SYNTHESIS AND EVALUATION OF ANTIBACTERIAL ACTIVITIES OF BIOGENIC SILVER NANOPARTICLES FROM BACTERIAL ISOLATES OF LONAR LAKE

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ABSTARCT

Lonar lake is a unique ecosystem formed by meteorite impact, which is located at Deccan Plateau of West-central of India. In the present study, water and sediment sample were collected from different sites of Lonar Lake. All these samples were streak on Horikoshi media B, cultures were maintained as stocks The pure bacterial isolates were grown in nutrient medium containing $AgNO_3$ substrate for the biosynthesis of silver nanoparticles. The antibacterial activity of these crude silver nanoparticles produced by alkaliphilic bacteria were studied against pathogenic bacteria such as Staphylococcus aureus and Escherichia coli. The zone of inhibition shown by isolates code no. W1A, W1B, W1C, and W2B against S. aureus was found to be 28 mm, 28 mm, 25 mm and 25 mm, and against E. coli all 4 isolates shown 20 mm of zone each. The zone of inhibition shown by silver nanoparticles is significant in comparison with traditional antibacterial agents. The 16S rRNA sequencing of these bacterial isolates were carried out. After the 16S rRNA sequencing the studied isolate code W2B, W2C, W1B, W1C were confirmed as Bacillis cohnii strain D7048, Bacillis cohnii strain GUFBSS253-2, Bacillus polygoni, Bacillis siralis, respectively.

Keywords *- Biogenic silver nanoparticles, antibacterial activity, Lonar lake.*

Introduction

Lonar lake is a unique ecosystem formed by meteorite impact, which is located at Deccan Plateau of West-central of India. Lonar Crater is filled with saline water and the uniqueness of water is its salinity and high alkalinity[1]. Lonar lake harbors diverse microorganisms having potential to produce various biologically active compounds which have potential of pharmaceutical and biotechnological application[2].

Metal nanoparticles produced by nanotechnology have received global attention due to their extensive applications in the biomedical and physiochemical fields [3]. Metals shows antimicrobial potential against the pathogenic microorganisms. The ability of microorganisms to reduce the inorganic metal has opened up an exciting eco-friendly approach towards development of green nanotechnology. The microbial recovery of precious metals with the formation of their nanoparticles is a green alternative to the conventional method[4].. Although silver nanoparticles are widely used in a variety of commercial products. There have been several studies that describe the in vitro toxicity of silver nanoparticles to a variety of different organs, including the lung, liver, skin, brain, and reproductive organs [5]. Antibiotic resisitance is burning problem all over the globe and these bacterial AgNPs shows strong antibacterial activity against pathogenic bacteria [6].

Lonar lake is wonder jewel of the earth. We are blessed to have Lonar crater in our country especially in Maharashtra. Microbiologist have found variety of microorganisms like silver nanoparticles producing bacteria, magnetic bacteria in the lake. The biosynthesis of silver nanoparticles from bacteria which is used as antibacterial agent is cost effective and environmental friendly process.

Materials and methods

A) Enrichment, Isolation and identification of bacterial isolates – Two water and 1 sediment were collected from different sites of Lonar lake. Horikoshi medium A, B & C were used for enrichment of the cultures[7]. Isolated Bacillus species were identified by cultural, morphological, biochemical tests. From these three sample morphologically different bacterial isolates were isolated. The bacteria isolated from water were designated as water sample 1 and water sample 2, for water sample 1-W1A, W1B, W1C, for water sample 2 - W2A, W2B, W2C and bacteria isolated from sediment designated as SA and SB.

B) Synthesis, Characterization and Antibacterial activity of crude silver nanoparticles (AgNPs) - Total 8 isolates collected from Lonar lake were sub-cultured in test tube containing 10 mL of nutrient broth containing 3.5 mM $AgNO_3$. The inoculated broth incubated at dark condition at room temperature for 15 days. After incubation period upon visual observation, the culture incubated in presence of silver nitrate. Along with these the control experiment was also run without $AgNO_3$.The biosynthesized silver nanoparticles from bacteria isolated from Lonar lake were screened against one Gram – positive and one Gram - negative bacteria such as *S. aureus and E. coli* respectively. The method used for antibacterial potential was well diffusion method on nutrient agar. Zone of inhibition showed by silver nanoparticles against pathogenic bacteria were measured.

