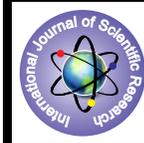


Expeditious Synthesis of Silver Nanoparticles By A Novel Strain *Sporosarcina pasteurii* SRMNP1 and Patrocladogram Analysis For Exploration of its Closely Related Species



Engineering

KEYWORDS : *Sporosarcina pasteurii* SRMNP1, Silver Nanoparticles, SEM-EDAX, Patrocladogram Analysis, Expeditious synthesis

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ABSTRACT

Sporosarcina pasteurii is known for its calcite precipitation property and is being explored in biological cementing industries. The present study involves biological expeditious synthesis of silver nanoparticles by the bacteria in cost effective manner in less than 18 hours. The property of silver tolerance and reduction of *Sporosarcina pasteurii* SRMNP1 (Accession number KF214757) strain is explored for the first time. The bacteria were subjected to different concentrations of silver nitrate and maximum tolerance was observed at 20 mM. The reduction of silver nitrate was indicated by the colour change of solution from whitish to brown. The resultant biomass after 18 hours of incubation was dried and lyophilized and characterized using SEM and EDAX. The analysis shows that the bacteria possess crystalline silver nanoparticles on their surface which shows the extracellular synthesis of silver by the bacteria. The study concludes that the *Sporosarcina pasteurii* SRMNP1 can be used as a potential source for biological synthesis of silver nanoparticles and further studies can also be made to explore the metal tolerance property of the organism. The evolutionarily close related species can also be explored for the bio-synthesis of silver nanoparticle in near future.

Introduction:

The bio-synthesis of nanoparticles has received increasing attention due to a growing need to develop environmentally benign technology in material synthesis. The availability of silver nanoparticles has ensured a manifold application in molecular diagnostics, anti microbial activity and therapeutic uses etc [1]. Therefore attempts have been made to synthesize silver nanoparticles using both chemical and biological methods. Microorganisms are recently found as possible Eco friendly nano factories [2]. The biosynthesis of silver nanoparticles employs use of biological agents like bacteria, yeast, and fungi [3]. The ability of microorganisms to grow in the presence of high metal concentration might result from specific mechanism of resistance, such as efflux system, alteration of solubility and toxicity by changes in the redox state of the metal ions and extracellular complexation [4]. This study presents data on the synthesis of silver nanoparticles by *Sporosarcina pasteurii* (Accession number KF214757) formerly known as *Bacillus pasteurii*, a gram positive, aerobic endospore forming soil bacteria. It is known for its property of precipitating calcite and solidifying sand through the process of biological cementation. This work led to the exploration of silver reducing property of *Sporosarcina pasteurii* and its active role in extracellular synthesis of silver nanoparticle. The extracellular bio-synthesis of silver nanoparticles can be an attractive method for large scale synthesis because it offers the advantage of easy downstream processing [5]. The bacteria can synthesize Ag particles in less time compared to other microorganisms. Thus it can be used as a potential source in silver nanoparticles biosynthesis in future applications.

Partrocladogram construction is an *insilico* approach to find the evolutionarily close related species, which may exhibit maximum similar characteristic features. This study was also aimed in finding the closely related species of *S.pasteurii* and to identify the applications of the related species in various fields as that of *S.pasteurii*.

2. Materials and methods:

2.1 Culturing of *Sporosarcina pasteurii* SRMNP1

Sporosarcina pasteurii SRMNP1 was inoculated into 100 ml of LB broth (pH 7.0) in Erlenmeyer flask and the culture was grown with continuous shaking on shaker at 150 rpm, 37°C overnight. The grown culture was harvested by centrifugation at 8000rpm, 4°C for 10 min and washed thrice with double distilled water under aseptic conditions to remove the remaining media content.

2.2 Extracellular synthesis of Ag nanoparticles

1gm of the biomass was taken and added with filter sterilized Silver Nitrate solution with varying concentrations 0.17g and 0.34g (10mM and 20mM) to two different flasks containing 100 ml sterile double distilled water each respectively. These flasks were covered with aluminium foil and incubated at 37°C in shaker at 150 rpm. The colour of the culture turned from white to brown indicating the reduction of silver nitrate. Controls were kept to ensure that the change is mediated only by the extracellular enzymes of the bacteria. The bacteria showed maximum tolerance at 20mM and reduced the silver nitrate solution to crystalline silver nanoparticles. The biomass was harvested by centrifugation and lyophilized for further characterization studies.

