ANTIBACTERIAL POTENTIAL OF SILVER NANOPARTICLE PRODUCED FROM LONAR LAKE BACILLI

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ABSTRACT

Lonar Lake originated meteorite impact creator. The uniqueness of the lake is its salinity and alkalinity. The microbial ecosystem prevailing in the Lonar Lake has been study but antibacterial activity of silver nanoparticles produced from bacillus sp. has not been studied in details. In the present study, biosynthesis of silver nanoparticles from *bacillus* sp. from Lonar Lake is performed and antibacterial potential of silver nanoparticles against the pathogenic bacteria was carried out by using disc diffusion method.

Keywords: Silver nanoparticle, *Bacillus* sp., Lonar Lake, antimicrobial activity

INTRODUCTION

Lonar Lake (19°59' N, 76°34' E) is a unique feature in the Deccan traps in Buldhana District of Maharashtra State, India. The lake was first noticed in 1829 by British officer CJE Alexander. In 1896, American geologist, G. K. Gilbert conducted studies to prove that the Lonar was created due to meteor strikes (Newsom *et al,* 1986). Nearly 50,000 years ago, a meteorite of about 100m diameter and weighing about 2 million tones, hit the Deccan plateau. Traveling at a velocity of 19 km per beyond, the collision of this meteorite generated such an intense heat that it instantly created a molten pool of rocks. The molten mass of rocks, together with some non-melted material, was thrown out by the rebound force created by the impact (Shoemaker, 1963; Bhandari, 1984). It is the third largest meteorite crater in basaltic rock in the world, with a diameter of 1,800m. It comes after Bosmatvi Lake in Ghana, which has a diameter of 10,000 m and New Quebec in Canada with diameter of 3,500 m (Nandy and Deo, 1961).

Due to its uniqueness and its larger size it has become one of the most important craters in the world (Taiwade, 1995; Malu *et al,* 2000). Lonar crater is filled with saline water. The uniqueness of the lake water is it salinity and high alkalinity. Alkalinity environments show diverse flora of alkaliphilic microbial culture growing at pH 8 to 10 and some at high salt concentration (haloalkaliphiles requiring up to 33% NaCl along with Na_2CO_3). The alkaliphilic bacteria isolated from various alkaline environments include those belonging to the genera of *Bacillus* sp., *Flavobacterium* sp., *Pseudomonas* sp., *Aeromonas* sp., *Corynebacterium* sp., *Micrococcus* sp., *Archenohacteria* as *Natronobacterium* and *Natrococcus, anopxygenic phototrophic* bacteria as *Ectothiorhodospira, cynobacteria* are responsible for bloom formation as *Anabenopsis* sp., *Pleurocapsa spirulina* sp., *Arthospora* sp., etc. Many of alkaliphilic cultures capable of producing Silver nanoparticals which have medical application as antibacterial agent, Pharmacology, human and veterinary medicine, food industry, water purification, catalyst in a chemical reaction, biosensors, Drug formulation etc. (Silambarasan and Abraham, 2012; Shahverdi *et al.*, 2007). Hence attempt was made to isolate the bacillus species. In the present study, biosynthesis of silver nanoparticles from *bacillus* sp. from Lonar Lake is performed and antibacterial potential of silver nanoparticles against the pathogenic bacteria was carried out by using disc diffusion method.

MATERIALS AND METHODS

Bacterial strain and growth condition: Isolated *bacilli* collected from Lonar Lake, Maharashtra, were grown on nutrient agar substrate containing 3.5 mM $AgNO_3$ under dark condition at 37^0C. The isolated bacteria were identified as *Bacillus* sp. On the basis of morphological and biochemical characteristics using standard methods, the bacterial identifications were performed (Murray, 2005).

Preparation of silver nanoparticles: The isolated colony was sub-cultured into 50 ml of nutrient broth containing 3.5 mM $AgNO_3$.

The broth was inoculated with a loopful of bacteria and incubated for period of 7 days in darkness at room temperature. After 7 days upon visual observation, the culture incubated in the presence of silver nitrate showed a color changes from yellow to brown (Pugazhenthiran *et al.*, 2009).

For comparison, Petri dishes containing only the culture without silver nitrate solution and only silver nitrate without culture were incubated under similar experimental condition. These control experiments indicate that no color change could be observed in culture without silver nitrate and silver nitrate solution without the culture. This control experiment indicates that the Ag^+ ion reduction is not just a thermal process it is also a biological process (Gong *et al.*, 2007).

