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Antibacterial Activity Of A Novel Silver Nanoparticle Mediated By A Marine Actinobacterium Isolated From Marine Sediments

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ABSTRACT

Present study was investigated the antimicrobial activity silver nanoparticles mediated by marine actinobacteria obtained from mangrove creeks of Andaman island. Totally 12 actinobacterial strains were screened for silver nanoparticle synthesis, in which, only one strain NMSA 7 showed a stable synthesis of silver nanoparticles was belongs to the genus Streptomyces sp. The AgNPs was characterized by UV-visible spectrophotometer, XRD analysis, FT-IR and SEM. SEM study of the synthesized AgNPs showed that the nanoparticles were spherical and polydisperse in nature and ranged in size from 3.57 nm to 27.42 nm with an average size of 6.56 nm, respectively. Biosynthesized AgNPs have demonstrated a good antimicrobial activity against the human pathogen Staphylococcus aureus strain. The higher inhibition zone (12 mm) was recorded at a concentration of 500 µl. Silver biosynthesis in accordance with actinobacteria against clinical pathogens will be an efficient approach compared to other chemotherapeutic applications.

Keywords: Streptomyces sp. Silver nanoparticles (AgNPs), Staphylococcus aureus and Antibacterial activity

INTRODUCTION

Owing to its wide-ranging application potential and economically friendly method to explore new pharmaceutical materials, nanotechnology has increased in prominence in the therapeutic field in recent days (Patil et al., 2018). In addition, nano-based medications have been the key driving force behind new advances in the drug delivery system and antibacterial treatment due to their small size and proven efficacy (Yeh et al., 2020). Due to the increasing need to establish environmentally sound technology for developing and testing nanomaterials, the biosynthesis of nanoparticles has been provided considerable importance (Kulkarni & Muddapur, 2014). In specific, nanoparticles with microorganisms were a fantastic initiative. Due to its versatility and eco-friendliness, biosynthesized nanoparticles have become more important in last years (Ovais et al., 2018).

Due to the use of moderate experimental temperature, pH, and pressure conditions, biological synthesis of these nanomaterials has become important (Mittal et al., 2014). AgNPs have gained substantial interest from all other NPs because of their wide variety of applications such as catalytic activity, antibacterial activities and anti-cancer behaviour (Hasan et al., 2018). A desirable and environmentally friendly alternative approach for preparation of larger quantity could be the extracellular biological synthesis of AgNPs

because it provides the benefit of fast recovery processing (Thakkar et al., 2010). Microorganisms are used for the bioproduction and synthesis of various nanometer-size compounds as an environmental-friendly nanofactory (Prasad et al., 2018). The nanoparticles are microbially synthesised and are notable in their bioactivity, as they show a stable antagonism to many emerging diseases (Busi & Rajkumari, 2019). Taking these advantages into account, microbial synthesis could prove an excellent alternative for AgNP synthesis extracellular (J. Singh et al., 2018).

The use of antimicrobials has improved and the pathogenic bacteria's resistance mechanism has also become more complex (Santajit & Indrawattana, 2016). In hospitals with compromised immune patients with a greater risk of infection acquired in the hospital, microbes may present a threat. Efforts to explore and apply nanotechnology are also required for the hour (Ordonez et al., 2019). The use of bacteria, fungi and plants has already been well-documented in biosynthetic silver nanoparticles (AgNPs) (Guo et al., 2015). In reality in recent years, AgNPs, actinobacteria from marine sediments were synthesised by a possible new antibiotic producer. AgNP was the most promising source for the very efficient destruction of microbes because both intracellular and extracellular (Möhler et al., 2018). Silver is used as a bacterial antibacterial agent, incorporated in wound dressing, to treat pathogenic bacteria and as a coating material for implant and medical equipment (Sivasankar et al., 2019). Marine actinobacteria will be the useful resource for experimental drug and medical metabolites (Santos et al., 2020).

MATERIALS AND METHOD

2.1 Collection and isolation of marine sediment actinobacteria

The marine sediments were collected from mangrove creeks of Andaman island using sterile container and were transported to the laboratory under cold condition. The collected sediment samples were air-dried to remove the unwanted bacterial forms. The sediment was serially diluted to 10 times and roughly 1 ml of the sample was applied to actinomycetes isolation agar (AIA) media complemented by cyclohexamide 50 µg/ml and nystatin 50 µg/ml. After inoculation, plates were incubated at 28℃ for 7-15 days (Claverías et al., 2015).

