INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
17–1434/2018/20–5–1230–1234
DOI: 10.17957/IJAB/15.0598
http://www.fspublishers.org

Full Length Article

Antimicrobial Effects of Silver-Phyconanoparticles from *Sargassum vulgare* against Spoilage of Fresh Vegetables Caused by *Bacillus cereus*, *Fusarium solani* and *Alternaria alternata*

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Abstract

Fresh vegetables production in Mexico is adversely affected by fungal and bacterial diseases associated with *Fusarium solani*, *Alternaria alternata* and *Bacillus cereus* that are associated with postharvest decay and diarrheal syndrome. Hence, this study was conducted to investigate the synthesis and characterization of AgNPs using the aqueous extract of *Sargasssum vulgare*. Antimicrobial activities of biosynthesized AgNPs were evaluated against *B. cereus*, *F. solani* and *A. alternata* to demonstrate the bionanotechnological potentialities of these brown seaweeds. The UV–visible spectroscopic analysis showed the absorbance peaked at 460 nm, which indicated the synthesis of silver nanoparticles. The zeta potential of the biosynthesized AgNPs was found as a sharp peak at 27.5 mV with polydispersity index of 2.17 and polarity negative, while the mean particle size was 21.18 nm. The results of dual plate assays revealed that AgNPs showed broad spectrum antagonism ($p \leq 0.05$) against *F. solani* (70.9%) and *A. alternata* (55.05%) after nine days of incubation. On the other hand, different concentrations of AgNPs (25, 50, 75 and 100 mg/mL) showed antibacterial activity against *B. cereus* in comparison with the control after 24 h of incubation. Finally, further studies are required to confirm their potential of AgNPs from *S. vulgare* in the control of the *F. solani*, *A. alternata* and *B. cereus* under field conditions.

Keywords: Phycosynthesis; Silver nanoparticles; Seaweed; Antimicrobial activity

Introduction

In the actuality spoilage of fresh vegetables caused by fungal and bacteria diseases are a limiting factor in their production, and they are one of the main causes of the reduction of the planting area in Mexico and others countries (Fraire-Cordero *et al*., 2010; Al-Najada and Gherbawy, 2015). *Fusarium solani* and *Alternaria alternata* are common pathogens of a wide range of plants pre- and post-harvest. These phytopathogens cause postharvest decay, damping-off symptoms, and reduction in size of leaves and fruits, which affect a wide variety of vegetables causing devastating losses (You *et al.*, 2005; Lee *et al*., 2015). Additionally, wide varieties of vegetables have often served as vehicles of *B. cereus* that is associated with emetic and diarrheal syndrome (Tournas, 2005; Ceuppens *et al*., 2011). Several studies have reported the use of different control measures for the control of both diseases in fresh vegetables these measures include the application of chemical control (Al-Najada and Gherbawy, 2015; Pérez-Hernández *et al*., 2017). However, drawbacks such as promotion of tolerance and environmental toxicity associated with these chemicals have stimulated the search of different options (Shuping and Eloff, 2017).

Currently green nanotechnology has great importance due to the presence of inhibitory action against different fungal species (Anasane *et al*., 2016). In recent years, the exploration of plants as source of bioactive compounds for reduction of silver nanoparticles has drawn attention, due to the elimination of harmful reagents and effective synthesis of expected products through an economical method (Baharara *et al*., 2014; Arrieta *et al*., 2017). *Sargassum C.*

To cite this paper: González-Mendoza, D., B. Valdez-Salas, M. Carrillo-Beltran, S. Castro- López, V. Méndez-Trujillo, F. Gutiérrez-Miceli, L. Rodrígez-Hernández, D. Duran-Hernandez and N. Arce-Vazquez, 2018. Antimicrobial effects of silver-phyconanoparticles from *Sargassun vulgare* against spoilage of fresh vegetables caused by *Bacillus cereus, Fusarium solani* and *Alternaria alternata*. *Int. J. Agric. Biol*., 20: 1230–1234

Aagardh (Phaeophyceae, Fucales) is a frequent macroscopic alga of the marine sublittoral zone of tropical and plays a fundamental role in the life cycle of the associated fauna and other algae (Széchy *et al*., 2006; Camacho and Hernandez-Carmona, 2012).

Seaweeds as *S. vulgare* have been the focus of scientific interest mainly because of their strong antitumor and antiviral activity (Sousa *et al*., 2008; Plouguerné *et al*., 2013), making this seaweed an interesting alternative for possible nano-biotechnological applications. Therefore the aim of the present study was evaluated the antimicrobial potential of silver nanoparticles obtained from *S. vulgare* to demonstrate the bionanotechnological potentialities of these brown seaweeds in the biocontrol of *B. cereus*, *F. solani* and *A. alternata*.

