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Biofabrication of silver nanoparticles from *Pseudomonas fluorescens* to control tobacco mosaic virus

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Abstract

A microbe-based silver nanoparticle could inhibit the tobacco mosaic virus. In the present study, $AgNO_3$ reacts with *Pseudomonas fluorescens* CZ strain fermentative broth and formed silver nanoparticles. The crystallinity and purity of silver nanoparticles (AgNPs) were examined by an X-ray diffractometer. Number of Bragg reflection was indexed in the face-centered AgNPs to show a cubic structure. Observation of scan electron microscope (SEM) confirmed the formation of AgNPs. Antiviral effects determined by percentage inhibition of local lesion of tobacco mosaic virus (TMV). Spray of AgNPs and inoculation with TMV assayed analysis revealed percentage inhibition of local lesion 92.70. Effects of AgNPs on the morphology of TMV were observed by a transmission electron microscope (TEM). Micrograph images confirmed the significant effects on the morphology of TMV. Biogenic NP from $AgNO_3$ and fermented broth from *Pseudomonas fluorescens* could be a potent approach to control the TMV.

Keywords: Silver nanoparticles, Tobacco mosaic virus, X-ray diffractometer, Transmission electron microscope, *Pseudomonas fluorescens*

Background

Understanding of the virus disease outbreak has an emerging influence on horticultural crops and as well as on natural flora as climate alteration (Jones, 2016). There is an alarming situation that plant-associated viruses could affect the production and quality of the crops, while it is challenging task to manage plant viruses (Worrall et al. 2019). Tobacco mosaic virus (TMV) affects 125 species of important horticulture plants, such as tomato, tobacco, cucumber, and pepper (Islam et al., 2018). There are several approaches in practice to combat plant viruses, like the application of nanoparticles to detect and manage the plant viral diseases. Possibly fabrication of nanomaterials could be framed to deliver the genetic makeup, and as fertilizer and biosensors for plant disease detection. From decades, the applications of nanoparticles (NPs) in crop disease management have evolved significantly (Elmer and White, 2018). Several nanoparticles manufactured by the addition of harmful chemicals, which involved the low conversion of composition and high demand for energy. There is an urgent need to replace hazardous chemicals by developing ecofriendly nanoparticles. Biogenic approaches utilize synthesized nanomaterial from microbes and as well as from the plants (Huang et al. 2007). The application of nanomaterial with biocontrol antagonists could be more significant (Mallaiah, 2015). Several fungal- and soil-borne plant diseases were controlled by silver nanoparticles (Cromwell et al., 2014). Only a few reports of antiviral nanoparticles were investigated by using the nano-clay particles (Mitter et al. 2017), while recently several studies reported that bacterial species are biological control agents against

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TMV, such as *Pseudomonas fluorescens* CZ (Shen et al. 2017). By keeping in view the above studies, the present study was carried out by manufacturing nanomaterial from strain *P. fluorescens* with silver nitrate to combat TMV.

Material and methods

Strain *P. fluorescens* was supplied by College of Plant Protection, Shenyang Agricultural University, China. The strain was identified and reported as a potent antagonistic agent against TMV (Shen et al., 2017). Submerged fermentation broth was produced and centrifuged it. Broth was used for antimicrobial activity. Make 30% dilution of the active fraction with distilled water. *Bacillus subtilis* was used as a test pathogen. Silver nitrate and extracted broth from *P. fluorescens* strain were mixed. The reaction was set up by a mix of 90 ml of $AgNO_3$ with 10 ml of bioactive substance at 80 c by keeping at 5 pH and stirring it for 2 h and then put it over 1 day. The upper layer was removed by centrifuge to get rid of the impurities. Separated substance further dried at 80 c for 12 h and then crushed into a fine powder, and finally, at 700 c for 3 h calcinated to remove all the impurities (EL-Moslamy Shahira, 2018). X-ray diffractometer was used to calculate the diameter of nanoparticles. SEM analysis was done to observe the shape of the synthesized NPS. TMV obtained from the Lab of Shenyang Agricultural University, Shenyang, China. The viral inoculum was maintained on infected tobacco plants. Humidity and temperature were maintained. *Nicotiana glutinosa* was transplanted into a small pot in the greenhouse after seedling cultivation, and the same growth of tobacco seedlings was selected for reserve in 5–6 leaf stage.

1) Silver nanoparticles (AgNPs) (100 μl) treatment was given 24 h before TMV inoculation on the leaf.
2) Mix the TMV supernatant with 100 μl AgNPs for 10 min and rub the leaf with the mixture
3) Inoculate the plant leaf with TMV and after 24 h treated with AgNPs

Five seedlings per treatment were inoculated with 3 leaves per seedling. After the onset, the number of the lesions was investigated and the inhibition rate of the lesions was calculated. To observe the effects of AgNPs and TMV, supernatant was sprayed. For control, TMV was mixed with a 1:1 ratio with distilled water. After 36 h, it was photographed. Later, the same plant sample was observed by using the transmission electron microscope (TEM), to determine the effects of nanoparticle on the morphology of TMV.

