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Biogenesis of silver nanoparticles by marine bacteria *Labrenzia* sp. Mab 26 associated with *Isochrysis galbana*

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The metabolic or chemical nature of algal–bacterial interactions has opened up unexpected new ways for finding novel microbes with potential for several biotechnological applications. Silver nanoparticles (AgNps) possess many unique and attractive properties which are beneficial to various industries. In this context, the present study was undertaken to explore the bacterial strain *Labrenzia* sp. Mab 26, isolated from marine microalgae, *Isochrysis galbana* for AgNp biosynthesis. The synthesized AgNps were further characterized by UV–Vis spectroscopy, XRD analysis, FT–IR spectroscopic analysis and electron microscopy. This shows that microalgae-associated bacteria can be explored for efficient synthesis of AgNps.

Keywords: Algal–bacterial interaction, biogenesis, *Isochrysis galbana*, *Labrenzia* species, silver nanoparticles.

THE marine environment has proven to be an untapped reservoir of diverse natural products with potent biological activities[1,2]. Among marine organisms, microalgae represent one of the richest sources of valuable bioactive compounds for various industrial applications[3–5]. Bacterial communities in the algal phycosphere have been extensively studied[6–8]. It has been suggested that these microbial symbionts produce a wide range of bioactive compounds and thus create a suitable chemical microenvironment with their phytoplankton host. These metabolic associations thus make it challenging to understand which partner entity is accountable for the production of a particular metabolite[1]. Moreover, the associated bacteria may have a greater potential to produce bioactive agents than those of their free-living counterparts[9,10]. Thus, these symbiotic or commensal bacteria could be expected to be a remarkable source of valuable compounds with prospective benefits for humans. However, the biological wealth of bacteria associated with marine microalgae is relatively unexplored. In this background, the present study aims to explore the emerging application of culturable bacteria associated with marine microalgae for silver nanoparticle (AgNp) biosynthesis.

The bacterial strain, *Labrenzia* sp. Mab 26 (GenBank accession no KR004822; strain code of the isolate starts with MBTDCMFRI) which was isolated from marine microalgae culture, *Isochrysis galbana* (MBTDCMFRI S002) and preserved at the microbial culture collection of the Marine Biotechnology Division, Central Marine Fisheries Research Institute (CMFRI), Cochin, Kerala, India was used for this study[8]. The bacterial strain was grown in Zobell marine broth (ZMB, HiMedia, India) and incubated at room temperature for 48 h. After incubation, the biomass was separated by centrifugation (8000 rpm, 15 min; Remi, India). The culture supernatant was then mixed with an equal quantity of 5 mM silver nitrate solution (prepared in sterile distilled water) and kept in a shaker for three days at room temperature. Sterile ZMB mixed with silver nitrate solution was kept as control. Biosynthesis of AgNps was observed by a colour change[11].

The formation of AgNps was studied by UV–Vis spectroscopy (Thermo Scientific, USA). λ_{max} was determined within the range 300–800 nm. The sample was centrifuged (10,000 rpm, 20 min) and the pellet was freeze–dried for further analysis. The crystalline nature of the synthesized AgNps was determined by X-ray diffraction (XRD) analysis (Bruker, D8 Advance, Germany). Nicolet

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Avatar-370 Fourier transform infrared (FT–IR) spectrophotometer with the resolution of $4\ cm^{-1}$ was used for FT–IR spectroscopic analysis. Freeze–dried samples were analysed using potassium bromide (KBr) window (1–2 mg of AgNps with approximately 100 mg of powdered KBr). On an average, 32 scans were taken in the spectral region of 400–4000 cm^{-1}. The size and shape of the obtained AgNps were also studied using Scanning electron microscopy (SEM) and transmission electron microscopy (TEM). A slightly turbid solution of freeze–dried sample was prepared by suspending minute amounts of AgNp in ethanol and the particles were dispersed by ultrasonication. A drop of this solution was casted on carbon-coated grids (200 mesh) and observed under TEM at various magnifications (Jeol-Model JM 2100). For SEM analysis, the solution was mounted on specimen stubs with carbon conductive tape and coated with gold in a sputter coater (TESCAN VEGA 3).