2.2 Silver nanoparticle synthesis and characterization

The chosen actinobacterial strain was inoculated in the yeast malt broth and incubated at 28 °C for 7 days. The cultured broth was centrifuged to remove cell debris at 5000 rpm between ten to 20 minutes. In 100 ml of $AgNO^3$ 1 mM solution, roughly 10 ml of cultured liquid was added and incubated into the dark shaker at room temperature (Deljou & Goudarzi, 2016). The synthesis of AgNPs from $AgNO^3$ was periodically monitored by UV/visible spectrophotometer (Arya et al., 2018). Synthesized AgNPs have been obtained at 15,000 rpm and are categorized by XRD, FT-IR, and SEM (Sivasankar et al., 2018; Zhang et al., 2016). Fourier transform spectroscopy (FTIR), has been observed for the functional group. Morphology and distribution of the particle size of AgNPs under the scanning electron microscope is examined (SEM) (P. Singh et al., 2015).

2.3 Assessment of AgNP's antimicrobial efficacy

Bacterial pathogen S. aureus was obtained from medical college repository and was inoculated in NB medium and incubated for 24 h at 37 ^{0}C. After incubation, test pathogen was inoculated on MHA using sterilized cotton swabs. Wells were cut using a sterile gel borer and 50 µl of AgNPs (different concentrations viz.100 µg, 200 µg, 300 µg, 400 µg and 500 µg) were poured into the wells. Inoculated plates were incubated at 37 ^{0}C for 24 h. After

incubation, the presence of the inhibition zone around the wells was checked for all plates (Feroze et al., 2020).

RESULTS AND DISCUSSION

3.1 Isolation and characterization of the silver nanoparticle synthesizing actinobacteria

A total of 12 actinobacterial strains were isolated from the samples of Andaman sediments in this analysis. All the 12 strains were screened for silver nanoparticle synthesis, in which, only one strain NMSA 7 showed a stable synthesis of silver nanoparticles. Hence, the strain NMSA 7 was further identified and selected for nanoparticle synthesis. The actinobacterial strain NMSA 7 was identified by morphological, physiological and chemotaxonomical methods. The strain was found to possess a creamy white coloured aerial mycelium with rectiflexible smooth spores (Fig. 1). In addition, the strain NMSA 7 was able to produce melanoid and soluble pigments in the test medium. Besides, the strain NMSA 7 was found to possess LL-DAP in their cell wall as cell wall amino-acid and do not possess any characteristic sugar pattern. Similarly, the physiological tests were also revealed that the strain NMSA 7 was able to utilize arabinose, xylose, mannitol, rhamnose and sucrose as the sole source of carbon for its growth and metabolism (Table 1).

Results of this analysis justifies that the actinobacterial strain NMSA 7 was belongs to the genus Streptomyces sp. Hence, based on the above findings, the strain was identified as Streptomyces sp. NMSA 7. In large part, the aquatic ecosystem is an untapped source of actinobacteria which can produce fresh, bioactive natural products (Ranjani et al., 2016). Actinobacteria make up approximately 70 percent of the compounds that actually have a natural derivative medicinal use and are the multiplicators of secondary pharmaceutically active metabolites (Palaniappan et al., 2013). Marine actinobacteria are exceptional in augmenting very diverse pharmacological activity, particularly anti - bacterial, anticarcinogenic, antiviral, larvicidal and enzyme inhibition activity (S. Alharbi, 2016).

3.2 Silver nanoparticle synthesis and characterization

The marine actinobacterium Streptomyces sp. NMSA 7 was found to be the suitable candidate for silver nanoparticle (AgNPs) synthesis. Actinobacterial metal nanoparticles synthesis involves finding the reduction of the cell components (Manivasagan et al., 2016). The biosynthesis of AgNPs was carried out using the cell free culture filtrate of the Streptomyces sp. NMSA 7. When the reductive processes of metal ions include cell-wall reduction enzymes or fermentable secreted cofactors, the metal nanoparticles are produced extracellularly. The reduction of silver nitrate to silver ions is light yellow to dark brown, suggesting the silver nanoparticles synthesis (Amooaghaie et al., 2015). The biosynthesized AgNPs was initially characterized by UV-visible spectrophotometer between the range of 300-800 nm. The sample absorbance was obtained at 422 nm which indicates the presence of AgNPs in the sample (Fig. 2a). Usually, the absorbance between 420-450 nm denotes the presence of consistent AgNPs (Chouhan et al., 2017).

XRD analysis of the Streptomyces sp. NMSA 7 nanoparticle sample showed the 2Ɵ values 111, 200, 220, 311 which represents the crystalline nature of the AgNPs present in the sample (fig. 2b). Roy et al. have documented the same findings and demonstrate that the silver nanoparticles are facial, cubic and crystalline in their existence (Roy et al., 2013). The extension of the peaks of Bragg along their cores suggests the progression of tiny nanoparticles of silver (Shankar & Rhim, 2015). The presence of certain bio-organic compounds/proteins on the surface of silver could contribute to a couple of unassigned peaks observed (Shanmugam et al., 2016).

