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Antimicrobial Activity of Biogenic Si-Ge Combined Nanocomposite Synthesized by Marine Diatom *Coscinodiscus* sp.

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Abstract: Living organisms are giving a major support in nanomaterial synthesis for various applications like drug delivery, biological imaging and sensors. Diatom is a well diverse marine microorganism. In this study aim to synthesis silicon (Si) and germanium (Ge) based diatom nanomaterial for antibacterial activity. *Coscinodiscus* sp. is unicellular microalgae that make microscale silica frustules with nanoscale two dimensional pore arrays. Diatoms are made the unique silica structure in well organized by micro to nanoscale. The Ge is metabolically slot in marine diatom *Coscinodiscus sp.* frustule by two steps culturing process in laboratory condition. First the diatom cultured in culture medium with silicon starvation state, then the silicon starved diatom was transferred into Si: Ge (2:1) mixed culture medium. Electron microscopic observation has been shows that the nanomaterial synthesis does not causes any structural changes in cell structure. But in diatom frustules carries some changes in its pore size. In XRD result patterns also shows that some peaks have been appeared in 2θ at 10.81, 27.33, 31.69, 45.42, 56.41, 66.27 and 75.21 are conformed the cubic phase of both Si and Ge present in diatom frustules. Moreover, Si and Ge was measured and found that it is a Si-Ge nanocomposite in diatom frustules. The biogenic Si-Ge nanocomposite was analyzed for its anti-bacterial activity and the result has also showed significant inhibition against both gram negative and positive bacteria. This is the first study to demonstrate the applications of biogenic Si-Ge nanocomposite in antibacterial activity study. This study might fulfill the biomedical applications in near future.

Key words: Diatom, Biosynthesis, Nanocomposite, Silica and Germanium

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Introduction

Biosynthesis of nanomaterials from living organisms is given as precious contribution in nanotechnology. In general some of the microorganisms are able to synthesis metallic nanoparticles and it could be used as different biomedical therapeutics and diagnosis applications. Biosynthetic methods are alternative one for the physical and chemical methods to produce novel nanomaterials with effective therapeutic properties [1]. Recently the researchers aim to produce the silicon nanomaterials in different concentration with various silicon nanostructures to generate numeric physicochemical properties, which are concentrated in many applications such as optical, photonic, sensors, energy storage, drug delivery and biological imaging [2-8]. The silicon is the most abundant element in our earth. It has totally changed human's life by its applications mainly in optical and electronic field. The considerations of diatoms are single-celled algae made of microscale silica shells. It is called as "frustules". The very complicated submicron scale, two dimensional pore arrays are present in whole [9]. The diatom frustules special properties are typically controlled synthesis of silica nanostructure pattern [10].Recent studies shows the germanium nanomaterial metabolic activity with diatom frustules with biosilica of *Pinnularia* species by two stage bioreactor cultures and it also confirms the integration of the germanium into the diatoms frustule [11]. The biomimetic approaches normally pointed on biomolecules integration and biological activities in these processes, commonly live cells have the possible integration properties on the production of the nanostructured materials, those are currently used for photonic device applications [12]. Previous studies has given the considerable improvement in antibacterial agents for inhibition of pathogenic bacteria to direct implications in human life and as developed resist bacteria [13]. Therefore, well understandings of the antimicrobial resistance mechanism are very important. So, the improved methods used for detection of resistance and new types of antimicrobial agents for resistant microorganisms [14]. The well characterized bacterial strains easily get more informational experiments about molecular mechanisms of antimicrobial resistant [15]. Here, we studied the metabolically introducing the germanium into marine diatom's silica frustule during the culture and examined the germanium induced diatoms frustule structures, physic-chemical and biological properties by XRD pattern and electron microscopy. Furthermore, we studied the antimicrobial activity of silica and germanium combined nanomaterial against some pathogenic bacteria.

Materials and Methods

Culture of Diatom

Phytoplankton was collected from Vellar estuary, Tamil Nadu, India. The collection net was made up of bolting silk cloth no 30 with mesh size 48 μm. The net was submerged in the seawater and towed horizontally from a mechanized boat with an outboard engine at a speed of 01 – 02 knots for one hour duration. Collected plankton was identified using light microscope. The identified diatom species isolated from collected samples with the help of micropipette for culture. The culture of diatoms was carried out by the standard methods as described by Anderson (1975) [16]. The F/2 Guillard's medium was used for diatom culture. Pure *Coscinodiscus* sp. were cultured for silicon – germanium nanomaterial, under standardized conditions of temperature at 25±0.5°C, 12/12 day/night cycles; photoperiods of 12 hours light (fluorescent lamps) in the algal culture laboratory room at Centre of Advanced Study in Marine Biology, Annamalai University of India. To achieve a stationary growth the culture was grown up to 4 weeks to carry out the further experiment.