Algal microhabitat has opened up unexpected ways for finding novel microbes with a potential for several biotechnological applications[1]. AgNps are the noble metal particles that have been studied extensively as they possess greater material properties and functional versatility[12,13]. They are widely used as antibacterial agents in food storage, water treatment, disinfecting medical devices and home appliances, and textile coatings[14,15]. Moreover, AgNps have been proposed to possess anti-permeability, anti-inflammatory and anti-fungal activities. So, they are used in surgically implanted catheters to reduce infections that may occur during surgery[16]. The nanoparticle synthesis can be done by chemical, physical or biological methods. Major problems with most of the physical and chemical methods of nanosilver production are the use of toxic chemicals and hazardous by-products (thio-glycerol, hydrazine, 2-mercapto ethanol). Often, these toxic chemical residues adhere to the surface that may restrict the use of synthesized nanoparticles in healthcare and medicine[11,13]. Hence, researchers are now interested in biogenic production of AgNps as it is a cost-effective method and an eco-friendly alternative[11,17,18]. Recently, biosynthetic methods have been developed as a viable and simple substitute to complex chemical synthetic processes to produce AgNps[15]. The metal nanoparticles have been successfully synthesized by microorganisms such as actinomycetes, fungi, yeast, bacteria, plants and algae[19]. The green synthesis of AgNps was done using marine microalgae, *I. galbana*[20]. However, from a perusal of the literature, we found no report on the synthesis of nanoparticles from heterotrophic bacteria associated with *I. galbana*. Therefore, the present study highlights the potential of *Labrenzia* sp. Mab 26 associated with *I. galbana* for green synthesis of AgNps (Supplementary Figure 1). Maximum peak at 420 nm in the UV–Vis spectrum might be due to the reduction of silver ions into silver particles by secondary metabolites produced by bacterial cells (Supplementary Figure 2). Combined vibration of electrons at the surface of AgNps in resonance with the light wave results in a surface plasmon resonance (SPR) absorption band with a peak at 420 nm (refs 11, 18). XRD analysis of the synthesized AgNps showed highly crystalline nature with strong peaks at 2θ values of 27.51°, 31.87°, 45.57°, 56.56°, 66.26° and 75.25° corresponding to XRD planes from (210), (113), (124), (240), (226) and (300) respectively, based on the fcc cubic structure of silver AgNps (Figure 1). Here the peaks are shifted to higher angles due to the small size of the particles. The Debye–Scherrer equation (size = $0.9\lambda/\beta\cos\theta$, where λ is the wavelength of the X-rays, β the width of the peak and θ is half of the Bragg's angle) was used to calculate the mean particle diameter of AgNps. The average crystallite size of the synthesized AgNps was found to be 20.79 nm. The FT–IR analysis of synthesized AgNps showed peaks at 3424.29, 1632.42, 1067.80 and 564.99 cm^{-1} (Figure 2). The intense absorption at 3424 cm^{-1} might be due to the stretching vibration of O–H group, whereas the absorption at 1632 cm^{-1} can be assigned to the presence of carboxylic (C=O) group[17].

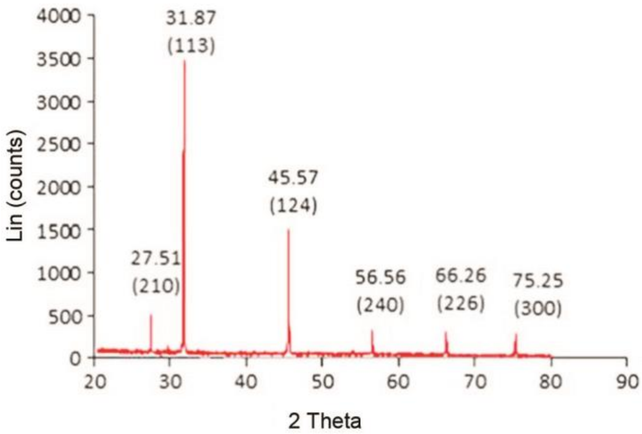

Figure 1. X-ray diffraction pattern of synthesized silver nanoparticles.

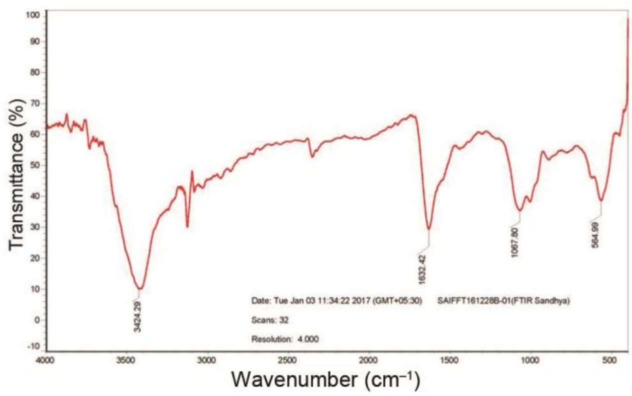

Figure 2. Fourier transform infrared spectroscopy of synthesized silver nanoparticles.

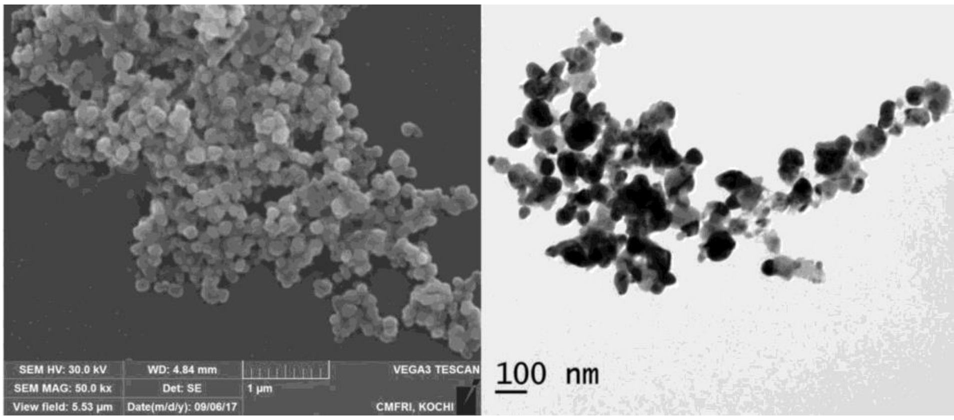

Figure 3. Scanning electron microscopy and transmission electron microscopy images of synthesized silver nanoparticles.

