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Research Article

Biodiversity of Actinomycetes Prevailing in Soil for the Synthesis of Gold Nanoparticles

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Abstract

Background and Objective: Actinomycetes are important microorganisms that have an important role in the production of bioactive molecules due to their ability to decompose many organic materials. Because of this the present investigation focus on the exploration of different goldmine soil for the isolation of potential actinomycetes which can be further used for the synthesis of gold nanoparticles. The study aims to explore gold mine soil to isolate and characterize the variety of actinomycetes prevailing in the soils of the Hutti gold mines and Kolar gold mines which can be further used for the synthesis of gold nanoparticles. **Materials and Methods:** Soil samples were subjected to the isolation of actinomycetes. The isolates of actinomycetes were screened for the synthesis of gold nanoparticles in starch casein broth using aurium chloride as a substrate. **Results:** The presence of gold nanoparticles in the solution was confirmed by visual observation of colour and UV-Vis Spectrum at 400-700 nm. Further, the size of gold nanoparticles was illustrated by scanning electron microscopy (SEM). **Conclusion:** Gold mines are considered as a good source for isolating biodiverse microorganisms and this isolated microorganism can be explored for the synthesis of various bioactive molecules among them Gold nanoparticles is considered to be one of the most important bioactive molecules because of their various applications in a different field.

Key words: Soil, gold mines, actinomycetes, streptomyces, gold nanoparticles, biodiversity, aurium chloride

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INTRODUCTION

Biodiversity deals with patterns of species distribution and the process that result in such patterns over space and time. It aims to reveal where organisms live, at what abundance and why. It is estimated that there are millions of microbial species on the planet, relatively few of which have been isolated in culture[1]. Despite the recognized importance of microorganisms, we still know little about the magnitude and variability of microbial biodiversity in natural environments relative to what is known about plants and animals. Out of necessity, microbial diversity studies usually examine the richness including abundance, range and distribution of microbes, thereby improving our understanding of both natural ecosystems and human health. Microorganisms constitute a huge and almost unexplained reservoir of resources likely to provide innovative applications useful to man. It has been estimated that only about less than five percent of all microorganisms on earth have been studied[2]. The Indian subcontinent is one the richest geographical area in the world allocated with microbial diversity. Diversity is the inconsistency of living beings and the ecosystem in which they occur. It is mainly represented by genetic, functional and taxonomic diversity. Microorganisms represent the richest inventory of all the three elements mentioned above as they underline the basic ecosystem process. Among microorganisms, Actinomycetes represents a very exotic as well as interesting and diverse group with about 56 families and 220 genera. They can be found in a variety of habitats and are particularly abundant in soil and aquatic bodies. These are filamentous, branching bacteria with a fungal type of morphology. Streptomyces is a familiar name to all microbiologists but few will recognize actinomycetes, the group to which it belongs. Identified from the 1870s onwards, these organisms confused their discoverers by sharing characteristics of both bacteria and fungi.

Actinomycetes are physiologically very diverse as evidenced by their production of numerous extracellular enzymes and thousands of secondary metabolites[1]. They have an unrivalled capacity to produce commercially significant bioactive compounds. Nanoparticle one of the bioactive compound has a wide range of applications[3]. Antimicrobial properties, anticancer activities, genotoxicity, diagnostic devices, drug delivery systems, detection of pathogens, purification of water, cosmetics and nano drugs, sensors in environmental cleanup strategies and food processing industries, nanocatalysts and oil industries and textile industries and decorative materials are some of the important and potential applications to mention. Among nanoparticles, gold nanoparticles were reported to have important applications. For the synthesis of nanoparticles, many methods are available, among all microbial nanotechnology is the eco-friendly process[4]. It is the science that deals with the use of microorganisms.

In the present study, an attempt was made to isolate and characterize the variety of actinomycetes prevailing in the soils of Hutti gold mines and Kolar gold mines for the synthesis of gold nanoparticles.

MATERIALS AND METHODS

Study area: The study was carried out in the A-DBT Research Laboratory, Gulbarga University, India from June-December, 2021.

Isolation and screening of actinomycetes: Isolation of actinomycetes was performed by serial dilution method[5] using starch casein agar[6]. Soil samples from Hutti Gold mines and Kolar gold mines were collected and subjected to the isolation of actinomycetes. The isolated colonies were further purified by subculturing and maintained at 40°C on SCA.

