Migration Letters

Volume: 20, No: S13(2023), pp. 27-34

ISSN: 1741-8984 (Print) ISSN: 1741-8992 (Online)
www.migrationletters.com

Antibacterial Effect of Silver Nanoparticles Synthesized from the Secondary Metabolites of Marine Actinomycete Species

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Abstract

Bioprospecting marine actinomycetes can yield biological solutions to many of the key human concerns. The primary reservoir of bioactive metabolites with diverse applications is found in marine actinomycetes. Bioactive secondary metabolites produced by marine actinomycetes are widely used as immunosuppressives, antimicrobials, enzymes, and cosmetics. Actinoplanes, Micromonospora, Saccharopolyspora, Streptomyces, and Amycolatopsis are the main taxonomic groups of actinomycete that are important to industry. Because of the effectiveness of actinomycetes and their bioactive secondary metabolites, modern research is concentrating more on marine actinobacteria to address current difficulties. Nanotechnology combines the concepts of physics, chemistry, and biology to produce particles smaller than 100 nm, or nanoparticles, with specific applications. When it comes to a diverse array of bacteria and fungus, silver nanoparticles have broad spectrum antibacterial characteristics. Within the medical community, silver nanoparticles have become more well-liked because of their antibacterial properties.

Keywords: *Nanoparticles, metabolites, enzymes, cosmetics, nanotechnology.*

Introduction

Significant scientific discoveries and numerous technical advancements occurred in the latter half of the 20th century, the implications of which are only now starting to become clear. Increased unification of major science disciplines like physics, chemistry, and biology on the nanometer scale has given rise to the field of nanoscience (Figure 1). This is due to improved understanding of material properties at the atomic level, evolution based on the modern molecular approach on living organisms, and the increase of information processing using advanced instrumental tools. Due of its anticipated influence on numerous critical fields, including health care, medicine, electronics, energy research, space industries, optics, food, mechanics, and environmental management, nanoscience is a young field that is receiving a lot of attention in the twenty-first century. Numerous technological domains have been encouraged in the wake of the moral implications and findings surrounding the progress of nanotechnology during the past two decades. [1]

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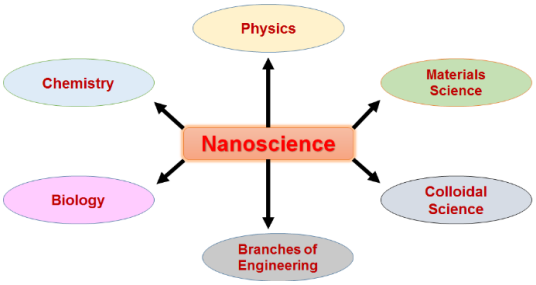


Figure 1: The multidisciplinary scientific approaches of nanoscience [2]

The Greek word "nano" (meaning dwarf) denotes one billionth (10-9 m) of a meter. Since people have been using Nobel metals like silver and gold nanoparticles as colorants for church windows for more than 2000 years, the concept of nanoscience is not a recent development. Gold nanoparticles were utilized to add a red hue to the well-known Lycurgus cup, which was created in the fourth century. It wasn't until the seventeenth century that the manufacture of large red glass containers known as "Purple of Cassius"—a precipitate of stannic hydroxide and colloidal gold—was unearthed, indicating that gold-ruby glasses had been produced during the Roman era. [2]. However, the earliest known scientific investigation into nanomaterials dates back to 1831, when Michael Faraday examined gold's ruby red colloids and revealed that the color was caused by particles. In 1959, Professor Richard P. Feynman presented the fundamental concept of nanotechnology during his talk titled "There's Plenty of Room at the Bottom" at the American Physical Society conference hosted at the California Institute of Technology.

There are two types of nanotechnology: "dry" nanotechnology and "wet" nanotechnology, which includes biological systems. In order to design man-made items at the nanoscale and integrate nanoscale assemblies into large-scale structures, as nature does, research on dry nanotechnology is now looking for systematic ways. Nanomaterials typically act at the nanoscale level and coexist with living things in the natural world. A nanotechnologist's job is to use naturally occurring and synthesized nanoparticles to create large amounts of nanomaterials in a narrow size. Nanoparticles are regarded as the fundamental advancement in nanotechnology and are used as starting materials to create a variety of nanomaterials and nanodevices. (3).