Materials and Methods

Biosynthesis of Silver Nanoparticles (AgNP) from *Sargasssum vulgare*

Thalli of *S. vulgare* were collected by free diving in the shallow subtidal zone from Ensenada Bay, Baja California, Mexico. The *S. vulgare* extract solution was prepared according to Plouguerné *et al*. (2013) using 30 g of biomass that had been rinsed with deionized water and cut into small pieces. Then *S. vulgare* biomass were blended with 300 mL of H_2O and heated at 60°C for 30 min. The samples were then cooled at room temperature and centrifuged at 4000 rpm for 20 min to remove particulate matter and to get clear solutions, which were store under refrigeration at 4°C until use.

The green production of Ag-nanoparticles was obtained by the reduction of AgN03 (40 mL) using *S. vulgare* extract (10 mL) as reducing and capping agent. The solution was stirred at 60°C for 15 min in an Erlenmeyer flask. The color of the reaction mixture was gradually changed from pale yellow to brown, which indicates the formation of silver nanoparticles. Finally, the AgNPs were purified by centrifugation at 10,000 rpm for 10 min to remove excess silver ions and transferred to freeze dryer (powder obtain was employed in the antimicrobial assays).

Characterization of AgNPs

The formation of AgNPs from *S. vulgare* was register trough spectral analysis (UV visible), between wavelengths of 400–500 nm in a spectrophotometer (DR6000™ UV VIS Spectrophotometer).

Zeta Potential and Dynamic Light Scattering (DLS)

The hydrodynamic sizes and the Zeta potential of biosynthesized AgNPs in solution were analyzed using a Nanotrac Wave instrument (Microtrac) according to proposed by Abdelmoteleb *et al*. (2017). Three milliliter of solution was placed in zeta cell for measured zeta potential and DLS at 25°C, at 780 nm with a scattering angle of 90°. The data obtain were examined using Microtrac software.

Effect of AgNPs against Phytopathogenic Fungi

Antifungal activity of AgNPs was performed by dual culture technique in individual culture plates. The plates were divided into two quadrants; the first quadrant was prepared with 15 mL of PDA-AgNPs and the second quadrants were only prepared with PDA medium. After an agar disc (6 mm) was taken from 4-day-old PDA culture plates of each fungus (*F. solani* and *A. alternata*) and placed at the periphery of the PDA-AgNPs plates. Another agar disc of the same size of each fungus was also placed at the periphery but on the opposing end of the same Petri dish (PDA with only *S. vulgare* extract, control). After 9 days of incubation at 27°C, the zone of inhibition of was recorded.

Antimicrobial Activity of AgNPs against *Bacillus Cereus*

The antimicrobial activity of AgNPs was evaluated on *B. cereus* according to proposed methodology by Bao *et al.* (2011). The impregnated disks with different concentrations from AgNPs- *S. vulgare* (25, 50, 75 and 100%) were placed on the agar surface, and the plates were incubated at 34°C for a day, after which time the zones of inhibition were determined. Control plate (discs with only *S. vulgare* extract) was separately prepared.

Statistical Analysis

Differences between the treatments were evaluated using one-way analyses of variance and the Tukey's test ($p \leq 0.05$), and SAS Version 9.0 (SAS Institute, 2002) was used.

Results

Biosynthesis of AgNPs- *Sargasssum vulgare*

The Fig. 1 shows the solution of silver nitrate change yellowish brown to colloidal brown after adding the *S. vulgare* extract as result of formation of silver nanoparticles. Later thus formation of AgNPs were followed and characterized by UV-Visible spectroscopy (Fig. 2) and the surface plasmon resonance of the AgNPs was centered at approximately 460 nm.

Zeta Potential and Dynamic Light Scattering (DLS)

The average hydrodynamic size and zeta potential of the AgNPs was determined by the DLS as shown in Fig. 3. DLS analysis gave two different peaks and these suggest that DLS measurement may not be accurate for polydisperse samples due to its nature to respond toward larger particles. On the other hand, zeta potential of the biosynthesized AgNPs was found as a sharp peak at 27.5 mV with