Biosynthesis of Si- Ge nanomaterial

The *Coscinodiscus* sp. culture was carried out in two steps culture for biosynthesis of silica – germanium nanomaterial [17]. In first step, the diatoms of *Coscinodiscus* sp. was inoculate in F/2 Guillard's medium contain silicon for synthesis a silicon nanomaterials at a duration of one week time. The same diatom culture was maintained in starved condition of silicon in culture medium for next one week. In the second step, the silicon: germanium solution was prepared in the ratio of 2:1 and the prepared solution was added into liquid culture medium for synthesis of silicon-germanium nanocomposites. After one week culture in silicon: germanium solution the diatom cells were harvested from culture medium and washed in demineralized water for removing the external germanium from diatom frustules.

Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM)

The synthesised nanocomposite was dried in hot air oven before the SEM and TEM analysis. Platinum was used as a coating material for SEM analysis.JSM-5610LV (JEOL Ltd., Tokyo, Japan) SEM instrument facility was provided by department of manufacturing engineering, Annamalai University for the analysis. TEM analysis was carried out by FEI Tecnai G2T2S-Twin TEM microscope (FEI Tecnai, USA) operating at 200 kV. TEM instrument facility was provided by VIT University for the analysis.

X-ray diffraction (XRD)

X-ray diffraction measurement was done by using Panalytical X’pert PRO powder X-ray diffractometer instrument using Cu Kα radiation and operating at a 15 KVA UPS support. XRD instrument facility was provided by Alagappa University.

Antibacterial activity

The biosynthesized silica germanium nanomaterial was used for antibacterial activity by agar well diffusion method. Both gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*) bacterial strains were used in this study. All the bacterial strains were cultured in nutrient broth at 35 °C for 24 h. The live bacterial culture was prepared before 24 h from the study period. The cultured live bacterial strain was inoculated in nutrient agar plates using sterile cotton. Three wells per plate were made. The each plates wells were filled with 25, 50, 75 µl of biogenic silica germanium nanomaterial using micropipette. Tetracycline TE-30 antibiotic disk are used for Control. Biogenic nanomaterial loaded agar plates were incubated at 35°C for 24 hours. After 24 hours incubation, the inhibition zone was measured around each loaded wells. The inhibition zones were called as antibacterial activity zone of the test material.

Results and Discussion

Marine diatom *Coscinodiscus sp*. (Fig-1(a)) was selected for biogenic Si-Ge nanomaterial synthesis for its well fine structure and low cost. In two steps culture was successfully carried out with silica and germanium compound in nutrient. The live *Coscinodiscus* sp. was uptake the silica with germanium. Soluble germanium in second step culture medium inhibits diatoms cell division [18].

Light microscope and SEM images of Coscinodiscus sp.

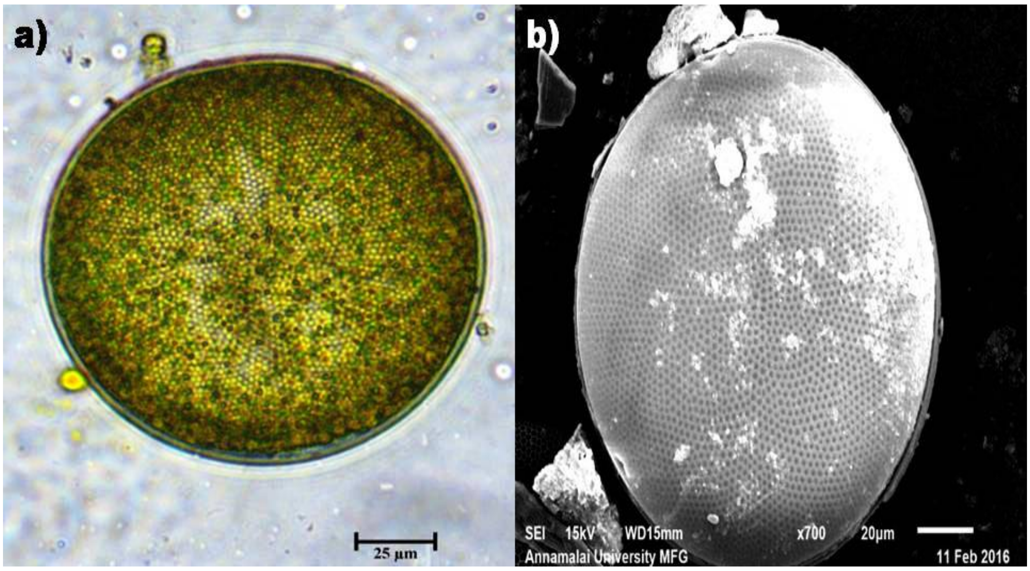


Figure-1: a) Shows the light microscopy image of *Coscinodiscus* sp. **b) SEM image of *Coscinodiscus* sp. frustule with Ge.**

