WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 12, 447-457. Research Article ISSN 2277– 7105

INTRACELLULAR AND EXTRACELLULAR BIOSYNTHESIS OF SILVER NANOPARTICLES BY EXTREMOPHILIC BACTERIA

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Article Received on
27 Sept. 2016,

Revised on 18 Oct. 2016,
Accepted on 08 Nov. 2016

DOI:10.20959/wjpr201612-7158

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ABSTRACT

The development of reliable processes for the synthesis of silver nanomaterials is an important aspect of nanotechnology today. There is enormous interest in developing green synthesis procedures for production of silver nanoparticles by using biomimetic approaches. There are few reports in literature on the biosynthesis of silver nanoparticles using microorganisms such as fungi. In our research focus has been given to the production of silver nanoparticles by an efficient, eco friendly viable process for the synthesis of silver nanoparticles using three halophilic and alkalihpilic bacterial strains Ra3, CD3 and CD13, isolated from different sources. A significant result of this study is that all the three bacterial strain are synthesizing silver nanoparticle intra and extracellularly. The shape and size of silver nanoparticle were characterized by scanning electron microscopy and UV- visible spectroscopy. The silver nanoparticle was effectively produced by all the three bacterial isolates. The method of extraction of intracellular and intracellular nanoparticles was inexpensive, simple and effective in large scale with no need to complex instruments. The bacteria work as a bio-nanofactory which continued to grow after synthesis of silver nanoparticles. This new approach of using non pathogenic bacterial strain for the successful synthesis of nanosized silvers could be easily scaled up which establishes its commercial validity.

KEYWORD: Silver nano particle, biosynthesis, extracellular, intracellular, microbial production.

INTRODUCTION

Nanoparticles are particles between 1 and 100 nanometers in size. In nanotechnology, a particle is defined as a small object that behaves as a whole unit with respect to its transport and properties. Nanoparticles are of great scientific interest as they act as a bridge between bulk materials and atomic or molecular structures. The size and shape of metal nanoparticles are typically measured by analytical techniques such as transmission electron microscopy, scanning electron microscopy or atomic force microscopy.

Nanoparticles have immense importance in bioremediation environmental protection. Researchers are using photocatalytic tungsten oxide nanoparticles to break down oil into biodegradable compounds.[1] The nanoparticles are in a grid that provides high surface area for the reaction, is activated by sunlight and can work in water, making them useful for cleaning up oil spills. The novel properties of nanoparticless have been exploited in a wide range of potential applications in medicine, cosmetics, renewable energies, environmental remediation and biomedical devices.[3] There is growing interest in understanding the relationship between the physical and chemical properties of nanomaterials and their potential risk to the environment and human health. Colloidal silver has been consumed for decades for its perceived health benefits[8] but detailed studies on its effect on the environment have just begun.[9] The medical properties of silver have been known for over 2,000 years. Since the nineteenth century, silver-based compounds have been used in many antimicrobial applications. Nanoparticles have been known to be used for numerous physical, biological, and pharmaceutical applications.[10]

The production of nanoparticles majorly involves physical and chemical processes.[13] But most of the chemical and physical methods of nanosilver production are extremely expensive and also involve the use of toxic, hazardous chemicals, which may pose potential environmental and biological risks.[2] Thus, there is a need for an environmentally and economically feasible way to synthesize these nanoparticles. The hunt for such a method has led to the need for biological production of silver nanoparticles whereby biological methods are used to synthesize the silver nanoparticles.[14]

In this study microbes are utilized for their unique capability to precipitate silver nanoparticles in the growth medium. It is thought the certain microbes may possess certain enzymes or any metabolic components which may cause separation and precipitation of silver nanoparticles from a silver compound $AgNO_3$. We have reported the efficiency of bacterial

strains to synthesize silver nanoparticles. Three concentrations, 25mM, 15mM and 10mM, of $AgNO_3$ is used for the study. For the measurement of size and shape of synthesized nanoparticles, scanning electron microscopy (SEM) was performed.

MATERIALS AND METHODS

PREPERATION OF BACTERIAL CULTURES

Three halophilic and alkaliphilic bacterial strains, Ra3, CD3 and CD13, were screened for the study. All the three cultures were grown in nutrient broth and kept at 37˚C for 48hrs.

