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Radiation degradation of (1 → 3)-β-D-glucan from yeast with a potential application as a plant growth promoter

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ABSTRACT

The (1 → 3)-β-D-glucan extracted from the yeast cell wall was irradiated by γ-rays from a Co-60 source at dose range of 100–300 kGy in a swelling condition of 10, 15 and 20% for degradation. The water-soluble contents of irradiated samples obtained by 10% (1 → 3)-β-D-glucan mixture increased from 25.89 to 66.71% by the increasing of irradiation doses from 100 to 300 kGy. While the molecular weight of the water-soluble (1 → 3)-β-D-glucan was found to be decreased from 48.13 to 10.77 kDa. In the UV-spectra of irradiated water-soluble (1 → 3)-β-D-glucan, a new peak appeared at 265 nm with the intensity increased by the increase of the dose. The IR spectra of irradiated (1 → 3)-β-D-glucan were recognized by a decrease of the peak intensity at 1156 cm^{-1} indicated to C—O—C glycosidic linkages with the increasing of irradiation dose. In addition, radiation degraded (1 → 3)-β-D-glucan with M_w about 18 kDa prepared at the dose of 250 kGy displayed a strongly promotion effect on the growth of mustard green and the optimum concentration of the degraded (1 → 3)-β-D-glucan was found to be about 75 mg l^{-1}. Thus, the degraded (1 → 3)-β-D-glucan prepared by radiation technique displayed as a promising, safety and high effective plant growth promoter for agriculture application.

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1. Introduction

(1 → 3)-β-D-glucan, composed of glucose units linked together to form a long polymer chain is a fiber-type homopolysaccharide contained in the cell walls of fungal, bacteria and plant [1–3]. Fungi, including yeast and mushrooms are the major source for production of (1 → 3)-β-D-glucan. Accroding to Klis et al. [4], yeast (1 → 3)-β-D-glucan has a long chain of approximately 1500 (1 → 3)-β-D-glucose units and consist of a backbone of β-(1 → 3)-linked D-glucopyranosyl units with β-(1 → 6)-linked side chains of varying length and distribution. In food industry, (1 → 3)-β-D-glucans isolated from yeast has been used in the production of salad toppings (dressings), frozen deserts, sauces, yogurts and other milk products, soft doughs and paning doughs, conditories and mixture for cake filling [5,6]. Yeast (1 → 3)-β-D-glucan is also known to possess antitumor and antimicrobial, activities by enhancing the host immune function. Stimulatory effects of this polymer on both specific and non-specific immune responses, antimicrobial activity, and tolerance to oral antigens have been demonstrated in mice [7], in fish [8], pig [9,10], broiler [11], shrimp [12]. In addition, these authors also informed that (1 → 3)-β-D-glucan has beneficial effects on growth performances in pig, fish, shrimp and chicken. In plant, laminarin and (1 → 3)-β-D-glucan extracted from fungus *Pyricularia oryzae* were found as elicitors for causing an accumulation of antibiotic phytoalexins to prevent infection from fungal diseases [13,14]. However, the activity of the polysaccharides is usually influenced by molecular weight (M_w) and low M_w products displayed stronger effects on stimulation of immune response, antimicrobial and phytoalexin activities than those of high M_w polysaccharides [15,16].

Polyshaccharides have been reported to be degraded by acidic hydrolysis [17,18] or enzymatic treatment [19,20]. Although these methods are effective in decreasing the molecular weight, they do exist some disadvantages such as high cost, low yield, long processing time, and chemical wastes from the treatment [21,22]. Gamma irradiation is a useful tool for degradation of alginate, chitosan, cellulose and so on by the cleavage of the glycosidic bonds [23–25]. The basic advantages of polymeric degradation by radiation include the ability to promote changes reproducibly and quantitatively, without the introduction of chemical reagents and without the need for special equipment/setup to control

Abbreviations: ANOVA, analysis of variation; FTIR, fourier transform infrared; GPC, gel permeation chromatography; LSD, the least significant difference; NMR, nuclear magnetic resonance; M_w, molecular weight; UV, ultraviolet.

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for temperature, environment and additives [23]. Therefore, this technology is unique and more environmentally friendly than conventional methods.

Although the acid and alkaline hydrolyses, enzymatic digestion, and ultrasound irradiation have been applied as methods for degradation of (1 → 3)-β-D-glucan [26–29], ionizing radiation was rarely used in the research of (1 → 3)-β-D-glucan degradation. So far, there have been several reports on stimulation of immune response of yeast (1 → 3)-β-D-glucan, but the research on plant growth promotion effect of this natural polysaccharide has not been carried out. Hence, the aim of the present study is to apply radiation degradation method to prepare low M_w and water-soluble (1 → 3)-β-D-glucan for producing natural plant growth promoter.

2. Materials and methods

2.1. Plant and chemical

The seeds of mustard green namely *Brassica juncea* var. *rugosa* used in this study were supplied by Trang Nong Ltd. The Kit for analyzing of (1 → 3)-β-D-glucan (K-YBGL) was supplied by Megazyme International Ireland Ltd.

