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Salicylic acid loaded chitosan microparticles applied to lettuce seedlings: Recycling shrimp fishing industry waste

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ABSTRACT

Shrimp fishing industry wastes are still a main problem with high environmental impact worldwide. In this study, chitosan with ultra-high molecular weight and deacetylation degree ≥85% was obtained from shrimp fishing industry waste from Argentinean Patagonia. Chitosan based microparticles capable to entrap salicylic acid, a phytohormone known to play major role in the regulation of plant defense response against various pathogens, were prepared using TPP as crosslinker. Unloaded microparticles and microparticles loading several salicylic acid amount were fully characterized exhibiting a size between 1.57 μm and 2.45 μm. Furthermore, a good PDI, entrappment efficiencies from 59% to 98% and salicylic acid sustained release over 24 h were achieved.

Chitosan based microparticles were non toxic in most of the doses applied in lettuce seedlings. Instead, microparticles can positively modulate plant growth and have the potential to improve plant defense responses. In particular salicylic acid loaded microparticles effect was very promising for its application as activators of salicylic acid dependent plant defense responses in lettuce as a model of horticultural plant species.

1. Introduction

Chitosan (CS) is the second most abundant polysaccharide in nature after cellulose, and it is a deacetylated derivative of chitin found mainly in the exoskeletons of crustaceans (Kanmani, Jeyaseelan, Kamaraj, Sureshbabu, & Sivashanmugam, 2017). CS is a polycationic heteropolysaccharide consisting of two monosaccharides, N-acetyl-glucosamine and D-glucosamine which relative amounts could varies in a wide range, yielding CS of varying degrees of deacetylation ranging from 75% to 95% (Tharanathan & Kittur, 2003). In addition, CS has functional groups which play an important role in its functionalities. The most important one is the amino group, specially in acidic conditions, which allows CS to interact with negatively charged molecules due to the protonation phenomenon (Bellich, D'Agostino, Semeraro, Gamini, & Cesàro, 2016).

Since CS could be produced from chitin obtained from fishing waste by exhaustive alkaline deacetylation, many commercial applications of CS and its derivatives have been explored (Bellich et al., 2016). This polymer combines several properties such as biocompatibility, biodegradability, nontoxicity and bioadhesion, which make it valuable compound for biomedical (Dash, Chiellini, Ottenbrite, & Chiellini, 2011), pharmaceutical (Yang et al., 2017), food (Atay et al., 2017; Prashanth & Tharanathan, 2007), waste water treatment (Kanmani et al., 2017), and agricultural applications (El Hadrami, Adam, El Hadrami, & Daayf, 2010) among others.

In agriculture, the use of toxic pesticides to control plant diseases is a common practice which cause hazards to the human health and environment (Mishra, Keswani, Abhilash, Fraceto, & Singh, 2017). Despite the valuable contribution of new pesticides to control plant diseases, smart and sustainable options to develop a bioplaguicide as an alternative to agro-chemicals still remains as a challenge in phytopathology. In this sense, CS has been proved as an environmental-friendly polymer for agricultural uses due to its wide spectrum biological activity including antimicrobial effect against many bacteria,

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fungi and yeasts, with a lower toxicity toward mammalian cells (Kong, Chen, Xing, & Park, 2010) and induce host defense responses in both monocotyledons and dicotyledons (El Hadrami et al., 2010). CS is also considered to be an applicable elicitor by triggering host defense respones in both mono and dicotyledon plants (Ryan, 1987). Elicitors in plant biology are extrinsic or foreign molecules often associated with plant pests, diseases or synergistic organisms. The term plant elicitor applies to a group of compounds, which triggers physiological and morphological responses within the natural defense mechanisms. Plant elicitors can help to reduce the amount of chemicals applied to crops in order to reduce infections.

CS in combination with other chemicals has been successful for the control of foliar diseases in cucumber, cantaloupe, pepper, and tomato (Abdel-Kader, El-Mougy, Aly, & Lashin, 2012). The plant defenses can be constitutive or induced and exogenous administration of different phytohormones, are reported to induce plant defenses triggering local and systemic plant responses (Tocho, Lohwasser, Börner, & Castro, 2014). In this sense, salicylic acid (SA) is a phytohormone known to play major role in several physiological processes including the regulation of plant defense response against various pathogens. SA has been well associated with resistance to biotrophic pathogens revealing that a wide range of plant defense responses is dependent on SA signaling (An & Mou, 2011).

Taking into account the low solubility of SA in water (Shalmashi & Eliassi, 2007), the development of a SA delivery system based on biocompatible polymers is a markable strategy to gain efficient control in terms of its loading and rate ofdelivery in plants. In addition, the regulation of the application doses of SA is key in the modulation of growth and defense responses of plants (http://www.mcahonduras.hn/documentos/PublicacionesEDA/Manuales%20de%20produccion). Currently, in horticulture, the development and application of more stable, sensitive and appropriate bioactives to be applied in aqueous solutions are highly valued. Lettuce is one of the most important leafy vegetables cultivated worldwide and fungal diseases are one of the main factors which affects its productivity. The abusive use of fungicides which in many cases have already show resistance to many phytopathogens (Kurzawińska, 2007; Parra et al., 2016) remains still being the commonly used strategy. In this work, we try to provide emerging tools to further develop more sustainable-based strategies for pest-management and improve growth and yield of lettuce plants.