So diatom multiplications were totally affected in second step culture. The Ge is biologically inserted into naturally made Si nanomaterial contained diatom frustule. The two steps culturing processes are used for the Si-Ge nanocomposite synthesis by marine diatom. The Si-Ge is very complicated metabolism of Ge transported into the cell wall and Ge bonded with Si cell wall [19]. In previously, some of the study carried out for role of Ge incorporation into diatom frustule using radiolabelled^{68}Ge. Moreover, the Ge intake was metabolically similar to Si intake profile [20, 21]. Diatom frustules made by amorphous silica, so Ge also slotted into diatom frustule in amorphous [22].

TEM images of diatoms frustules mesopore structure

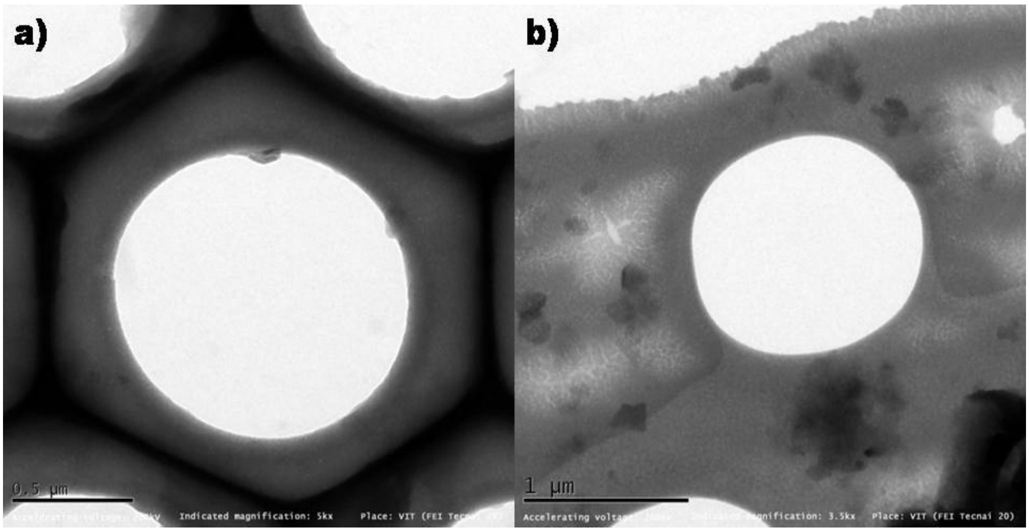
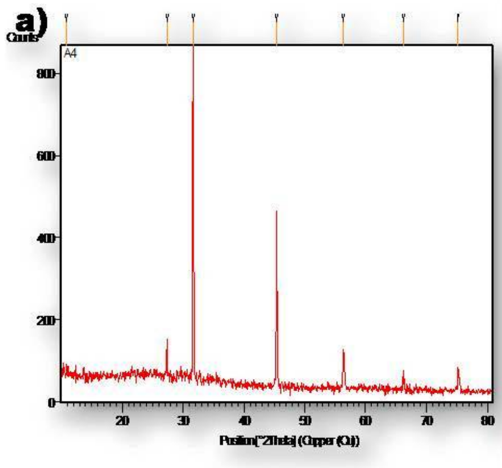


Figure-2: a) TEM image of normal diatoms frustules mesopore structure **b) TEM image of diatoms with Ge**