RESULTS AND DISCUSSION

Total twelve samples were collected from Lonar Lake comprising of eight water and samples and four sediment samples. From these samples ten morphologically different colonies were isolated. Out of these ten samples were isolated, eight bacilli were collected from water sample and two were isolated from sediment. Out of these ten bacilli nine were gram positive and one was gram negative , seven were long rod, two short rod and one was filamentous long rod, nine were spore forming and one was non spore forming. The position of eight bacilli was central, one was terminal, six bacilli were opaque in shape, two were ellipsoidal, one was spherical and the shape of one bacillus was unknown. All these ten bacilli were motile, all were catalase positive, and nine were oxidase positive and one oxidase negative. All were indole negative, MR negative eight were VP negative, two were VP positive, all ten bacilli were citrate negative, nine were glucose negative, one was glucose positive. All ten isolates were arabinose negative, nine were mannitol negative and one was mannitol positive, all ten bacilli were xylose negative.

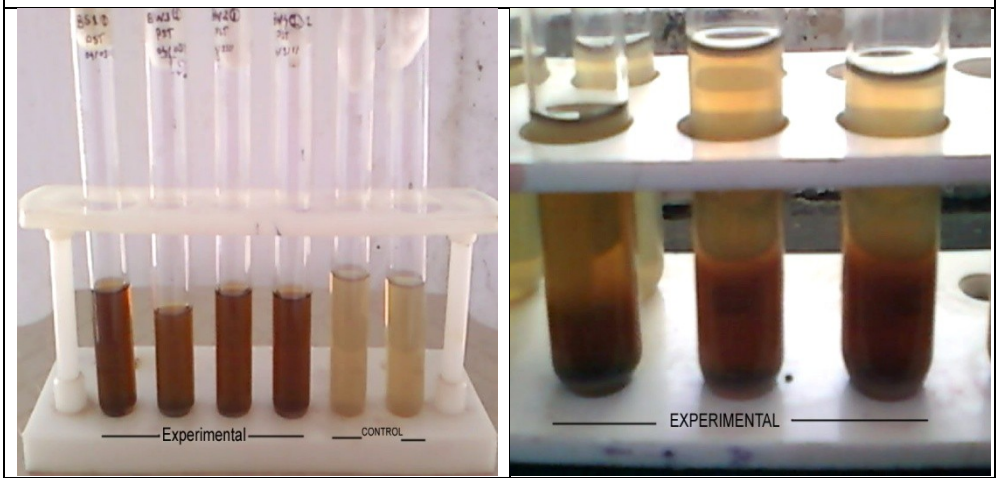
Table 1: Characteristics of isolated gram positive, spore bearing bacterial bacillus spp

Sample	Isolation code	Pigment	Colony Shape	Edge	Internal structure	Shape Of Bacteria	Spore		motility	Catalase	Oxidase	Indol	MR	VP	Citrate	glucose	Arabinose	Mannitol	Xylose	Identification of Culture based on 16S rRNA
							Position	Shape												
Water	BW2(1)	White	Circular	Entire	Wavy Interlaced	LR	C	O	+	+	+	-	-	-	-	-	-	-	-	*B. alkalophillous*
Sediment	BS1(2)	Colorless	Circular	Entire	Transparent	SR	C	O	+	+	+	-	-	-	-	-	-	-	-	*V. marismortui*
Water	BW1(1)	White	Circular	Entire	Transparent	LR	C	O	+	+	+	-	-	-	-	-	-	-	-	*V. marismortui*
Water	BW4(3)	Colourless	Circular	Entire	Wavy interlaced	LR	C	O	+	+	+	-	-	-	-	-	-	-	-	*B. halodurans*
Water	BW4(4)	Colorless	Circular	Entire	Transparent	LR	C	S	+	+	+	-	-	-	-	-	-	-	-	*V. marismortui*
Sediment	BS1(1)	Colourless	Circular	Entire	Transparent	LR	C	O	+	+	+	-	-	+	-	+	-	+	-	*B. pseudofirmus*
Water	BW3(2)	White	Circular	Entire	Transparent	LR	T	E	+	+	+	-	-	+	-	-	-	-	-	*B. clausii*
Water	BW4(1)	Colourless	Circular	Entire	Transparent	SR	-	-	+	+	+	-	-	-	-	-	-	-	-	*P. aerophilum*
Water	BW4(1)1	Colourless	Curled	cerenate	Wavy Interlaced	FLR	C	E	+	+	+	-	-	-	-	-	-	-	-	*B. okuhidensis*
Water	OBW3(2)	Colourless	Circular	Entire	Transparent	LR	C	O	+	+	-	-	-	-	-	-	-	-	-	*B. pseudofirmus*

After synthesis of silver nanoparticals the supernatant of these bacilli were used further for the antibacterial activity against *E. coli* by disc diffusion method. *Bacillus psuedofirmus* shows different zone of inhibition after antibacterial activity by disc diffusion method. These were 13, 15, 17, 20 and 21mm in Ag, Ag+culture, Culture Sup., Antibiotic disc and standard antibiotic streptomycin disc with Ag respectively. The zone of inhibition was maximum in culture supernatant that was 17mm. *B. clausii* shows different zone of inhibition after antibacterial activity by disc diffusion method. This were 10, 12, 15, 20 and 20mm in Ag, Ag+culture, Culture Sup., Antibiotic disc and standard antibiotic streptomycin disc with Ag respectively. The zone of inhibition was maximum in culture supernatant that was 15mm.