INTRACELLULAR ANALYSIS OF SILVER PRECIPITATION

Three concentrations of $AgNO_3$ were used: 25mM, 15mM and 10mM for all the three bacterial strains. All are allowed to grow for 48hrs. After the growth of cultures, each were supplied with silver nitrate solution of respective conc. i.e. 25mM, 15mM and 10mM. All the flasks were kept for incubation at 35^{o}C with 80rpm until precipitation is observed.

EXTRACELLULAR ANALYSIS OF SILVER PRECIPITATION

Already grown bacterial cultures were used for the extracellular analysis. The culture was centrifuged at 5000 rpm for 10 mins to remove all kind of cell debris. Then the supernatant was transferred to the fresh vessels. Three batches of vessels having half of the culture broth were supplied with 25mM, 15mM and 10mM concentrations of silver nitrate. These vessels are as well kept in incubator at 35^{o}C with 80rpm until precipitation is observed.

ESTIMATION OF AMOUNT OF PRECIPITATE

When compared to the control, the cultures for intracellular and extracellular study showed precipitates and colour change. The milky or light grey in coloured precipitate was observed. In order to establish the occurrence of such precipitate the quantitative estimation is required. The precipitates were weighed and dry weight was calculated.

SCANNING ELECTRON MICROSCOPY OF THE PRECIPITATE

A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning it with a focused stream of electrons. The precipitates obtained by the intracellular and extracellular activities of the respective bacteria Ra3, CD3 and CD13 were scanned through SEM to observe any silver nanoparticles produced.

RESULTS AND DISCUSSION

Pure bacterial cultures halophilic and alkaliphilic were screened and used for the study (Fig.1). Remarkable colour changes were observed with the bacterial cultures kept for intracellular (Fig.2) and extracellular (Fig. 3) synthesis of silver nanoparticles batch.

Weight of the dry precipitate was measured for both the intracellular and extracellular broths. Ra3 was the best in producing precipitate at any concentration as compared to others. It gave best results at all the three concentrations i.e. 25mM, 15mM and 10mM (Table 1). At 25mM its extracellular broth produced even better result among the best with 1.934gm of precipitate. CD3 was next in performance with 1.817gm of precipitate in extracellular broth at 25mM of concentration. CD13 also produced its best result at 25mM of concentration with 1.677gm of precipitate.

Since Ra3 strain showed good precipitation of nanoparticles, the sample was proceeded for scanning electron microscopy of the intracellular and extracellular study batches of the respective culture. Figures showed the presence of crystals which indicate the production of silver nanoparticle by the bacteria (Fig. 6).

Legends of Figure

Fig. 1: Cultures of Respective Bacteria Ra3, Cd3 and Cd13.

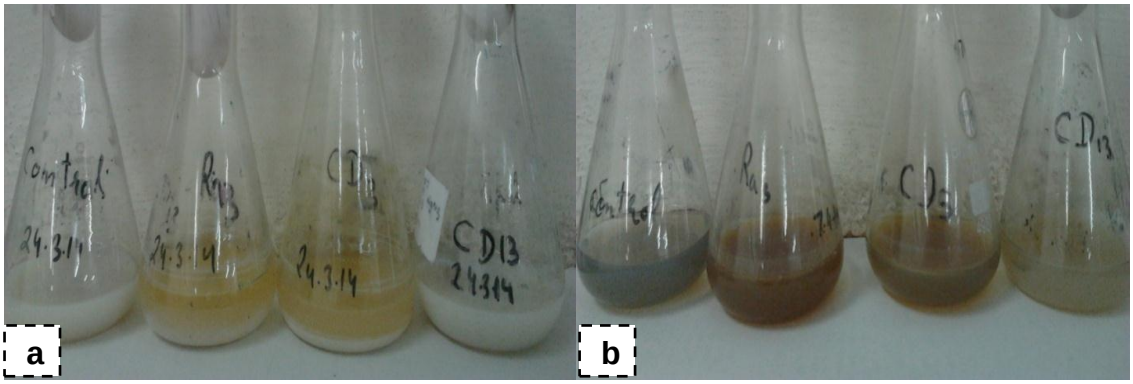


Fig. 2: Study of Intracellular activity by the bacteria. (a) the batch of 25mm $AgNO_3$ and (b) batch of 10mm $AgNO_3$ of respective cultures.

