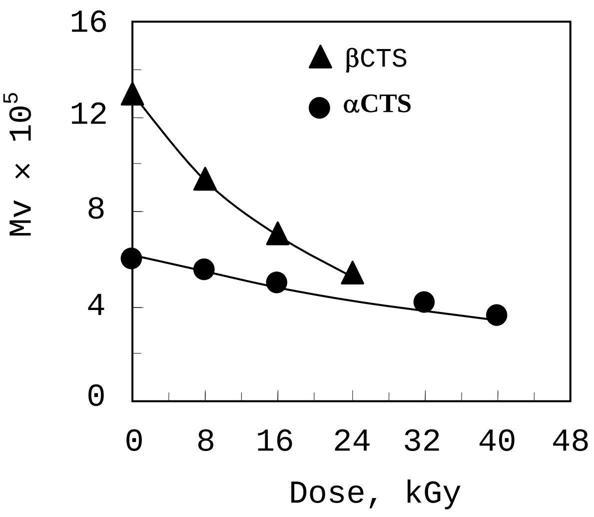

FIG. 2. The relationship of chitosan Mv with dose.

Results on degradation of chitosan by gamma Co-60 radiation were shown in Fig. 2. Gs - value can be used to estimate the susceptibility of polymer to radiation degradation. The Gs- value was calculated from Fig. 1 to be of 0.20 and 0.38 scissions/100eV for alpha and beta chitosan, respectively. That means beta chitosan is also more susceptible to radiation degradation as in the case with H_2O_2.

3.3. Preparation of oligochitosan

TABLE 2. THE CORRELATION OF WATER SOLUBLE CHITOSAN CONTENT WITH DOSE

Dose, kGy			4	8	12	16	24	32	48
WSC, %	βCTS	0 % H_2O_2	-	45,0	-	61,9	69,8	78,2	-
		1 % H_2O_2	74,9	86,0	93,9	100,0	-	-	-
	αCTS	0 % H_2O_2	-	21,3	-	29,2	-	43,4	74,9
		1 % H_2O_2	66,3	74,6	87,6	91,2	-	-	-

Results in Table 2 indicated that irradiation of chitosan solution containing 1% H_2O_2, the required dose for obtaining 75% water soluble oligomer was reduced from 48kGy to 8kGy for alpha chitosan and from 32kGy to 4kGy for beta chitosan. The use of H_2O_2 together with radiation is very effective for degradation of chitosan in solution.

3.4. Field test of elicitation effect of oligochitosan for sugarcane and rice

3.4.1. Field test for sugarcane

TABLE 3. THE EFFECT OF OLIGOCHITOSAN ON DISEASE INDEX AND PRODUCTIVITY FOR SUGARCANE

Samples		Leaf DI		Trunk DI		Productivity	
		Index, %	% for Ctrl	Ratio, %	% for Ctrl	Tons/ha	Increase, %
Kinds of oligochitosan	BOC70	9.32 b	78.72	-	-	50.86 a	11.02
	BOC90	6.85 b	57.85	-	-	51.62 a	12.68
	AOC70	6.19 b	52.28	-	-	53.15 a	16.02
	Control	11.84 a	100	-	-	45.81 b	-
Conc. of oligochitosan	15 ppm	33.52 a	95.28	19.73 ab	86.95	67.67 ab	7.01
	30 ppm	27.97 b	79.51	16.41 b	72.32	71.38 a	12.87
	60 ppm	30.44 ab	86.53	17.58 a	77.48	69.52 a	9.93
	Control	35.18 a	100	22.69 a	100	63.24 b	-

Results in Table 3 showed that for all treatments with oligochitosan the disease index on leaf was reduced to 53.2 to 78.7% compared to control 100%. It can be concluded that oligochitosans (alpha and beta) with the degree of deacetylation in the range of 70-90% exhibit elicitation effect for sugarcane to reduce the disease index on leaf to 50-80% for the control 100%. Results in Table 3 also showed that the oligochitosan concentration of 30ppm was optimal for sugarcane. The disease index was reduced to 50-80% compared to control 100% and the productivity of sugarcane was increased to 11-13% (6-8 tons/ha).