Utilizing biological methods that involve microorganisms like bacteria, actinobacteria, molds, yeast, and algae, in combination with plant or plant-derived extracts, is being proposed as a viable alternative to chemical and physical approaches in the synthesis of metal nanoparticles. This recommendation is based on the dependability, cost efficiency, environmentally friendly nature, and energy effectiveness inherent in biological processes. Moreover, these approaches adhere to the principles of green chemistry, connecting the fields of microbial biotechnology and nanotechnology. Over the last five years, there have been comprehensive discoveries extensively documenting the biosynthesis of a variety of nanoparticles. These include silver, gold, copper, selenium, palladium, platinum, titania, silica, zirconia, quantum dots, magnetite, uranite, and their alloys, all facilitated by various microorganisms (see Figure 2)[4].

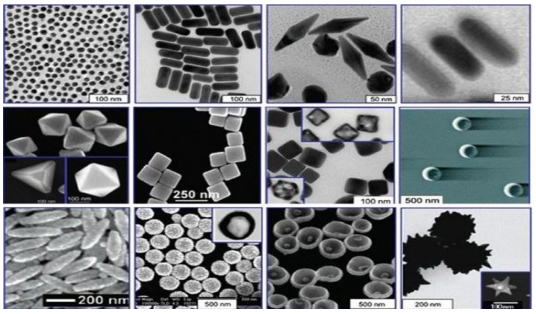


Figure 2: Different types of metal and its alloy nanoparticles [4]

Marine Actinomycetes

The majority of Earth's surface, exceeding 70%, is comprised of oceans, and marine organisms exhibit distinct physiological and metabolic traits that differentiate them from their terrestrial counterparts. The marine environment harbors a remarkably diverse and untapped population of marine actinomycetes, which are a relatively recent discovery. These marine actinomycetes produce a wide range of unique secondary metabolites exhibiting biological activity, suggesting their potential future use in medicinal applications.

An excellent source of bioactive secondary metabolites is the actinomycetes. Over the past five decades, substantial efforts have been dedicated to the efficient extraction of novel actinomycetes from terrestrial sources for drug screening. However, a recent upswing in the discovery of new compounds from terrestrial actinomycetes has been noted. While the rediscovery of known substances has accelerated, there has been a decrease in the overall diversity of life. The oceans harbor the highest biodiversity, whereas the terrestrial environment is notably exceptional. Given the distinct environmental conditions between sea and land, it is presumed that marine actinomycetes differ from their terrestrial counterparts in both characteristics and the types of bioactive substances they produce. Actinomycetes prove to be an excellent source of bioactive secondary metabolites [5]. Throughout their evolutionary history, marine actinomycetes have undergone adaptations to thrive in a wide array of living environments.

- Extremely high pressures (with a maximum of 1100 atmospheres)
- Anaerobic conditions at temperature just below ocean deep seafloor.
- At temperature over 100°C (near hydrothermal vents at the mid-ocean ridges).
- Highly acidic conditions (PH as low as 2.8)]

Actinomycetes from marine environments possess significant potential, yet their exploration for the identification of new secondary metabolites remains largely untapped [7].

1. Antibiotics
2. Anti-tumor agents.
3. Immunosuppressive agents.
4. Enzymes.

Distribution of Marine Actinomycetes

Culture-dependent and culture-independent approaches reveal the widespread occurrence of recently identified actinomycetes in diverse marine settings, encompassing depths ranging from the ocean floor to coral reefs, sediments, invertebrates, and plants. Actinomycetes are found throughout the entire depth spectrum of ocean ecosystems, spanning from near-shore and intertidal areas to the surface of the ocean. The identification of indigenous marine actinomycetes underscores their broad distribution across a variety of marine environments and habitats. [8]

Actinomycetes, including Dietzia maris, Rhodococcus erythropolis, and Kocuria erythromyxa, were detected in a sediment core extracted from a depth of 1225 meters near the coast of Hokkaido. Similarly, actinomycetes were also identified in samples collected from the Challenger Deep, the deepest trench in the Marianas, reaching a depth of 10923 meters. In certain marine habitats, such as deep-sea gas hydrate reservoirs and marine organic aggregates, actinomycetes have been identified as the predominant constituents of the microbial communities. In the microbial community of the Wadden Sea, actinomycetes obtained from marine organic aggregates exhibit notable antagonistic activity [6].