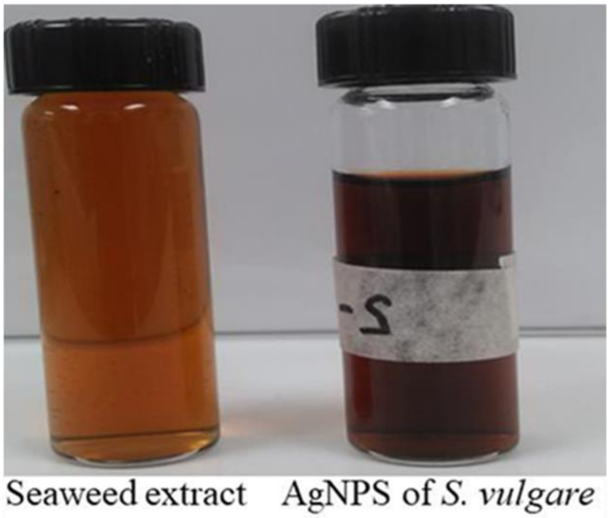

Fig. 1: Synthesis of silver nanoparticles using seaweed extracts of *S. vulgare*

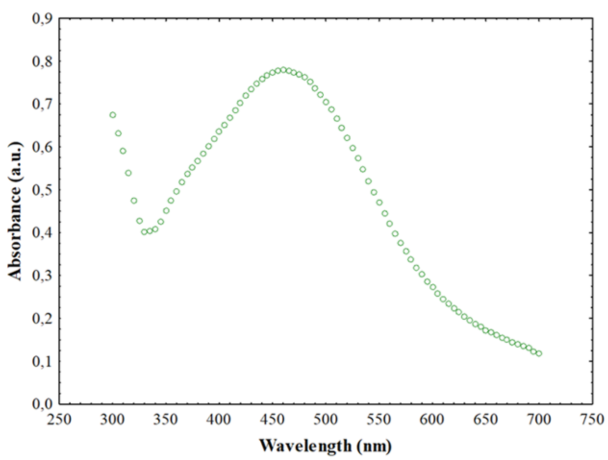

Fig. 2: UV-vis spectra of the synthesized silver nanoparticles

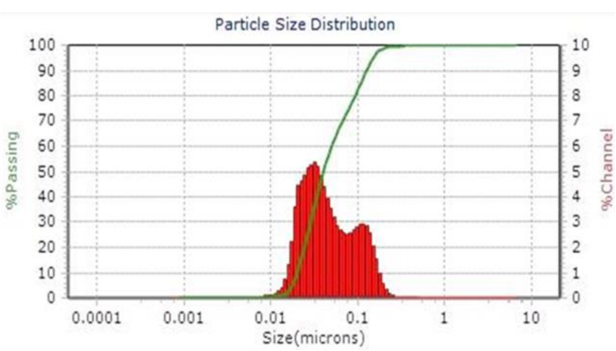

Fig. 3: Particle size distribution of silver nanoparticles from dynamic light scattering measurements

polydispersity index of 2.17 and polarity negative, while the mean particle size was 21.18 nm.

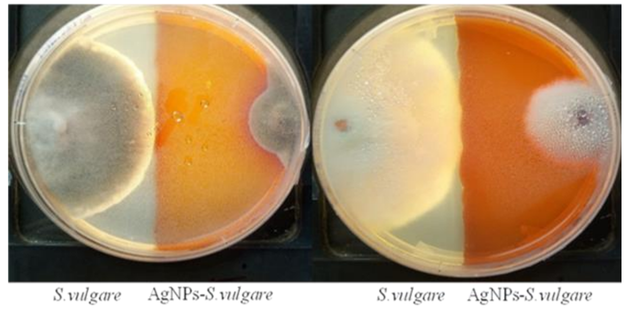

Fig. 4: Inhibition of the growth of *Fusarium solani* (a) and *Alternaria alternata* (b) by AgNPs-PDA and PDA-*S. vulgare* extract (control) after 9 days of exposure

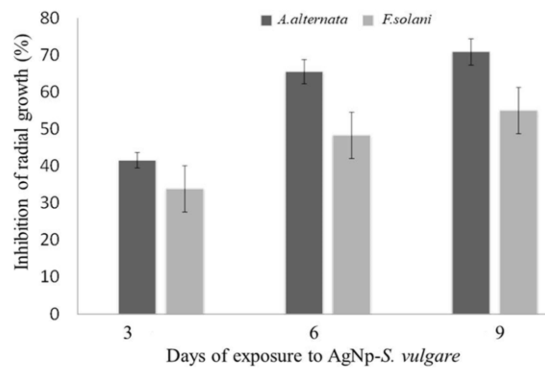

Fig. 5: Antifungal activity of AgNPs from *S. vulgare* against *Fusarium solani* and *Alternaria alternata* after 3, 6 and 9 days of treatment

Antifungal Activity of Synthesized Silver Nanoparticles

The results of dual plate assays revealed that AgNPs from *S. vulgare* had antagonistic effect on *F. solani*, and *A. alternata* after 9 days incubation (Fig. 4). The AgNPs showed broad spectrum antagonism ($p \leq 0.05$) against *F. solani* (70.9%) and *A. alternata* (55.05%) when compared with the control after nine days of incubation (Fig. 5).