2.2. (1 → 3)-β-D-glucan preparation

(1 → 3)-β-D-glucan was prepared from brewer's yeast cell wall by the method of William et al. [30]. The water-insoluble β-D-glucan was obtained by means of twice extraction of *Saccharomyces carlsbergensis* cells using 3% NaOH at 90 °C followed by triple digestions with hydrochloride acid (2.45, 1.75 and 0.94 mol l^{-1}) at 90 °C. The residue was then washed in turn by diethylether, ethanol and deionized water. After the removal of all soluble components, (1 → 3)-β-D-glucan was left as the insoluble residue. The final extracted sample contains about 92% (1 → 3)-β-D-glucan (analyzed by a K-YBGL Kit).

2.3. Degradation of (1 → 3)-β-D-glucan

The degradation of (1 → 3)-β-D-glucan by radiation was conducted as follows: the (1 → 3)-β-D-glucan was suspended in deionized water and incubated overnight at room temperature to induce swelling, and then stirred for 3 h to obtain 10% (w/v) mixtures. Irradiation of (1 → 3)-β-D-glucan mixtures was carried out using a Co-60 irradiator for degradation. The doses applied in this study were in range of 100–300 kGy at room temperature with a dose rate of 3 kGy h^{-1}.

2.4. Water-solubility determination

The water-solubility of irradiated (1 → 3)-β-D-glucan was determined by the method of Byun et al. [31], the samples after irradiating were first lyophilized. Two grams of sample powder were put into a 50-ml glass tube with a cap, vortexed with 10 ml deionized water for 20 min, and centrifuged at 3500 g for 20 min. The supernatant was separated and dried at 100 °C for 2 h, and the weight of the dried products obtained from the supernatant was determined. The water-solubility was calculated as follows: water-solubility (%) = 100 × (weight of dried supernatant)/(weight of initial (1 → 3)-β-D-glucan powder).

2.5. M_w estimation

Gel permeation chromatography (GPC) was carried out to monitor the changes in the average molecular weight (M_w) of (1 → 3)-β-D-glucan by gamma irradiation. GPC was implemented using a Agilent 1100 GPC system (USA) equipped detector RID G1362A and a Bin pump G1312A. Ultrahydrogel columns model 250 and 500 from Waters (USA) (7.8 id × 300 mm) equipped with a guard Ultrahydrogel column from Waters (USA) (6 id × 40 mm) were operated at 40 °C and eluted with distilled water at a flow rate of 1.0 ml min^{-1}. The (1 → 3)-β-D-glucan sample concentration was 0.1% (w/v) and 20 μl of sample solution was loaded into the GPC system. The column was calibrated using six pullulan standard samples with M_w values of 7.78, 12.2, 23.7, 48, 100 and 380 kDa (Polymer Laboratories, USA).

2.6. FTIR (Fourier-transform IR) spectrometry

UV–visible spectroscopy of irradiated (1 → 3)-β-D-glucan solution was performed at 25 °C by a Shimazu spectrophotometer UVmini-1240 in the range of 200–600 nm. All spectra were collected from aqueous solutions containing 0.025% (w/v) of water-soluble (1 → 3)-β-glucan.

Fourier transform infrared (FTIR) spectra of degraded (1 → 3)-β-D-glucans were obtained on a Shimadzu FTIR-8100A spectrophotometer, which was linked to a Shimadzu DR-8030 computer system, in the wavelength region between 4000 and 400 cm^{-1}. Samples were prepared in KBr pellet formed by well-dried mixtures of 3 mg sample and 100 mg KBr. All spectra obtained were the results of 128 scans at ambient temperature and a spectrophotometer resolution of 4 cm^{-1}.

2.7. ^1H and ^{13}C-NMR spectrometry

^1H and ^{13}C-NMR spectra of degraded (1 → 3)-β-D-glucan were carried out by a Fourier transformation NMR (Utrashield 500 plus, Brucker Bioscience Corporation, USA). The water-soluble (1 → 3)-β-D-glucan obtained from the 250-kGy-irradiated mixture was dissolved in D_2O (Cambridge Isotope Laboratories, Inc., USA) with a concentration of 5 mg l^{-1}. ^1H and ^{13}C spectra were measured at 500 MHz for ^1H and 125 MHz for ^{13}C under proton decoupling conditions with 10,000 scans.

2.8. Growth promotion test

The effects of the degraded (1 → 3)-β-D-glucan on growth promotion were evaluated using ten 14-day-old seedlings of mustard green (*B. juncea* var. *rugosa*). A seedling was cultivated in 2000 ml solutions containing 0.1% hyponex and 75 mg l^{-1} (1 → 3)-β-D-glucan degraded by a dose range of 0–300 kGy. The controls were performed under identical conditions without supplementation of (1 → 3)-β-D-glucan. All cultures were in a standardized greenhouse at the Saigon Thuy Canh Corporation. The shoot height, root length, fresh biomass (root and shoot), and dried matter content (root and shoot) were determined after 28 days of cultivation. All experiments were repeated three times. Data were statistically analyzed using the ANOVA test. The means were compared using the least significant difference (LSD) at a 5% probability level, and the standard deviations were calculated.

3. Results and discussions

3.1. Change in water-solubility and M_w of (1 → 3)-β-D-glucan by irradiation

(1 → 3)-β-D-glucan extracted from brewer's yeast cell wall in this study is water insoluble. To induce water-soluble (1 → 3)-β-D-glucan, several researchers have modified by carboxylmethylation [26,30]. Beside these methods, degradation to reduce the molecular size is also a way for preparation of water-soluble (1 → 3)-β-D-glucan. The results in Fig. 1 indicated that the lower concentration of (1 → 3)-β-D-glucan used, the higher water-soluble content was

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