FT-IR measurement of the sample revealed that 3462.78 corresponds to the OH stretched hydrogen bonds. In addition to that, a peak at 1637.50 was detected for secondary and primary amine group (N-H) (Fig. 3). This analysis revealed that the COOH functional group was found in the sample and was significantly correlates to the AgNPs. The amino acid residue and peptides carbonyl groups, the free amine or cysteine groups in protein have the capacity to bind to the silver ions (Balaji et al., 2009).

SEM analysis of the synthesized AgNPs revealed that the nanoparticles were spherical and polydisperse in nature and their size was ranging from 3.57 nm- 27.42 nm with an average size of 6.56 nm (Fig. 4a & b). The biomolecules present on nanoparticles' surface create a structure of agglomeration (Auría-Soro et al., 2019). Bacillus licheniformis mediated AgNPs reported similar findings (Kalimuthu et al., 2008).The SEM analysis revealed that the Streptomyces sp. NMSA 7 mediated AgNPs were relatively small and might possess great penetration potential on the bacterial cells. This smaller in size will pave a way in developing much efficient nano based drug delivery system (Rizvi & Saleh, 2018).

3.3 Antimicrobial potential of AgNPs

In the present study, the biosynthesized silver nanoparticles were tested for antimicrobial activity against clinical pathogen Staphylococcus aureus strain. The results revealed that the actinobacteria mediated AgNPs possess good antimicrobial potential. The higher zone of inhibition 12 mm was recorded at 500 µl concentration followed by 10 mm at 400 µl concentration (Fig. 4c). The least concentrations though have rare or nil zone of inhibition. As stated in the characterization part, the size of the nanoparticle is very smaller, and hence the potential inhibition against the Staphylococcus aureus could be due to the smaller size. In health, medicine and environmental uses, silver nanoparticles are commonly utilized (Ahmed et al., 2016). A positive current on the Ag+ ions is attributable primarily to the function by which silver nanoparticles exhibit antibacterial action (Siddiqi et al., 2018). This is due to an electrostatic attraction in microorganism cell membranes between positive charges for silver nanoparticles and negative charges

1. Conclusion

In the present analysis, a total of 12 actinobacterial strains were isolated and the strains were screened for silver nanoparticle synthesis, in which only one strain NMSA 7 displayed a stable silver nanoparticle synthesis belonging to the genus Streptomyces sp. The biosynthesized AgNPs was initially characterized by UV-visible spectrophotometer and the absorbance was obtained at 422 nm which indicates the presence of AgNPs in the sample. The XRD study of the Streptomyces sp. NMSA 7 nanoparticle sample revealed 2Ɵ values 111, 200, 220, 311, reflecting the crystalline structure of the AgNPs present in the sample. FT-IR measurement of the sample revealed the presence of OH stretched hydrogen bonds and amine groups corresponding to silver nanoparticles. SEM study of the synthesized AgNPs showed that the nanoparticles were spherical and polydisperse in nature and ranged in size from 3.57 nm to 27.42 nm with an average size of 6.56 nm, respectively. Biosynthesized AgNPs have demonstrated a good antimicrobial activity against the human pathogen Staphylococcus aureus strain. The higher inhibition zone of 12 mm was recorded at a concentration of 500 µl. The study concludes that the strain Streptomyces sp. NMSA 7 was found to be a good source for the synthesis of biologically active silver nano leads and thus paved the way to the development of eco-friendly nanomedicine.