Screening of actinomycetes for the synthesis of gold nanoparticles: The isolated cultures of actinomycetes were screened for the synthesis of gold nanoparticles. A loop full of 3 days old culture of actinomycetes were inoculated into 100 mL starch casein broth individually and incubated at 40°C for 5 days on a shaker (200 rpm). After the period of incubation, the broth cultures were centrifuged at 5000 rpm for 20 min at 20°C. The biomass obtained was washed 2-3 times with sterile distilled water. The washed biomass was resuspended in 100 mL of Aurium chloride solution and kept for incubation at 40°C on a shaker (200 rpm) for 3 days. Visual observation for the development of colour and UV absorbance at 400-700 nm was recorded[7].

Identification of streptomyces: The screened potential isolates for the synthesis of gold nanoparticles were characterized by morphological, microscopic and biochemical tests. The observation was carried out by the method and media proposed by Ahmad *et al.*[8].

Synthesis of gold nanoparticles: Gold nanoparticles were synthesized by the potential isolates DBZ-29 and DBZ-33 employing the same procedure mentioned under screening for gold nanoparticles synthesis. The synthesized gold nanoparticles were characterized by visual observation and

UV-Vis spectrophotometric analysis. The size of gold nanoparticles was analyzed by scanning electron microscopy[8].

RESULTS AND DISCUSSION

Totally 38 colonies of actinomycetes were isolated from the soil samples of Hutti gold mines and Kolar gold mines (Fig. 1) and were screened for the synthesis of gold nanoparticles (Fig. 2). It was found that about 8 isolates synthesize extracellular gold nanoparticles and these potential isolates were characterized by microscopical (Fig. 3), morphological and biochemical tests (Table 1) for the identification of *Streptomyces* sp. Microscopic observations reveal gram-positive bacteria with aerial mycelium while the morphological characteristic reveals the cemented, soily odour colony with aerial mycelium. Eight biochemical tests were performed as per the prescribed protocol by Shirling and Gottlieb where catalase (+ve), nitrate reduction (+ve), H_2S production (-ve), gelatin liquefaction (+ve), Milk peptonization (+ve), tyrosinase production (+ve), Starch hydrolysis (+ve) and cellulase production (+ve) to identify streptomyces (Table 1). Among these 8 isolates, DBZ-29 was found to be potential for the synthesis of extracellular gold nanoparticles (Fig. 4). The development of colour from yellow to deep purple after challenging with aurium chloride solution confirms the synthesis of gold nanoparticles. The presence of gold nanoparticles in the solution was confirmed by recording UV-Vis spectrum at 400-700 nm absorbance (0.19 optical density at 550 nm) (Fig. 5). Further, the size of gold nanoparticles was illustrated by scanning electron microscopy (SEM) which reveals the mono dispersion pattern of gold nanoparticles (Fig. 6). The result of presence studies reveals that the maximum absorbance of the aurium chloride solution treated with streptomyces DBZ-29 was recorded at 550 nm and the size of the gold nanoparticles was found to be 48 nm shown by scanning electron microscopy. This confirms the presence of gold nanoparticles in the solution treated with the Streptomyces DBZ-29. Thus the soil samples of Hutti gold mines and Kolar gold mines can be explored further to know the biodiversity of microorganisms and their bioactive molecules for different applications.

Several reports are available on the screening of biological entities for the synthesis of extracellular gold nanoparticles, especially including plants[9-14], bacteria[15-20] and fungi[21-27]. However, the investigation on screening of actinomycetes for the synthesis of extracellular gold nanoparticles is very meagre except few reports by[28-38]. The change in or development of wine red colour in the test solution within 18 hrs reveals more physiological capability of

Table 1: Biochemical properties of actinomycetes

Biochemical test	Results
Catalase	Positive
Nitrate reduction	Positive
H_2S production	Negative
Gelatin liquefaction	Positive
Milk peptonization test	Positive
Tyrosinase production	Positive
Starch hydrolysis	Positive
Cellulase production	Positive

