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Characterization, antimicrobial and antioxidant property of exopolysaccharide mediated silver nanoparticles synthesized by *Streptomyces violaceus* MM72

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ABSTRACT

Green synthesis of silver nanoparticle (SNPs) has gained considerable attention in nano-biotechnology and nano-medicine owing their potency and eco-friendliness. In this, we account the exopolysaccharide and its mediated synthesis of SNPs and their biological activities. Exopolysaccharide of the *Streptomyces violaceus* composed of total carbohydrate (61.4%), ash (16.1%), moisture content (1.8%) and NMR confirmed their structural composition. SNPs synthesized by the exopolysaccharide, confirmed using UV–vis spectral analysis and characterized by TEM and XRD analyses. Further, the SNPs evaluated for its antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis* using disc diffusion method. The SNPs has shown promising antibacterial activity were evaluated for MIC. Furthermore, the SNPs were tested for antioxidant activities and was found to have promising antioxidant activity over the standards. The above results prove that SNPs can be considered as a potent antibacterial and antioxidant drug in future.

1. Introduction

Polysaccharides of marine origin are the most important components of marine microorganisms, algae, and crustaceans (Raveendran, Poulose, Yoshida, Maekawa, & Kumar, 2013). There has been a great interest in applications of in biomedical engineering and nano-biotechnology (Raveendran, Yoshida, Maekawa, & Kumar, 2013). Desirable characteristics of the polysaccharides of marine origin and mostly hydrophilic forms which are stable, non-toxic, safe, are readily biodegradable and easily reproducible (Lee, Park, & Robinson, 2000). Marine polysaccharides, especially microbial exopolysaccharides (EPS) are highly complex bio-polymers which contain proteins, lipids, and nucleic acids along with polysaccharides (Laurienzo, 2010). Nowadays, polysaccharide mediated synthesis of nanoparticles has gained importance since they are highly biodegradable, biocompatible, cheaper to produce and non-toxic (Raveendran, Poulose et al., 2013). The polysaccharide-based nanoparticles are routinely used in tissue engineering, cancer treatment, drug delivery as a drug carrier, wound dressing, waste water treatment etc. (Jayakumar, Chennazhi, Nair, Furuike, & Tamura, 2010).

In this context, marine actinobacteria deserve a special mention as they seamlessly produce novel biologically active metabolites, such as immunosuppressant, anti-prolific, anti- oxidant, and anti-HIV substances (Chater, 1993) which are commercially used for various treatments (Manivasagan, Venkatesan, Sivakumar, & Kim, 2014). Among them, *Streptomyces* contributes > 50-60% of the marketed antibiotics (Manivasagan et al., 2014). Hence, the utilization of marine actinobacteria have been gaining in the nano-biotechnology in recent times, and the actinobacterial synthesized nanoparticles are eco-friendly, cheaper to produce and can be used for various nano biomaterial production (Manivasagan, Venkatesan, Senthilkumar, Sivakumar, & Kim, 2013). Keeping importance of marine actinobacteria and their biologically active metabolites in mind, in the present study, to the synthesis of silver nanoparticles by exopolysaccharide from *Streptomyces violaceus* and evaluate their antibacterial and antioxidant activities.

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2. Materials and methods

2.1. Isolation and identification of strain

The *Streptomyces violaceus* MM72 was isolated from the marine coastal sediment of Tuticorin, Southeast coast of India. Isolation was done on Zobell marine agar plates (10% NaCl). The strain was identified based on morphological and biochemical characteristics according to the Bergey's manual of determinative bacteriology and molecular identification by Manivasagan et al. (2013).

2.2. Production of EPS

EPS production was carried out according to the method of Manivasagan et al. (2013). The strain was inoculated in the production medium g/L (10 g glucose, 5 g tryptone, 5 g yeast extract, 3 g NaCl, 3 g K_2HPO_4, 1 g KH_2PO_4, 0.5 g $MgSO_4$, 0.5 g $CaCO_3$, pH 7.0) and incubated for one week at 37 °C in a rotary shaker at 200 rpm. After the incubation, broth culture was centrifuged at 10,000 rpm for 10 min and the supernatant was collected.

2.3. Extraction of EPS

The EPS extraction was done followed by the method of Sun et al. (2016). The supernatant was dialyzed against distilled water and the collected supernatant was precipitated by adding ice-cold acetone at overnight incubation at 20 °C. The precipitate was centrifuged at 5000 rpm for 15mins and obtain the EPS. The EPS was further dissolved in distilled water and adding ethanol for precipitation again centrifugation at same and finally washed with acetone to obtained EPS for further study.

2.4. Chemical characterization of EPS

2.4.1. Determination of total carbohydrate

The total carbohydrate content was estimated calorimetrically by the phenol-sulfuric acid method using d-glucose as a standard (Dubois, Gilles, Hamilton, Rebers and Smith 1956).