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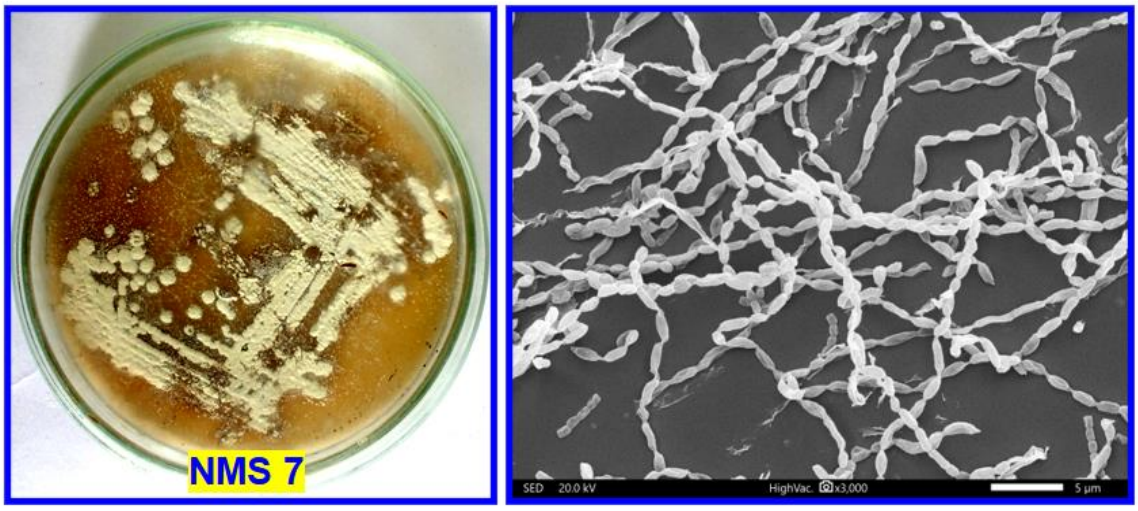

Fig. 1. Displays the cultural morphology and spore chain morphology of marine actinobacterium Streptomyces sp. NMSA 7

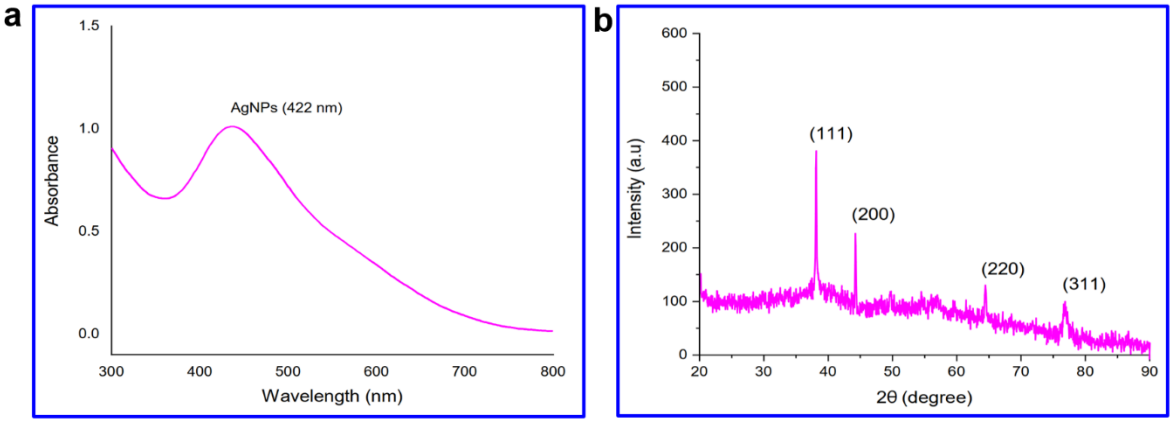

Figure 2. Represents the UV-visible absorbance (a) and XRD analysis (b) of the AgNPs synthesized by Streptomyces sp. NMSA 7