3.4.2. Field test for rice

Results in Table 4 indicated that the disease index on rice leaf was reduced significantly by treatment of all kinds of oligochitosan studied. Results in Table 4 also showed that the optimum concentration of oligochitosan was of 15ppm for rice. The disease index on leaf for instance was reduced to 63-70% for control 100% and the productivity of rice was increased to 11-26%.

TABLE 4. THE EFFECT OF OLIGOCHITOSAN ON DISEASE INDEX AND PRODUCTIVITY FOR RICE

Samples		Leaf DI		Panicle DI		Productivity	
		Index, %	for Ctrl, %	Ratio, %	for Ctrl, %	Tons/ha	Increase, %
Kinds of oligochitosan	BOC-8600	1.57 b	48.16	50.67 b	63.87	3.27 a	32.39
	BOC-5700	0.96 b	29.45	56.00 ab	70.59	3.13 a	26.72
	BOC-3600	1.70 b	52.15	55.33 ab	69.75	2.40 a	-
	AOC-8400	1.12 b	34.36	52.00 b	65.55	2.53 a	2.43
	Ctrl (H_2O)	3.26 a	100	79.33 a	100	2.47 a	-
Conc. of oligochitosan	15 ppm	14.4 b	62.88	10.00 c	34.13	6.80 bc	25.93
	30 ppm	15.3 b	66.81	12.00 c	40.90	6.00 c	11.11
	60 ppm	16.0 b	69.87	15.30 c	52.22	6.30 bc	16.67
	Ctrl (H_2O)	22.9 a	100	29.30 a	100	5.40 a	-

In general, oligochitosan is very effective to induce defense response of disease infection for rice and sugarcane. In addition, treatment of oligochitosan for sugarcane and rice not only made disease index reduction but also productivity increase.

3.5. Procedure for production of biotic elicitor oligochitosan

- 10kg chitosan (DDA 65-75%, MW~ 100-200kDa)
- Soaking with water in plastic tank
- Adding 6kg lactic acid and stirring
- Adding 4litters of H_2O_2 50% and water up to 200litters while stirring
- Filtering through stainless steel net (~100mesh)
- Packing in plastic can (25litter/can)
- Irradiation with gamma Co-60 at 4kGy for beta chitosan (8kGy for alpha chitosan)
- Neutralizing irradiated chitosan solution with NaOH to pH 5
- Adding ethanol with final concentration of 40% v/v for preservation
- Quality control and packaging
- Product: "biotic elicitor oligochitosan".

The characteristics of biotic elicitor oligochitosan product are as follows:

- Concentration of oilgochitosan in solution:	30g/L
- Content of water soluble oligomer pH7:	70-80%
- Mw:	5,000 - 10,000
- Ethanol concentration:	40% v/v
- Expire date:	2 years min.

4. CONCLUSIONS

- Preparation of alpha and beta chitosan with degree of degradation of about 70% and Mv in the range of 6 - 10 × 10^5 Da by deacetylation of chitin at room temperature.
- Reduction regulation of chitosan Mv to 1 - 2 × 10^5 Da by degradation with H_2O_2.
- Preparation of oligohitosan with molecular weight of 6 - 10 × 10^3 Da by irradiation of chitosan solution at dose 48kGy for alpha chitosan and 32 kGy for beta chitosan.
- Irradiation of chitosan solution containing 1% H_2O_2 to reduce the required dose to 8kGy for alpha chitosan and 4kGy for beta chitosan.
- Setting up production procedure for biotic elicitor oligochitosan with characteristics: oligochitosan: 30g/L; water soluble oligomer: 70-80%; Mw: 5,000-10,000; ethanol: 40% v/v, pH 5 and expire date: 2 years.
- Optimum concentration of biotic elicitor oligochitosan was of 30ppm for sugarcane and 15ppm for rice. The disease index (*U. scitaminea and C. falcatum*) for sugarcane was reduced to 45-72% compared to control 100% and sugarcane productivity was increased up to 13% (8 tons/ha). The disease index (*P. grisea*) for rice was reduce to 34-53% compared to control 100% and the productivity of rice was increased 11-26% (0.6-1.4 tons/ha).

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