C) Identification of bacterial cultures – Silver nanoparticles producing bacterial cultures were identified by using 16S rRNA of bacterial small subunit rRNA genes were amplified by PCR using primers. The rRNA gene insets were sequenced on AB1 sequencer which could be viewed by using softwares like Finch TV, BioEdit, ChromasLite, SeqScanner, etc. Quality of obtained sequence observed through Electropherogram peaks. Sequence analysis was done using BLAST server or server related to specific database at Chromgene pvt Ltd. Banglore .

Result

In the present study, total three samples comprising of two water and one sediment were collected from different sites of alkaline Lonar Lake, India. In the winter season December 2018. From these samples 8 morphologically different colonies were isolated. The isolates coded as W1A, W1B, W1C, W2A, W2B, W2C and SA, SB. Out of 8 isolates only 4 isolates were found to be efficient for production of silver nanoparticles were isolated from water and it was found that the isolates which were isolated from soil not able to produce silver nanoparticles. The isolate code W1A, W1B, W1C, W2B were biosynthesized the silver nanoparticles by reducing silver nitrate. The preliminary identification of biosynthesized silver nanoparticles was carried out by visual detection of color change from yellow to brown, which indicates the formation of silver nanoparticles.

The supernatant collected after centrifugation was used further for antibacterial activity against *E.coli* and *S.aureus* by Well Diffusion Method. All four isolates shows the zone of inhibition against *E.coli* and *S.aureus*.

For comparision, along with these control experiment also carried out by using only bacterial suspension & only silver nitrate but no zone of inhibition was observed against *S.aureus* & *E. coli*.

Table 1 - Zone of inhibition against *S.aureus* & *E. coli*

Culture code	Zone of inhibition showed by Bacterial AgNPs (mm) against studied bacteria	
	S.aureus	*E. coli*
W1A	28 mm	20 mm
W1B	28 mm	20 mm
W1C	25 mm	20 mm
W2B	25 mm	20 mm

16S rRNA sequencing and phylogenetic analysis of isolate code W1B, W2B, W1C & W2C

Identified species (BLASTn comparison):

1) W1B

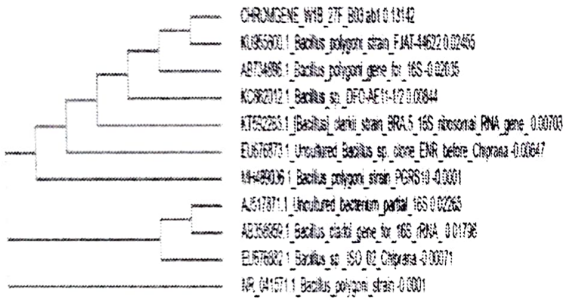
Hit 1: *Bacillus polygoni gene for 16S rRNA, partial sequence, strain: U0305*
Identity: 90.95%
Coverage: 82%
E-value: 5e-68
Accession No: AB734898.1

2) W1C

Hit 1: *Bacillus siralis strain FJAT-45146*
16S ribosomal RNA, partial sequence
Identity: 97.61%
Coverage: 92%
E-value: 1e-159
Accession No: KR514577.1

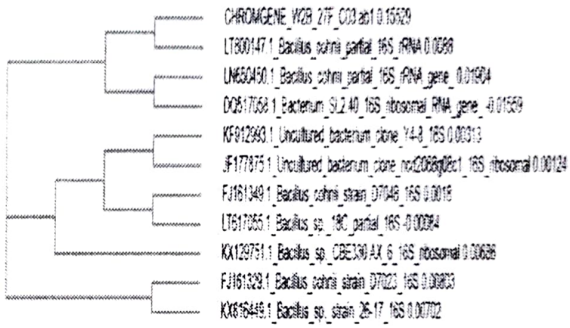
3) W2B

Hit 1:*Bacillus cohnii strain D7048*
16S ribosomal RNA gene, partial sequence
Identity: 89.54%
Coverage: 94%
E-value: 0.0
Accession No: FJ161349.1