Silver Nanoparticles

The metallic element silver, with the chemical symbol Ag (derived from the Latin argentum), is a dazzling white color that is ranked 47th on the periodic chart. Of all the metals, pure silver has the lowest contact resistance and the maximum thermal and

electrical conductivity. Silver has been utilized extensively for thousands of years throughout human history together with other precious metals like gold and platinum. It has been used in jewelry, coins, cutlery, dental alloy, explosives, and photography, among other things (Nordberg and Gerhardsson, 1988). Although the exact mechanism is still unknown, silver is widely used in many well-known and traditional applications, such as medicine and hygiene, where its disinfecting qualities are used. Ancient people preserved wine and water in silver vessels. Hippocrates, the Father of Modern Medicine, prescribed a powder made of silver because he thought it had restorative characteristics. The powder was also used to treat ulcers. Until the discovery of antibiotics, compounds containing silver were a prominent treatment for wound infections during World War I. The first known medical application of silver may have been launched in 1884 when German obstetrician C.S.F. Crede offered 1% silver nitrate (AgNO3) as an eye treatment to avoid Gonnococcal ophthalmia neonatorum. Additionally, a standardized topical silver sulfadiazine cream was developed for the antibacterial treatment of severe burn injuries.

Techniques for the Characterization of NPs

Following the successful synthesis of NPs, the next essential step involves determining their sizes, distributions, shapes, surface areas, and surface morphologies. Variable diffractographic and spectroscopic methods can be used to achieve these parameters. The quantification of nanoparticle diffraction patterns using X-ray diffraction (XRD) measurement provides structural information that may be compared to a standard crystallographic database like JCPDS. All things considered, XRD analysis can produce a variety of data on the crystalline size, geometry, purity, and orientation and phases. Nanoscale surface morphology and dispersion can be studied using a scanning electron microscope (SEM). Nevertheless, the dimensions, shape, and quantity of material layers can be ascertained through the use of a transmission electron microscope (TEM), which provides data at various resolutions. To obtain detailed information regarding the metals present in nanoparticles (NPs), a combination of scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) is employed. SEM offers a resolving power of approximately 2 nm and a maximum magnification of about 100,000x, while transmission electron microscopy (TEM) provides a resolving power of around 0.2 nm and a maximum magnification of approximately 1,000,000x. In cases of intracellular NP synthesis, SEM and TEM can be used to determine the location of generated NPs. For precise measurements of nanoparticle dimensions, shape, and crystalline configuration, the use of high-resolution transmission electron microscopy (HR-TEM) is essential. Atomic force microscopy (AFM), which provides three-dimensional information on NPs, is crucial for examining the surface topography of nanoparticles. Metallic nanoparticles (NPs) with unique optical properties resulting from surface plasmon resonance (SPR), ideally in the 190–1,100 nm range, are monitored using UV–Vis spectroscopy. The interaction of radiation with metallic NPs facilitates the measurement of their remarkable properties.

Aims and Objectives

The present study aims to accomplish the following objectives:

- To isolate and purify marine actinomycetes from marine sediment samples.
- To synthesis silver nanoparticles from marine actinomycetes.
- To characterize the synthesis of marine actinomycetes.
- To assess the antibacterial impact of silver nanoparticles produced by the marine actinomycete.

Literature Review

A novel antitumour antibiotic called sapurimycin was identified by Hara et al. It differs from kapurimycin from *Streptomyces* sp. DO-116 in structure but is structurally connected to it. The high porus polymer resin supplied the fermentation medium in which the antibiotic was created, causing the antibiotic to adsorb in the culture and raising titre. *Sapurimycin* shown anticancer effect against leukemia P388 and sarcoma 180 in mice, and

it was active against germs, especially Gram-positive bacteria. In vitro, *sapurimycin* produced single strand breakage in the DNA of supercoiled plasmids. Novel glycopeptide antibiotics called helvecardins A and B were discovered by Takeuchi et al. from a strain of Pseudonocardia compacta sub sp. *Helvitica*.[9]

Selva et al isolated a novel antibiotic GE-2270 from the broth culture of *Planobispora rosea. GE-2270* is active against Gram-positive bacteria and anaerobes. Tomitta et al found five unusual actinomycetes, producing a family of antiviral antibiotics consisted of seven components fluvirucins Ai, A2, Bl, B2, B3, B4 and B5. All strains possess meso-2, 6-diamino-pimelic acid in their cell walls. Among them, four strains were classified as Maduromycetes, while the fifth one was identified as a nocardioform actinomycete. In research conducted by Karwowski et al. (1971), a distinctive set of spiroketal 24-membered macrolides known as dunaimycins was extracted from the culture filtrates of two actinomycetes, precisely recognized as strains AB 16910-321 and AB 1711J-452 of Streptomyces diastatochromogenes. Importantly, dunaimycins demonstrate dual properties, functioning as both immunosuppressants and antimicrobial agents. [10]