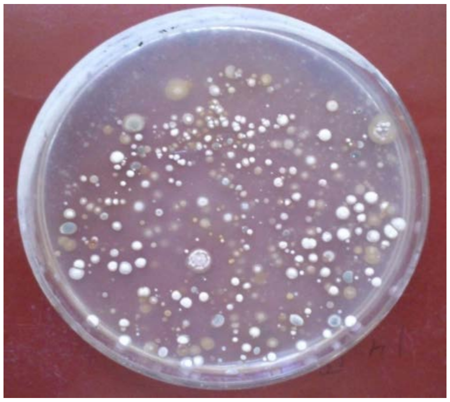

Fig. 1: Plate showing the biodiversity of actinomycetes in soil

Fig. 2: Screening of actinomycetes for the synthesis of gold nanoparticles

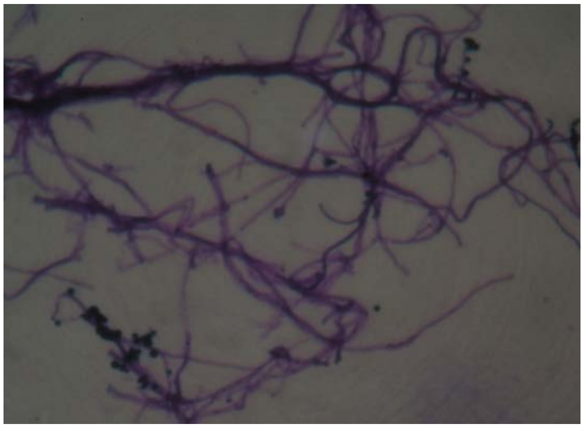

Fig. 3: Microscopic feature of actinomycetes

Fig. 4: Visual observation of gold nanoparticles synthesis

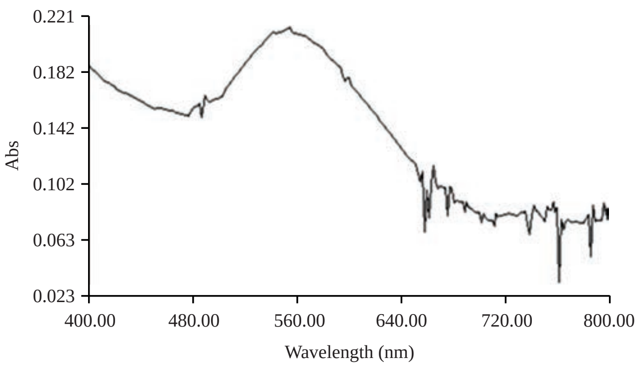

Fig. 5: UV-vis spectra for the synthesis of gold nanoparticles

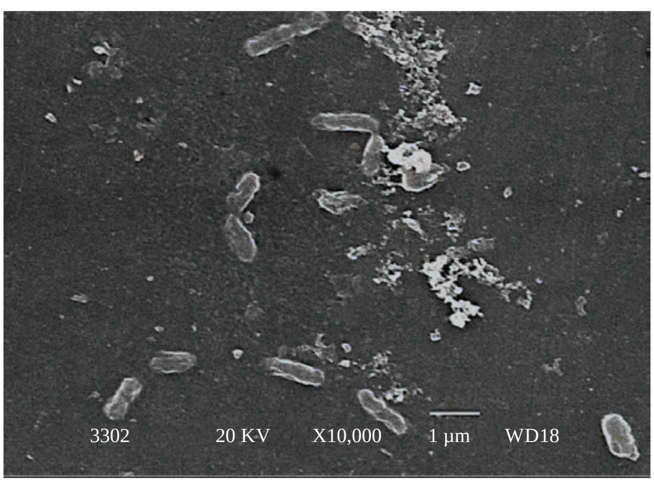

Fig. 6: Scanning electron microscopy of gold nanoparticles

Streptomyces tritolerans for the better synthesis of extracellular gold nanoparticles. Nikajima[39] presented a research account on the integration of various process variables as mentioned above for the extracellular synthesis of gold nanoparticles by an isolate of actinomycete, *Thermomonospora* sp. A novel alkali thermophilic actinomycetes *Thermomonospora* sp., isolated from self-heating compost was reported to have pH 9.0, temperature 50°C and 1 mM substrate concentration as optimum conditions for the extracellular synthesis of gold nanoparticles. It was also reported that the use of extreme biological conditions in the synthesis could be a contributory factor in the size and mono dispersal control using actinomycetes as the biological source. Microorganisms are the natural biofactories for the manufacture of various bioactive molecules[40]. They minimize the toxicity in the environment by reduction of the metal ions Thus, the employment of several microorganisms in the synthesis of nanoparticles is relatively a recent phenomenon and is an attractive field of research.

CONCLUSION

In the present study soil samples of Hutti gold mines and Kolar gold mines were explored for the isolation of actinomycetes. The isolated actinomycetes were screened for the synthesis of gold nanoparticles. Based on visual observation of the colour from yellow to deep red compared to the control solution indicates the presence of gold nanoparticles in the test solution treated with actinomycetes. Further, the presence of gold nanoparticles in the treated solution was confirmed by UV-vis analysis and scanning electron microscopy. Thus, the soil of the hutti gold mine and kolar gold mine proved to be a good source for isolating a diverse group of microorganisms.

SIGNIFICANCE STATEMENT

This study discovers the important role of actinomycetes presents in soil that can be beneficial for the production of many important bioactive molecules which are known to have myriad applications in Biotechnology, Medicine and Pharmaceutical Industries.

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