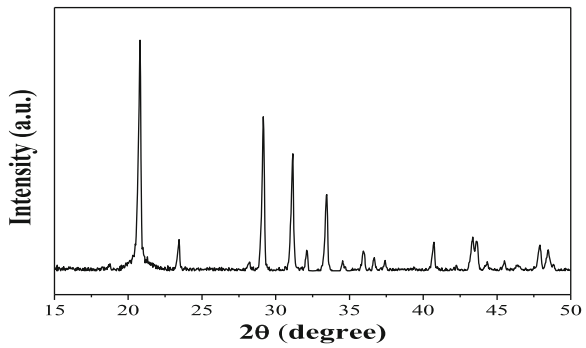

Fig. 1 X-ray diffractometer analysis of AgNPs that biofabricated by *Pseudomonas fluorescens*

Results and discussion

In the present study, *P. fluorescens* and $AgNO_3$ was used to synthesize AgNPs. This strain was previously reported as a potent antagonist against TMV (Shen et al. 2017). The fermented broth was bioassay against the (test pathogen) *Bacillus subtilis*. Strain *P. fluorescens* had a strong antibacterial activity and indicated that this strain had a potent efficacy against the microbes. So it could be an efficient agent to produce antiviral nanoparticle with $AgNO_3$. Several microbes, such as fungus, yeast, and bacteria, have applications in the synthesis of silver- and gold-based nanoparticles (Huang et al. 2007). Crystallinity and purity of NPs were examined by X-ray diffraction (XRD). The XRD pattern of AgNPs was face-centered cubic (Fig. 1). Diffraction peaks at 2 theta Bragg reflection, which indicated the crystalline nature of the nanoparticles. Sharpness of peaks indicated the purity of the synthesized NPs. Biological synthesis of silver nanoparticles (AgNPs) from the *Bacillus brevis* (NCIM 2533) had crystalline nature (Saravanan et al. 2018). The result is supported by the

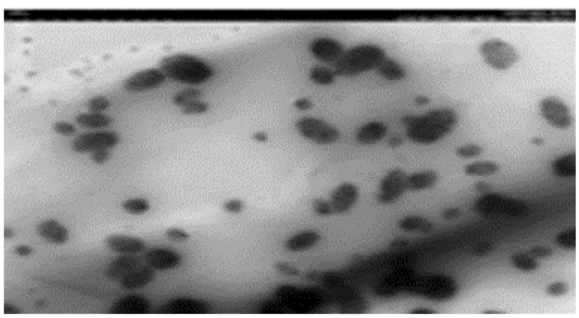

Fig. 2 Scan electron microscopic analysis of AgNPs that are biofabricated by *Pseudomonas fluorescens*

Fig. 3 Control effect of the AgNPs on *Nicotiana glutinosa*, (**a**) represented the control. Inoculated only with TMV broth, while (**b**) treated with AgNPs and inoculated with TMV. Control showed maximum lesion but the treated plant showed maximum reduction of TMV

argument of SEM observation indicated that there were slight variations in the size of AgNPs. The shape of the NPS was spherical and irregular in pattern (Fig. 2). The black dot indicated the capping of the materials. Size of the AgNPs could be between 10 and 100 nm. Silver nanoparticles mostly have a size range from 8 to 100 nm (Ahmed et al. 2016). The physiochemical characteristics of nanoparticle significantly influence toxicity and efficacy. AgNPs was assayed against TMV. Results showed that NPS could control TMV. Inhibition percentage on the lesion, in 24h before inoculation treated the sample, was 86.54%, while the sample treated and inoculated at the same time had more potent inhibitive effects. Before it was introduced to the plant, it was mixed for 10 min. The inhibition rate of the lesion was 92.70% and post 24-h inoculation treatment had 41.43% inhibition effect. The results showed that the NPS had good inhibition effects on TMV. Microbe-based nanoparticle have good antimicrobial affects (Saravanan et al. 2018). In control figure, (Ck) showed lesion on the leaf, while treatment with NPs reduced the lesion on the leaf (Fig. 3). The results showed that after 30 min of mixing the purified virus sap with 20 times diluted solution of extract, it changed the morphology of virus particles and changed significantly than the control, indicating that extract had certain destructive effects on virus particles (Fig. 4). Electron microscopic analysis revealed morphological abnormalities in the treated sample. While in the control sample, there were non-significant changes that occurred. Silver-based nanomaterials are stated as antimicrobial substances since the last 10 years (Divya et al. 2019). So the overall results indicated that AgNPs had potent effects on TMV. According to our knowledge, this is the first report to control RNA-based viruses by the microbe-based silver nanoparticle. A detailed study should be brought up by using PCR or ELISA to make a strong evidence of action of AgNPs from *P. fluorescens*.

Conclusion

Biological fabrication of AgNPs was carried out by mixing extracts of *P. fluorescens* and silver nitrate. Characterization of NP indicated a balanced and cost-effective natural substance. Antiviral activity of AgNPs showed potent effects against RNA-based plant virus. This finding could help to meet the emerging need of environment-friendly compounds for the disease management of the crop.

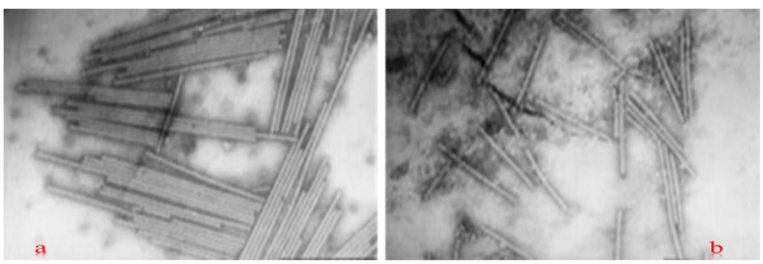

Fig. 4 Analysis of transmission electron microscope by the activity of AgNPs on morphology of TMV, **a** controlled and **b** treated with AgNPs. In the control, morphology of TMV is smooth and normal, while in the treated sample, the morphology of viroid is irregular

Abbreviations
AgNPs: Silver nanoparticles; SEM: Scan electron microscope; TMV: Tobacco mosaic virus; TEM: Transmission electron microscope; NPs: Nanoparticles

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Author's contributions
TA designed the study, did the analysis, and wrote the manuscript. The author read and approved the final manuscript.

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