4) W2C

Hit 1: *Bacillus cohnii strain GUFBSS253-2*
16S ribosomal RNA gene, partial sequence
Identity: 97.04%
Coverage: 92%
E-value: 0.0
Accession No: JN315891.1



Discussion

Yanhe et al.[8] worked on Baer Soda Lake located in the Hulunbeir area of inner Mangolia, Region of China number of diverse bacteria of Baer Soda Lake was characterized using culture and molecular methods. In present study isolation of bacteria was done from alkaline Lonar Lake. 16S rRNA sequencing and phylogenetic analysis isolates were done. Kanekar et al.[9]worked on the Lonar Lake, India to identified the bacterial diversity present in the lake. They collected water and sediment sample from the various sites of lake. Isolation of bacteria from samples was technique. 16S rRNA sequencing and phylogenetic analysis were carried out.
Alkalibacillus haloalkaliphilus was the first report of obligately alkaliphilic organism from Lonar lake. In the present study, 3 samples were collected and from this samples total 8 isolates were isolated. Isolation of bacteria were carried out by using different alkaline medium. 16S rRNA sequencing phylogenetic analysis were also carried out. Shivakrishna et al.[10]worked on synthesis of silver nanoparticles from Marine Bacteria *P. aerogenosa,* the marine sample was collected from Nellore Coast, Andhra Pradesh, India. Bacterial stain was grown in Zobell Marine broth for biosynthesis of silver nanoparticles with various concentration of $AgNO^3$ The synthesized silver nanoparticles was observed through UV- visible spectroscopy analysis. In present study samples were collected from alkaline Lonar Lake and subjected for synthesis of silver nanoparticles. The production of silver nanoparticles were detected by visual detection. In the work of Tayde, [11] Antibacterial Potential of silver nanoparticles produced from Lonar Lake *Bacilli, Bacilli* collected from Lonar Lake were studied. The isolates were grown on nutrient agar containing 3.5 mM $AgNO_3$ under dark condition. For the preparation of silver nanoparticles isolated *Bacilli* were grown on

nutrient broth containing $AgNO_3$ and incubated for 7 days at 37°C. After 7 days upon visual observation, the culture incubated in the presence of silver nitrate showed a color changes from yellow to Brown. After the synthesis of silver nanoparticles the supernatant of *Bacilli* were used further for the antibacterial activity. In the present study, four silver nanoparticles synthesizing bacteria were isolated. The bacterial isolated were incubated in the presence of $AgNO_3$ for 15 days after 15 days the colour of broth were changed yellow to brown. The broth were centrifuged at 3000 rpm for 20 minutes. The supernatant was used further for antibacterial activity against pathogenic bacteria. Rathod et al.[12] the actinobacterium *Nocardiopsis valliformis* OT 1 strain isolated from soil collected from the rim of Lonar Lake. In present study silver nanoparticles producing bacterium were isolated from water sample of Lonar crater. The four isolates were showed efficient production of AgNPs. 16S rRNA sequencing and phylogenetic analysis of isolates were carried out.

Conclusion

From above results, it was concluded that the bacterium were present in alkaline Lonar Lake has great potential. These isolates were capable for production of silver nanoparticles.. Out of 8 isolates 4 were gives very predominant antimicrobial activity in which W1A, W1B, W1C, W2B showed strong antimicrobial activity. Our study provides primary verification that Lonar Lake isolates were promising source for silver nanoparticles as antimicrobial substance. Alkaline bacteria producers of potent metabollic compounds used commercially as antibiotics and other novel drugs. Lonar Lake has the capacity to provide active and high amount silver nanoparticles which have potential in medicinal and pharmaceutical industry. The biosynthesis of silver nanoparticles from Lonar Lake bacteria is eco- friendly and commercially easy process and it can potentially recovers the severe problems caused by chemical antimicrobial agents. It is need to be further studied for its potency and broad spectrum activity for improvement in the antimicrobial activity and production of new drugs.

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