In electron microscopy analysis, insert of the germanium into the diatom cells was realized without any changes in morphology shows in SEM image (Fig-1(b)). The TEM images fig 2(a) and fig-2(b) shows the mesopore structure of diatom frustules.Fig-2(a) is a TEM image of normal mesopore structure of diatom frustules. TEM image fig-2(b) differs from Fig-2(a).Both TEM images offer lot of information about diatom frustule with Ge. From the fig-2(b), the Ge dark spots are present in the diatom frustule. Ge is given the some deviation in mesopore diameters and strength of diatom frustule. Normally the mesopore diameter is 200nm but Ge presented diatom frustule mesopore diameter reduced to in 100nm. Previously, some of the studies demonstrate with assimilation of Ge interested into biosilica made frustule of the diatom abbreviated the size of the pores but the pores did not combine each other [11]. In fig-2(b) some of the abnormal small pore are present in the diatom frustule and also strength of the diatom frustule weaker then compared to normal one. Because unavailability of adequate silica for frustules development. Particularly, the nanostructured silica attached together and formed primary pore arrays like a hexagonal structure, the creation of secondary pore arrays filled the bottom of each primary pores [23].Some deference in angles and strength of the bond between SiO_2 and GeO_2 probably cause the weakness in exoskeleton and easy broken the frustule [24]. In previous observation, the diatom cells were very high aberration in high volume of Ge compared to low Ge in culture medium at Si-Ge nanocomposite synthesis [25].

XRD analysis and antibacterial assay



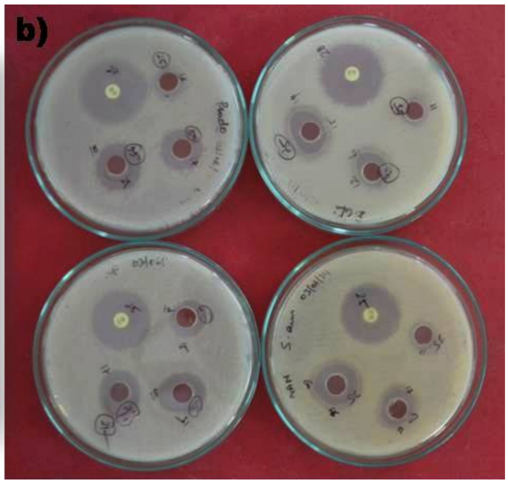


Figure-3: a). XRD pattern of biosynthesized Si-Ge. **b). Antibacterial activity study of biogenic Si-Ge nanocomposite.**

Biosynthesized Si-Ge nanocomposite was analyzed by XRD. The figure 3(a) shows the XRD pattern displays the cubic phases of Si and Ge. From the above (Figure-3a), the Ge matrix with silicon phase. The seven peaks were appeared in 2θ at 10.81, 27.33, 31.69, 45.42, 56.41, 66.27 and 75.21respectively. These peaks were conformed the cubic phases of elemental Si and Ge. The biogenic amorphous Si-Ge nanocomposite present in crystalline form [26].

Table-1: Antibacterial activity of biogenic Si-Ge nanomaterial.

Bacterial strain	Inhibition zone in mm			
	TE 30	Biogenic Si-Ge nanomaterial		
	control	**25 µl**	**50 µl**	**75 µl**
Staphylococcus aureus (+)	25	11	17	21
Escherichia coli (-)	28	11	15	19
Pseudomonas aeruginosa (-)	27	13	17	21
Salmonella typhi (-)	25	12	18	21

The antibacterial activity of biogenic Si-Ge nanomaterial was studied by disk diffusion method. Fig-4 clearly shows that the biogenic Si-Ge nanomaterial demonstrates antibacterial effects to the formation of inhibition zones in bacterial strains inoculated agar plates. Table-1 shows the diameter of inhibition zones. Very high inhibition zones of 21 mm, 19 mm, 21 mm and 21 mm formed in *S. aureus, E. coli, P. aeruginosa and S. typhi* respectively in 75 µl of biogenic Si-Ge nanomaterial. Si-Ge nanomaterial acts as a good inhibitor against bacterial infections. The inhibition of bacterial growth may be depended on the concentration of nanocomposite and organic matters from diatom. Interaction between bacterial cell wall and nanomaterial are very easy by abundance of bacterial charge [27]. Previously lot of experiments was explaining the antibacterial activity from microalgae in both gram negative and gram positive bacteria [28].

Conclusion

The biosynthesis of Si-Ge nanocomposite potentially available by tow stage diatom culture. Ge was very easily induced in diatoms frustule. Si and Ge bond affinity more or similar to Si and Si. So unavailability of Si in culture medium, diatom was intake the Ge for his frustule development. This diatom Si metabolic process is used for Si-Ge nanocomposite biosynthesis. In my knowledge, first time we report the biosynthesised Si-Ge nanocomposite by marine diatom used for antibacterial study. Biogenic Si-Ge nanocomposite is given the significant antibacterial activity against both gram positive and negative bacteria. From the above, biogenic Si-Ge nanocomposite are used for lot of application in biomedical filed near future.

Conflict of interest

The authors declare no conflict of interest.

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