2.3 Sample preparation for SEM-EDAX analysis

The lyophilized sample was dissolved in autoclaved PBS and smeared it on autoclaved aluminium foil and dried overnight under aseptic conditions. Then the thin bacterial smear with silver nanoparticles was coated with gold and subjected to SEM-EDAX analysis, which confirmed the presence of silver nanoparticles.

2.4 Patrocladogram construction

To find the close relatedness between the species, 16s rRNA sequence of all the species under the genus *Sporosarcina* was obtained from NCBI and multiple sequence alignment (MSA) was made using plug-in MAFFT v7.017 [6], E-INS-i algorithm and BLOSUM62 scoring matrix. Phylogenetic estimation was made by constructing maximum likelihood tree using PhyML 3.0 plug-in with Tamura-Nei substitution model. Based on the data, patrocladogram was constructed using the software MEGA 4.0 phylogenetic software [7].

3. Results:

3.1. Biosynthesis and characterization of silver nanoparticles

Sporosarcina pasteurii can synthesize extracellular silver (Ag) nanoparticles by reducing the silver nitrate ($AgNO_3$) which was provided as an external stress. The SEM image shows the formation of crystalline silver nanoparticles on the surface of the bacterium compared to control bacteria which were not subjected to any silver nitrate stress (Figure.1. A&B). The bacterium was known for precipitating calcite and also for solidifying sand given calcium source and urea [8]. For the first time the property of synthesis of silver nanoparticles by *Sporosarcina pasteurii* SRMNP1 is being explored in this study. The microorganism showed silver tolerance up to 20mM (data not shown) concentration of silver nitrate. To substantiate the formation of Ag silver nanoparticles, the sample was analysed by X ray Diffraction studies and the result shows the characteristic peak of crystalline silver which is absent in control sample (Figure.2a & 2b). The table.1a & 1b shows the elements present in control and treated samples, since it is a biological sample the occurrence of C, N, O and P are common. In addition to that the silver is seen in treated samples which confirm the presence of AgNPs. The Ag L in the table.1 indicates the diffraction of X ray by the electrons in the L shell of the silver atom, which is also a characteristic feature of AgNP.

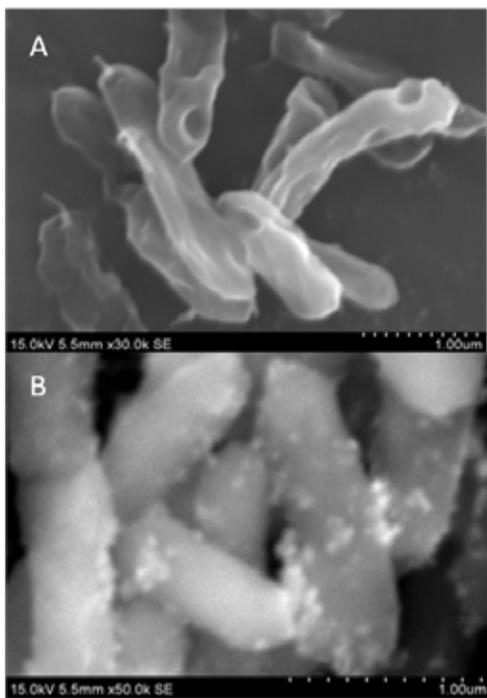


Figure.1. (A) SEM image of Control - *Sporosarcina pasteurii* SRMNP1 (B) SEM image of $AgNO_3$ treated *Sporosarcina pasteurii* SRMNP1.

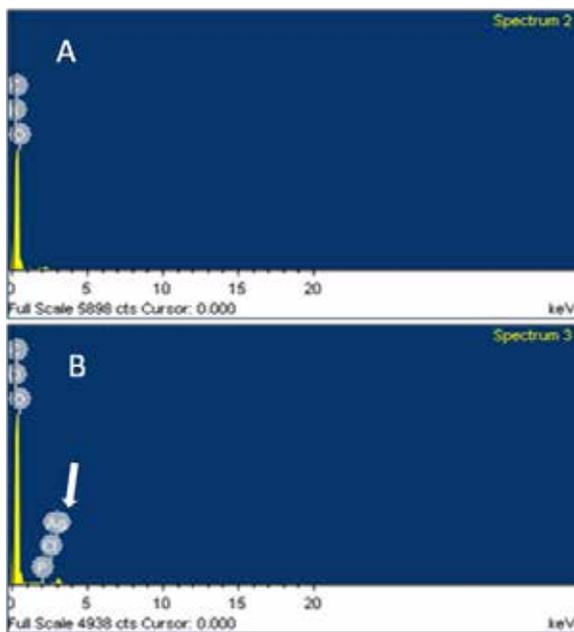


Figure.2. (A) - Energy-dispersive X-ray spectroscopy analysis of Control - *Sporosarcina pasteurii* SRMNP1 showing elemental peaks. (B) - Energy-dispersive X-ray spectroscopy analysis of $AgNO_3$ treated *Sporosarcina pasteurii* SRMNP1 showing Ag L peak.