Biologically, diverse bacteria have effectively generated metal nanoparticles. Actinomycetes are recognized as valuable reservoirs for innovative pharmaceutical and commercial goods, such as antimicrobials. As environmentally friendly nanofactories, they are actively employed [11]. Actinomycetes stand out as promising options for producing metal nanoparticles through both extracellular and intracellular means. The stability and polydispersity of nanoparticles produced by actinomycetes are noteworthy. Actinomycetes showcase significant biocidal activity against a range of diseases. Furthermore, their genetic manipulability allows for enhanced control over particle size [12]. Optimizing growth conditions, such as media composition, pH, temperature, substrate concentration, and inoculum size, not only enhances growth but also boosts productivity. Moreover, it allows for monitoring enzyme activity rates, influencing the synthesis of silver nanoparticles.

Methodology

Sample Collection

Core samples from the western coastal regions of India were utilized to systematically screen actinomycetes in marine sediments. A random sample collection, taken at a distance of 200 meters and a depth of 5 meters, was conducted. In June 2013, the middle section of the marine sediments was transferred to sterile bottles under aseptic conditions and then conveyed to the laboratory in an ice bag. The sediment sample exhibited a sandy texture and a slightly brown color.

Isolation of Actinomycetes

To reduce bacterial contamination, air drying was done on all the sea sediments. One gram of sediments underwent serial dilution, reaching a dilution of 10-6. Subsequently, one milliliter of the diluted material was dispensed onto a Petri plate. The medium was then supplemented with 50 micrograms each of cycloheximide and nystatin. Following solidification, the plates were placed in an incubator at 28°C for a period of seven to fifteen days to promote the growth of colonies(figure 3).

Figure 3: Isolation of actinomycetes

Spore Morphology

The identification of all isolates was carried out using the slide culture method. A glass slide was coated with a 0.1% trypan blue stain droplet, and a cover slip was delicately placed over the stain. Following this, the slide was examined using a bright-field microscope, and the spores were accurately documented.

Biochemical Characterization of Actinomycetes

After the preliminary examinations, the isolates were subjected to identification through biochemical analysis. This analysis encompassed assessments such as starch hydrolysis, casein hydrolysis, urea hydrolysis, the methyl red and Voges-Proskauer tests, the citrate utilization test, the catalase test, and the oxidase test.

Biosynthesis of Silver Nanoparticles (AgNPs)

Actinomycete supernatants (50 ml) were mixed with a 1 mM aqueous solution of silver nitrate, and the pH was adjusted to 8.5. The resulting mixture was incubated in darkness at 37 °C on a rotary shaker (200 rpm) for five days. Control experiments, lacking inoculation, utilized a silver nitrate solution to validate the bacteria's involvement in nanoparticle formation. Sampling approximately 2 ml of the solution at regular intervals, the reduction of silver ions was assessed by monitoring UV-Vis spectra with a Double Beam Spectrophotometer 6800 JENWAY. Upon introducing actinomycete supernatant to the silver nitrate solution, the color of each reaction vessel transitioned to a yellowish-brown hue. (figure 4)

Figure 4: Silver nanoparticles obtained from marine actinomycetes

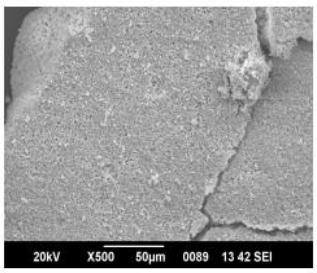
Scanning electron microscopy (SEM)

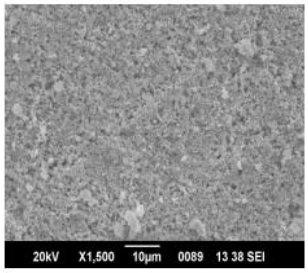
The analysis utilized the JSM-6390 scanning electron microscope (SEM) with an accelerating voltage of 20 kV and a working distance of 13 mm. The magnification ranged from 500X to 10000X. The samples were placed in the sample holder, which was then vacuum-dried before examination under the SEM. The study focused on examining the sample's size through SEM analysis.