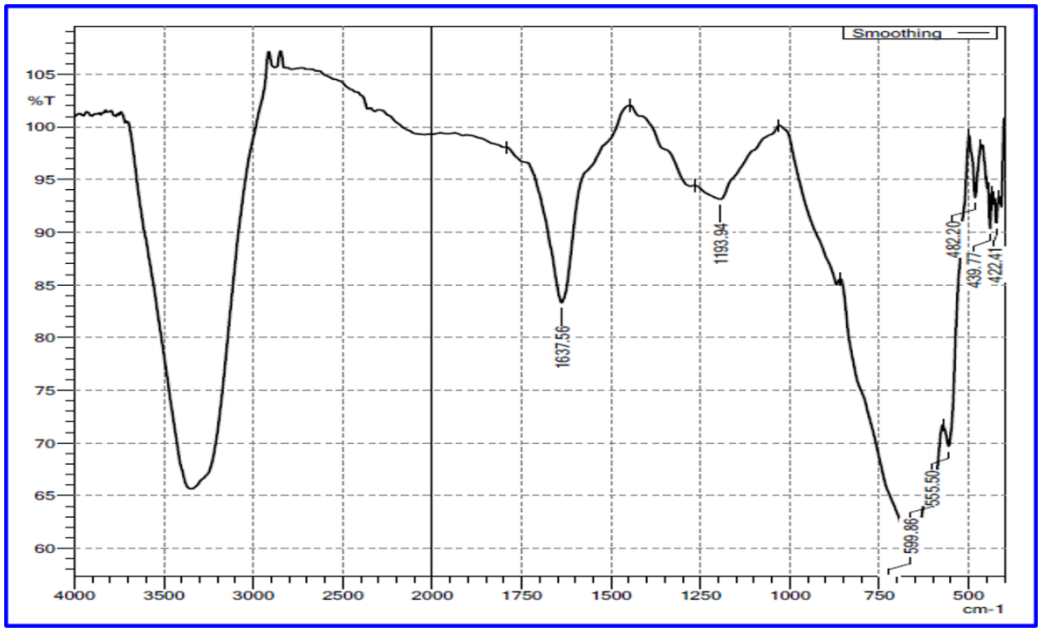

Figure 3. Displays the FT-IR analysis of the AgNPs synthesized by Streptomyces sp. NMSA 7

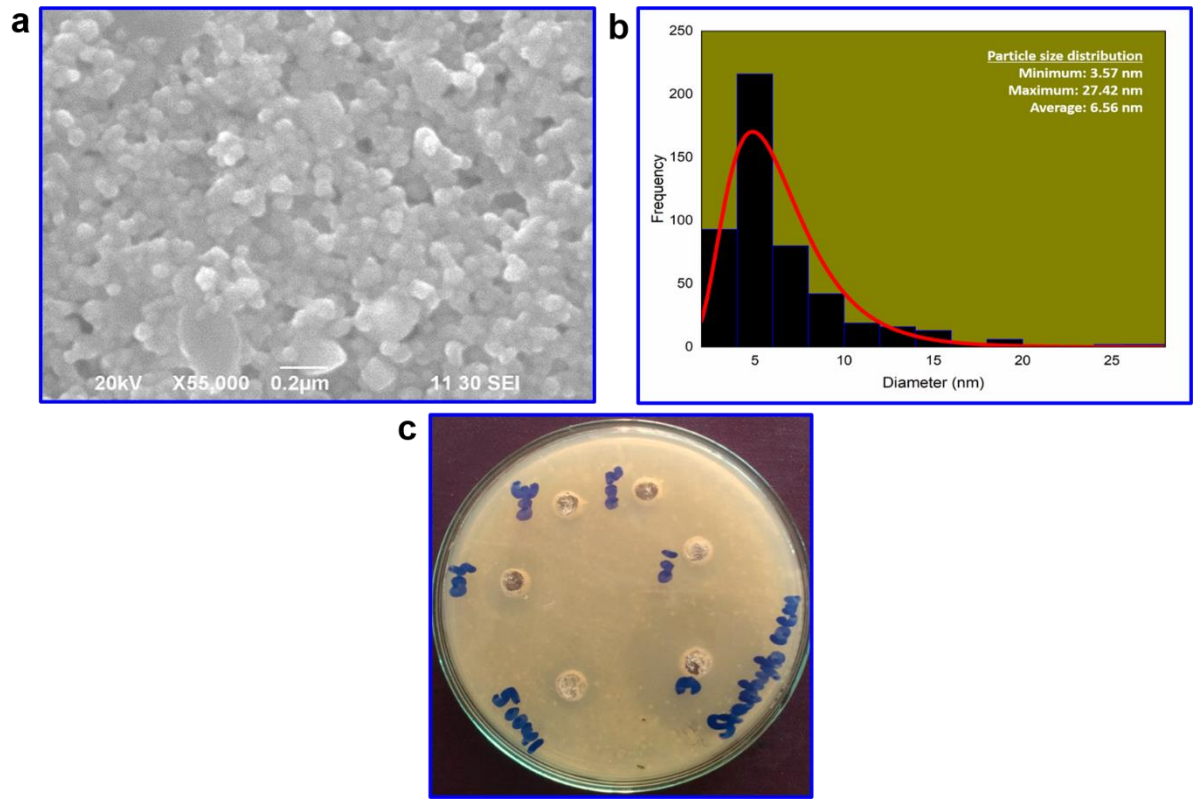

Figure 4. Represents the SEM analysis (a), particle size distribution (b) and well diffusion results of AgNPs synthesized by Streptomyces sp. NMSA 7

Table 1. Represents the morphological, physiological and chemotaxonomical characteristics of Streptomyces sp. NMSA 7

Characters studied	Streptomyces sp. NMSA 7
Cell wall amino acids	
LL-DAP	+
Meso – DAP	-
Whole cell suger	-
Color of aerial mycelium	Creamy white
Melanoid Pigment	+
Reverse side Pigment	-
Soluble Pigment	+
Spore chain	Rectiflexible with smooth surface
Carbone source assimilation	
Arabinose	+
Xylose	+
Inosital	±
Mannitol	+
Rhamnose	+
Sucrose	+
Raffinose	-