The peak at 1067 cm^{-1} can be attributed to the stretching vibration of C–O bond of the primary alcohol. The absorption peak at 564 cm^{-1} corresponds to metal-oxygen (silver stretching vibrations) vibrational mode[21]. The FT–IR analysis confirmed O–H stretching (around 3417 cm^{-1}), which could be accountable for reduction of metal ions into their corresponding nanoparticles[11]. TEM and SEM images illustrate that biosynthesized nanoparticles are small and spherical in shape (Figure 3). The size of synthesized nanaoparticles ranged from 14.04 to 37.04 nm and the average size was 28 ± 9 nm.

The present study demonstrates efficient synthesis of AgNps by a phycosphere bacterium, *Labrenzia* sp. Mab 26. The selected bacterial strain can synthesize AgNps extracellularly at room temperature.

Thus, the green synthesis of AgNps derived from microbial origin can be further explored for various biomedical, aquaculture and environmental applications. Moreover, additional research that takes into consideration the metabolic or chemical nature of algal–bacterial interactions would definitely trigger further exploration on the abundance of bacteria in phycosphere with bioactive potential.

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Late Quaternary monsoon and productivity variability in the northwestern Arabian Sea

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We studied the changes in planktic foraminiferal distribution along with some sedimentological and geochemical data at ODP Site 722B in the northwestern Arabian Sea to reconstruct monsoon upwelling and productivity variations during the last ~550 kyr. The higher relative abundance of *Globigerinita glutinata* and southwest (SW) monsoon assemblage along with increased Ba/Al ratio during most of the interglacial intervals, suggests lateral transport of eutrophic water due to strong upwelling causing enhanced productivity. A relatively higher test fragmentation and increased relative abundance of *Globorotalia menardii* during interglacial intervals indicate increased carbonate dissolution. The glacial–interglacial transitions are characterized by abrupt increase in the SW monsoon upwelling assemblage and Ba/Al ratio, suggesting more intensified upwelling and productivity due to accelerated SW monsoon and weak northeast monsoon conditions. Spectral analysis of the abundance data of SW monsoon upwelling assemblage and Ba/Al ratio demonstrates the 100-kyr cycle showing glacial–interglacial frequency and 23-kyr cycle indicating precession-driven insolation variability.

Keywords: Foraminifera, interglacial intervals, monsoon upwelling, productivity variability.

THE seasonal changes in the current direction, upwelling and mixed layer characters such as temperature, nutrient content and productivity in the Arabian Sea are the oceanic response of strong monsoonal winds[1,2]. The present-day ocean circulation pattern of the Arabian Sea is driven by monsoons. The monsoon is a result of inter-tropical convergence zone as well as the differential heating of the Asian continent and the Indian Ocean. Monsoon causes a semi-annual reversal of the current patterns in response to changes in wind direction. The southwest (SW) monsoon develops between June and September, whereas the northeast (NE) monsoon develops between November and March[3]. The differential (land–sea) sensible heating and heating of troposphere through latent heat induce the SW monsoon, which develops a characteristic circulation pattern in the atmosphere with changes in the wind direction[4]. The surface water of northwestern Arabian Sea is dragged by monsoonal winds. The coastal upwelling off Somalia, Oman and southwestern India is the result of clockwise circulation of surface waters during the SW monsoon, whereas a NE-trending narrow, low-level atmospheric Findlater Jet blowing across the Arabian Sea is responsible for open-ocean upwelling[5–9]. Naidu and Malmgren[10] suggested that the seasonal differences in sea surface temperature (SST) have a direct relation with the upwelling strength in the northwestern Arabian Sea. The upwelling leads to a major increase in productivity[11], which results into the development of characteristic assemblage of planktic foraminiferal species.

Earlier studies revealed that the changing northern hemisphere summer insolation is the major factor that influences the timing and strength of monsoonal winds[4,12,13]. The strong SW monsoon induced by insolation maxima is probably responsible for the enhanced productivity. Intensified wind and increased upwelling off the Somalia and Oman coasts, especially during interglacials, occurred due to intense SW monsoon[14]. A relatively weaker anticlockwise flow of surface current during the interval of the NE monsoon[15] results in surface water cooling and convective overturning[16]. The convective overturning injects nutrient-rich water into the surface waters[17], which is considered to dominantly control winter productivity that remains relatively low than the

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