The aim of this work was to prepared CS microparticles (MP), based on the polymer obtained from local shrimp industry waste, loading different amounts of SA in order to combine CS and SA actions triggering growth stimulation and plant defense responses with minimum impact to the environment, health and well-being.

2. Experimental

2.1. Materials

CS was synthesized in Gihon Laboratorios Químicos SRL (Mar del Plata, Argentina) from shrimp fishing industry wastes, NaOH 99% (Unipar Indupa, Argentina), glacial acetic acid 98.10% (Lotte BP Chemicals; Argentina), sodium tripolyphosphate (TPP) (Indaquim, Argentina) were used.

2.2. Synthesis of CS from local shrimp flour

CS was synthesized from exoskeleton from *Pleoticus muelleri* obtained from fish industry waste in Argentinean Patagonia through classic three step method of demineralization, deproteinization and deacetylation with modification (Puvvada, Vankayalapati, & Sukhavasi, 2012). Prior to use, CS was dissolved in 0.1 M glacial acid solution 1%(w/w) at room temperature for 2–3 h under severe stirring. Soluble CS obtained was purified by filtration to remove insoluble contaminants.

2.3. Determination of deacetylation degree (DD) by Fourier-transform infrared spectroscopy (FTIR)

The DD of independent batches of synthesized CS was determined by FTIR (Supplementary data). FTIR spectra were performed in the attenuated total reflection mode (DRS-FTIR) on a IRAffinity-1S FTIR spectrophotometer (Shimadzu, Japan). Samples were analyzed at room temperature by 16 scans, using a resolution of 4 cm^{-1}. DD was calculated according Eq. (1).

$$N-acetylation(\%) = 31.92 \times \left(\frac{A_{1318}}{A_{1380}}\right) - 12.20 \quad (1)$$

2.4. Size exclusion chromatography (SEC)

The weight-average molecular weight (M_w) and the number-average molecular weight (M_n) of CS samples were measured by SEC using a Shimadzu 20 A cromatographer (Japan), and the polydispersity was calculated from their coefficient M_w/M_n. A set of Agilent PL Aquagel-OH (30, 40 and 50) columns connected in series (300 × 7.5 mm and particle size of 8 μm) and conditioned at 28 °C was used to elute 2 mg mL^{-1} samples. Acetic acid solution (1%; pH 3.0) was used as the mobile phase at a flow of 1 mL min^{-1}. Calibration of SEC was carried out with pullulan polysaccharide standards (Polymer Laboratories, England UK) with sample injection volumes of 50 μL.

2.5. MP preparation

The CS based MP were prepared by the gelation method (Cerchiara et al., 2015) with modifications using TPP as crosslinker. Firstly, 1 L of a solution of CS (0.1 or 0.2% w/v, pH 3.0), prepared in an aqueous solution of 1% (v/v) acetic acid, was kept under vigorous stirring. When CS was completely dissolved, SA was added in different ratios (1, 5, 10 and 20% w/w with respect to CS) in order to obtain MP named as MP-1SA, MP-5SA, MP-10SA and MP-20SA, respectively, being MP-0 the unloaded MP system. After SA was totally dissolved, a TPP solution (10% w/w with respect to CS) was added dropwise (Table 1).

2.6. Obtaining of solid state MP by spray drying

Spray drying was performed in a laboratory scale Buchi Mini spray-dryer B-290 with a 1.5 mm diameter nozzle and main spray chamber of

Table 1
Physicochemical characterization: Main physicochemical properties of CS, SA and CS based MP. Size and PDI were determined by SEM analysis, and EE% was determined by UV, and thermogravimetric analysis of CS, SA and MP were determined by TGA; Peak temperatures (Tp) and final residual mass (M_r) for SA, CS and MP with different contents of SA.

Name	% SA amount (w/w respect to polymer)	Size (μm)	PDI	EE (%)	Tp(°C)	M_r800 °C (%)
CS	–	–	–	–	60.5 – 155.0 – 265.6	21.56
SA	–	–	–	–	189.9	0
MP-0	–	2.10 ± 0.78	0.14	–	53.8 –155.1 – 262.9	27.89
MP-1SA	1	1.99 ± 0.89	0.22	99	148.6–265.6	11.30
MP-5SA	5	2.45 ± 0.94	0.10	98	55.0 – 148.6 – 271.0	31.22
MP-10SA	10	2.05 ± 0.97	0.22	69	57.8 – 145.0 – 272.8	28.08
MP-20SA	20	1.57 ± 0.45	0.08	59	51.1 – 145.9 – 215.2 – 272.9	28.00