Results

SEM

In Scanning Electron Microscopy, it shows the size and shape of AgNP in different magnification power. The micrographs of nanoparticles obtained in filtrate showed that silver nanoparticle is spherical shaped well distribution without aggregation in solution shown in figure.5.





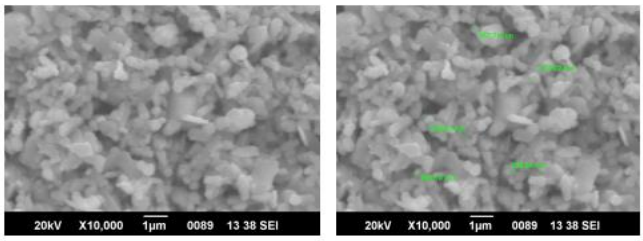


Figure 5: SEM analysis of silver nanoparticle produced by marine actinomycetes

By doing biochemical tests, the selected actinomycetes is confirmed as *Streptomyces* sp(figure 6)

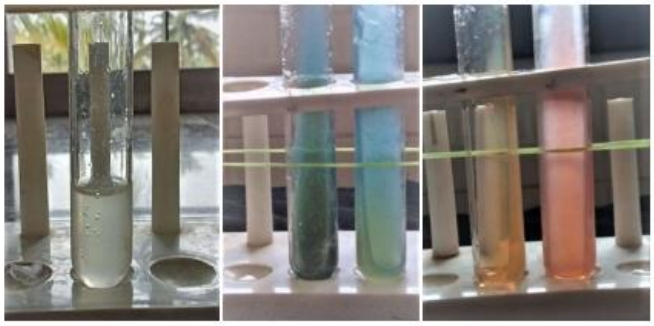


Figure 6: Biochemical tests such as catalase test, citrate test, Urease test

Antibacterial Activity of AgNPs

The antimicrobial efficacy of AgNPs was assessed through the agar well diffusion method. Multidrug-resistant pathogens, including E. coli, K. pneumoniae, Enterococcus sp., and S. aureus, were obtained from hospital-acquired infections. These pathogens were cultured in nutrient broth at 37°C for 24 hours. Following incubation, the test pathogens were applied to Mueller-Hinton agar (MHA) using sterilized cotton swabs. Wells were created in each plate using a sterile gel borer, and 50 µl of AgNPs were introduced against the clinical isolates. The inoculated plates were then incubated at 37°C for 24 hours. Subsequently, the plates were inspected for the presence of zones of inhibition surrounding the wells.

Table 1: Antibacterial activity of silver nanoparticles

SI No	Bacteria	Zone of inhibition(mm)
1	*Klebsiella*	7
2	*Enterococcus*	5
3	*Staphylococcus*	3

The synthesized silver nanoparticles, produced through the use of Streptomyces sp. cell-free culture filtrate, exhibited a noteworthy antibacterial activity profile when assessed using the well diffusion method. This was evident in their effective inhibition of the growth of human pathogens, as indicated by distinct zones of inhibition. Notably, the well diffusion method revealed the highest level of growth inhibition against Enterococcus sp. with a measurement of 5 mm each. 3 mm of growth inhibition was observed towards *Staphylococcus sp*. 7 mm zone was noted in *Klebsiella*. (Table 1) From the results obtained above, it was clear that the silver nanoparticle produced by marine actinomycete had positive effect against human pathogens

Conclusion

It is established that silver serves as a safe and harmless inorganic antibacterial agent, with a history of use spanning millennia. Silver, particularly in the form of nanoparticles, holds great potential for various biological applications. Organisms ranging from simple bacteria to sophisticated eukaryotes can be harnessed to produce nanoobjects with the desired size

and shape. Among the metallic nanoparticles applied in biomedical contexts, silver nanoparticles (AgNPs) stand out as particularly important and intriguing nanomaterials. AgNPs play a significant role in nanotechnology and nanoscience, especially within the field of nanomedicine. These nanoparticles have garnered attention for their potential applications in cancer treatment and diagnosis, even though other noble metals have been utilized for diverse reasons. The result of that actinomycete of marine origin of Southern Kerala cost are potential Sources of antimicrobial compounds and same can be explolited for the development of novel bioactive compounds and silver nano particle of pharmaceutical significance.

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