Antimicrobial Activity of Synthesized Silver Nanoparticles

To investigate the antibacterial activity of the synthesized AgNPs from *S. vulgare* against *B. cereus*, different concentration of AgNPs were used. In this aspect, our results showed that the antibacterial activity of disks prepared with the different concentration of AgNPs showed an important inhibition against *B. cereus* in comparison at the impregnated disk with only *S. vulgare* extract (control) (Fig. 6). However, the disks prepared with AgNPs (75 and 100 mg/mL) doses was more effective in comparison with the control after 24 h of incubation (Table 1).

Table 1: Inhibition of *Bacillus cereus* by AgNPs-*S. vulgare*

Treatments	Zone of inhibition (mm)			
	25%	50%	75%	100%
AgNPs from *S. vulgare*	10.06 ± 0.10[a]	10.06 ± 0.15[a]	10.53 ± 0.05[b]	10.83 ± 0.28[b]
S. vulgare extracts (control)	NA	NA	NA	NA

Results are expressed as mean ± standard deviation of values from triplicate experiments. Values with the same letter (a or b) within each line are equal according to the Tukey test at P≤0.5. NA: No antibacterial activity was found with the concentrations used in this experiment

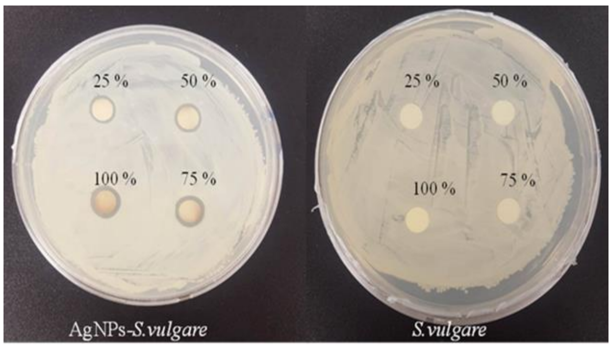

Fig. 6: Inhibition of the growth of *Bacillus cereus* by AgNPs- *S. vulgare* and *S. vulgare* extract after 24 h of exposure

Discussion

In the present study the phycosynthesis of silver nanoparticles from *S. vulgare* showed reddish brown color in aqueous solution as results of bio-reduction mechanism of metal nanoparticle in seaweed extracts. Though the mechanism of reduction of silver ions by the *S. vulgare* extract under study is not known yet, some studies suggests that this species can produce phenolic compounds, and sulfated polysaccharide that may be involved in the reduction and stabilisation process (Dore *et al*., 2013; Namvar *et al*., 2013). Therefore, the metabolites presents in extract of *S. vulgare* possibly will act as reducing and stabilizing agents for AgNPs synthesis (Prasad *et al*., 2013). Antimicrobial activity of silver nanoparticles from S. vulgare can be identified by inhibition zone formation when compared to seaweed extracts alone which does not show any inhibition zone (Fig. 3 and 4). The antimicrobial action mode of AgNPs against fungus and bacteria's is not yet fully explored. In this sense, certain authors reported that the antifungal activity of AgNPs results of free radicals which could cause disruption in lipids membrane of fungus cells causing it death (Dakal *et al*., 2016). The mechanism used by AgNPs-*S. vulgare* to cause bactericidal effect is not clearly known and is a debated topic. One possibility of growth-restriction may be a chance of the generation of free radicals by AgNPs positioned at surface, which may have been thrashed lipid membrane followed by destruction of microorganisms (Abdelmoteleb *et al*., 2017). Another possibility is that the interaction of the silver nanoparticles with the sulfur and phosphorus of the DNA can lead to problems in the DNA replication of the bacteria and thus terminate the microbes (Patra and Baek, 2017). In this study the zeta potential value of solution of AgNPs was evaluated to be 27.5 mV. It is suggested that the surface of the nanoparticles is negative charged indicating more stability and thus stability is probably due to the electrostatic repulsion between nanoparticles induced by the sulfated polysaccharide and deprotonated carboxylic groups on the surface of the nanoparticles (Cunha and Grenha, 2016; Kalliola *et al*., 2017). Today synthesizing metal nanoparticles using seaweed has been poorly explored although has been recognized as a green and efficient way for further exploiting these organism as convenient nanofactories (Satapathy *et al*., 2017). Therefore, further studies are required to confirm their potential of AgNPs from *S. vulgare* in the control of the *F. solani*, *A. alternata* and *B. cereus* under field conditions.

Conclusion

The present investigation concludes that AgNPs from *S. vulgare* is potential biocontrol agent against phyto-pathogens. It showed antimicrobial effects on *F. solani*, *A. alternata* and *B. cereus* in vitro. Though, evaluations in the field are necessary to know the biotechnological potential of AgNPs-*S. vulgare*.

Acknowledgements

The authors would like to thank Secretaria de Fomento Agropecuario de Baja California, Mexico (SEFOA) for their persistent encouragement and supports

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(Received 18 December 2017; Accepted 04 January 2018)