A			B		
Element	Weight%	Atomic%	Element	Weight%	Atomic%
CK	80.64	84.79	CK	80.71	87.36
K	19.20	15.15	OK	14.68	11.93
KK	0.16	0.05	PK	0.27	0.11
			CK	0.31	0.11
Total	100.00	-	AgL	4.03	0.49
			Total	100.00	-

Table.1. (A) - Shows the distribution of elements in the control sample of *Sporosarcina pasteurii* SRMNP1.(B) - Shows the distribution of elements in $AgNO_3$ treated sample of *Sporosarcina pasteurii* SRMNP1.

3.2. Phylogentic analysis

Patrocladogram shows the two very closely related species *S.luteola*, *S.thermotolerans* with the Patrocladogram distance of 0 and 0.007 respectively (Figure.3). Thus the two species might possess all major characteristic features of the *S.pasteurii* and can be explored for different properties like calcite precipitation and silver nanoparticle synthesis.

4. Discussion:

Several reports state that the silver nanoparticles can be synthesized in a time span of 24hours [9] to 72 hours [10], whereas *Sporosarcina pasteurii* SRMNP1 synthesized the nanoparticles in less than 18 hours. The particles are synthesized extracellularly and it makes the downstream processing easy. *Sporosarcina pasteurii* is a non pathogenic aerobic bacteria, which makes it easy to culture and thus can be exploited widely in large scale basis for the synthesis of Silver Nanoparticles.

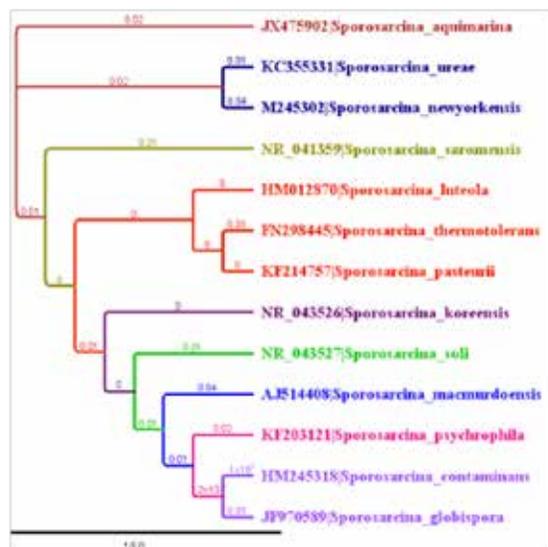


Figure.3 – Patrocladogram analysis of *Sporosarcina* species.

Silver nanoparticles exhibit anti-bacterial activity; they inhibit the cell division and damage the cell membrane and cellular contents of *Escherichia coli* and *Staphylococcus aureus* [11]. The antibacterial and antiviral actions of silver, silver ion, and silver compounds have been thoroughly investigated [12, 13]. A recent study reported the effect of biogenic silver nanoparticles in cancer treatment [14] and its wider application in imaging and targeted drug delivery [15,16] hence the organism can be explored for its role in all possible ways. *Sporosarcina pasteurii* also possess calcite-precipitating properties so based on patrocladogram the following *Sporosarcina* species, *S.luteola* and *S.thermotolerans* might have the same properties of reducing silver nitrate to silver nanoparticle and calcite precipita-

tion. These two species were identified recently and not much work has been carried out. *S.luteola* was first reported in the year 2009 [17] and *S.thermotolerans* in the year 2010 [18]. It is promising that the approach of Phylogenetic analysis may help in exploration of similar properties between closely related species.

5. Conclusion:

This study paves way to explore the properties of silver reduction and expeditious biosynthesis of Ag nanoparticles by a novel organism *Sporosarcina pasteurii* SRMNP1. The microorganism is very rapid in reducing the silver nitrate and forming AgNPs, so industries can consider this organism as a potential source to synthesize AgNPs in a quicker rate. The patrocladogram analysis finds two more closely related bacteria which may possess similar properties and they could be exploited in near future for further applications. These two species (*S.luteola* and *S.thermotolerans*) are recently reported and no extensive studies are carried out with them, this article provides an idea to explore what kind of properties can be

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