2.4.2. Determination of ash and moisture content

The ash and moisture content of exopolysaccharide was quantified gravimetrically according to the method of Seedevi, Moovendhan, Vairamani and Shanmugam (2016). 0.5 g of the dried polysaccharides taken in a porcelain crucible was burnt at 600 °C for 8 h in a muffle furnace. The weight of the residue, which represented the ash content and the results were given as a percentage. The moisture content of exopolysaccharide was determined through the micro-oven. 0.5 mg of sample was dried 130 °C for 2 h and the results were given as a percentage.

2.4.3. ^1H NMR analysis

^1H NMR spectrum for the EPS of *Streptomyces violaceus* MM72 was performed following the method of Seedevi, Moovendhan, Vairamani and Shanmugam (2017). Approximately 30 mg of sample was dissolved in 0.5 ml of D_2O (99.9%) in an NMR tube (5 mm diameter). The ^1H NMR spectra were taken at 27 °C and the chemical shift was expressed in parts per million (ppm).

2.5. Synthesis of silver nanoparticle

0.5 g of EPS was dissolved in 25 ml of distilled water and slowly added into 3 mM aqueous $AgNO_3$ solution with gentle stirring. The solution was stored in a dark place on a rotary shaker (120 rpm) for 1 h. During the reaction, the clear colorless solution turned to dark brown which indicated the formation of SNPs.

2.5.1. Conformation of silver nanoparticle through UV-spectral analysis

The absorbance of the reduced silver ions was measured periodically at 15 min intervals and were observed in UV–vis spectrophotometer (Evolution 201, Thermo Fisher Scientific, Waltham, MA, USA) to monitor the reaction rate (El-Rafie, El-Rafie, & Zahran, 2013).

2.5.2. Purification of silver nanoparticle

The synthesized silver nanoparticles were purified by dialysis and lyophilized (Manivasagan, Kang, Kim, & Kim, 2015).

2.5.3. Characterization of silver nanoparticle

The SNPs was analyzed for Transmission Electron Microscopy (TEM) imaging using JEOL-3010 (Voltage–300 kV). The SNPs was spotted on copper grids with carbon tape followed by dehydration at room temperature. Additionally, SNPs was analyzed in energy dispersive X-ray analysis (EDX) to confirm the presence of the silver nanoparticles in the sample. The SNPs was analyzed in XRD by using 30–80 thetas diffraction intensities at the angle of 2 theta (Pourali and Yahyaei, 2016).

2.6. Antibacterial activity

2.6.1. Disc diffusion assay

The SNPs was evaluated for the antibacterial activity by using disc diffusion method Nakkala, Mata, Gupta and Sadras (2014). Four bacterial pathogens viz. *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis* were procured from the Department of Microbiology, Rajah Muthiah Medical College Hospital (RMMCH), Annamalai University and used for the study. The 24 h broth culture of these pathogens was spread plated on nutrient agar and 100 µg/ml of SNPs impregnated filter paper discs (5 mm) were placed and incubated for 24 h at 28 °C. Standard Tetracycline discs were used as positive control. The antibacterial activity of the SNPs was determined by measuring the zone of inhibition.

2.6.2. Determination of minimal inhibitory concentration (MIC)

The SNPs was determined the MIC following the standard microdilution method according to Clinical and Laboratory Standards Institute Guidelines (CLSI) (Clinical and Laboratory Standards Institute, 2014). A stock solution of 1 mg/ml was prepared and was serially diluted to obtain various concentrations between 5 µg − 100 µg/ml. 0.5 ml of each of the dilutions containing 2.0 ml of nutrient broth were taken in test tube and to each of which 0.5 ml of old bacterial culture was inoculated. The test tubes containing broth alone was used as control. All test tubes and control were incubated for 24 h at 28 °C. After the incubation, the bacterial growth was recorded by measuring the optical density at 600 nm using UV–vis spectrophotometer (Evolution 201, Thermo Fisher Scientific, Waltham, MA, USA).

2.6.3. Bacterial viability assessment

Viability of the bacterial cells was assessed following the method of Verma, Hasan and Banik (2016). Bacterial pathogens (*E. coli, P. aeruginosa, S. aureus* and *B. subtilis*) were inoculated in nutrient broth and incubated at 28 °C overnight. Then, the bacterial strains were again sub cultured in fresh nutrient broth for about 4 h. The bacterial cells were collected by centrifugation and suspended in saline (isotonic) solution to bring the cell concentration to 10^7 CFU/ml. Then the SNPs were added to the bacterial cell suspension at their equivalent MIC levels. After that, colony counting method was employed to assess the cell viability by spread plating the diluted sample (100 µl) on nutrient agar plates, and incubated at 28 °C for 24 h. A control plate was maintained devoid of SNPs. The sample plates were compared with control plates by counting the colonies to determine the viability percentage of the bacterial cells.