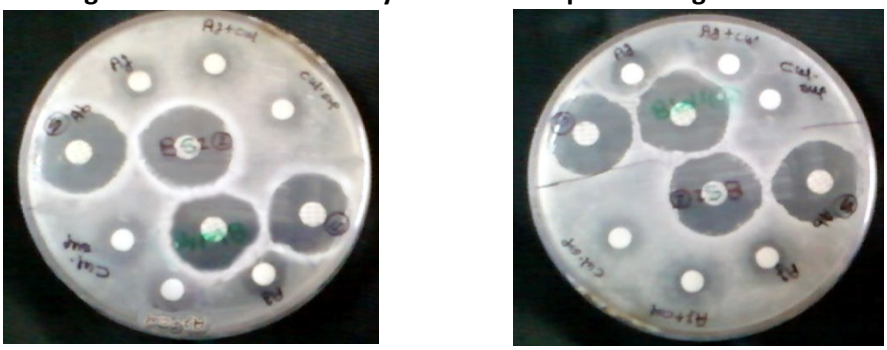
Pyrobaculum aerophilum shows different zone of inhibition after antibacterial activity by disc diffusion method. These were 8, 10, 13, 20 and 20mm in Ag, Ag+culture, Culture Sup., Antibiotic disc and standard antibiotic streptomycin disc with Ag respectively. The zone of inhibition was maximum in culture supernatant that was 13mm. *Bacillus alcalophilous* shows different zone of inhibition after antibacterial activity by disc diffusion method. These are 9, 9, 11, 17 and 23mm in Ag, Ag+culture, Culture Sup., Antibiotic disc and standard antibiotic streptomycin disc with Ag respectively. The zone of inhibition was maximum in culture supernatant that was 11mm. *Bacillus okuhidensis* shows different zone of inhibition after antibacterial activity by disc diffusion method. These were 13, 14, 16, 20 and 22mm in Ag, Ag+culture, Culture Sup., Antibiotic disc and standard antibiotic streptomycin disc with Ag respectively. The zone of inhibition was maximum in culture supernatant that was 16mm.

Fig1: Brown color shows synthesis of Silver nanoparticles while Yellow color broth indicated as control.



Virgibacillus marismortui shows different zone of inhibition after antibacterial activity by disc diffusion method. These were 10, 10, 15, 19 and 20mm in Ag, Ag+culture, Culture Sup., Antibiotic disc and standard antibiotic streptomycin disc with Ag respectively. The zone of inhibition was maximum in culture supernatant that was 15mm. *Bacillus halodurans* shows different zone of inhibition after antibacterial activity by disc diffusion method. These were 0, 12, 15, 23 and 25mm in Ag, Ag+culture, Culture Sup., Antibiotic disc and standard antibiotic streptomycin disc with Ag respectively. The zone of inhibition is maximum in culture supernatant that was 15mm. *Pyrobaculum aerophilum* shows different zone of inhibition after antibacterial activity by disc diffusion method. These were 8, 10, 13, 20 and 20mm in Ag, Ag+culture, Culture Sup., Antibiotic disc and standard antibiotic streptomycin disc with Ag respectively. The zone of inhibition was maximum in culture supernatant that was 13mm

Fig 2: Antibacterial activity of silver nanoparticles against *E.coli*



In conclusion we reported the antibacterial activity of Silver nanoparticals over multidrug resistant bacteria's. The biosynthesis route seems to be eco- friendly and easy to scale up the process. Thus, these Silver nanoparticals may prove as a better candidate for drugs and can potentially eliminate the problem of chemical agents because of their biogenic nature. All these eight bacilli that was *Bacillus pseudofirmus, Bacillus clausii, Pyrobaculum aerophilum, Bacillus alcalophilus, Bacillus okuhidensis, Bacillus pseudofirmus, Virgibacillus marismortui* and *Bacillus halodurans* after antibacterial activity the maximum zone of inhibition was 17mm, 15mm, 13mm, 11mm, 16mm, 13mm, 15mm and 15mm respectively in the bacterial culture supernatant.

Thus it was proven from the study that the silver nanoparticles synthesized from bacillus species from Lonar Lake seems to be promising and effective antibacterial agent against the human pathogenic bacteria. These silver nanoparticle producing bacilli may be useful for drug formulation in medicine.

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