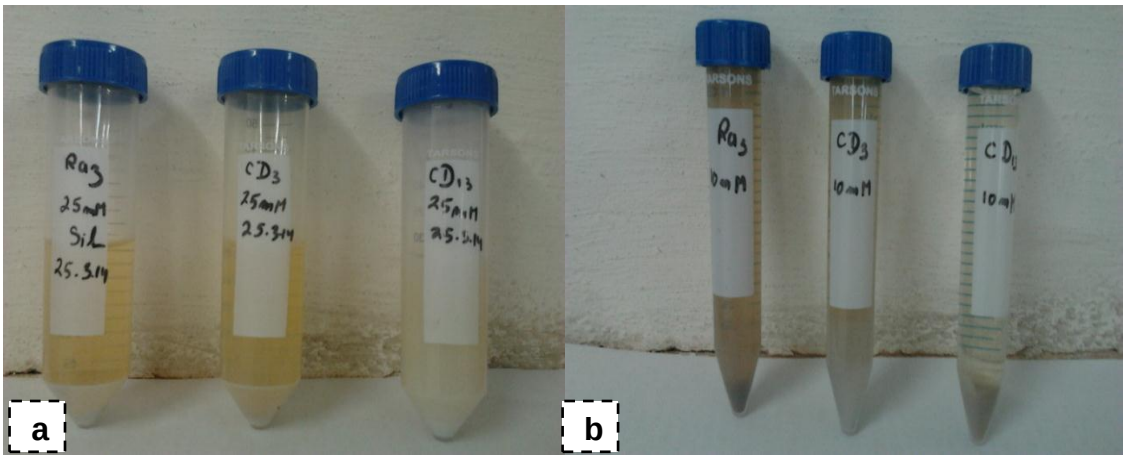


Fig. 3: Study of extracellular activity by the bacteria. (a) with 25mm $AgNO_3$ and (b) with 10mm $AgNO_3$ of respective cultures.

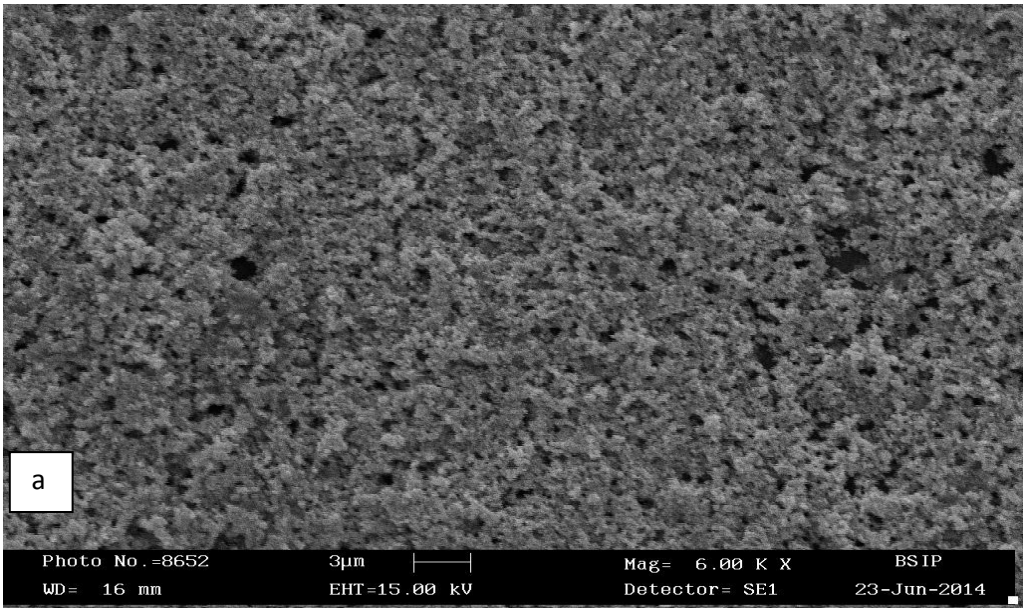
Fig. 4: Two Week Old Cultures of different bacterial isolates with 10mM, 15mM 25mM concentrations of AgNO3.

Ra3 (25mM) **Ra3 (15mM)** **Ra3 (10mM)**

CD3 (25mM) **CD3 (15mM)** **CD3 (10mM)**

CD13 (25mM) **CD13 (15mM)** **CD13 (10mM)**

Fig. 5: Dry Precipitate Of Different Concentrations (10,15 and 25 mM), Used For Extracellular Study.


a
Photo No.=8652
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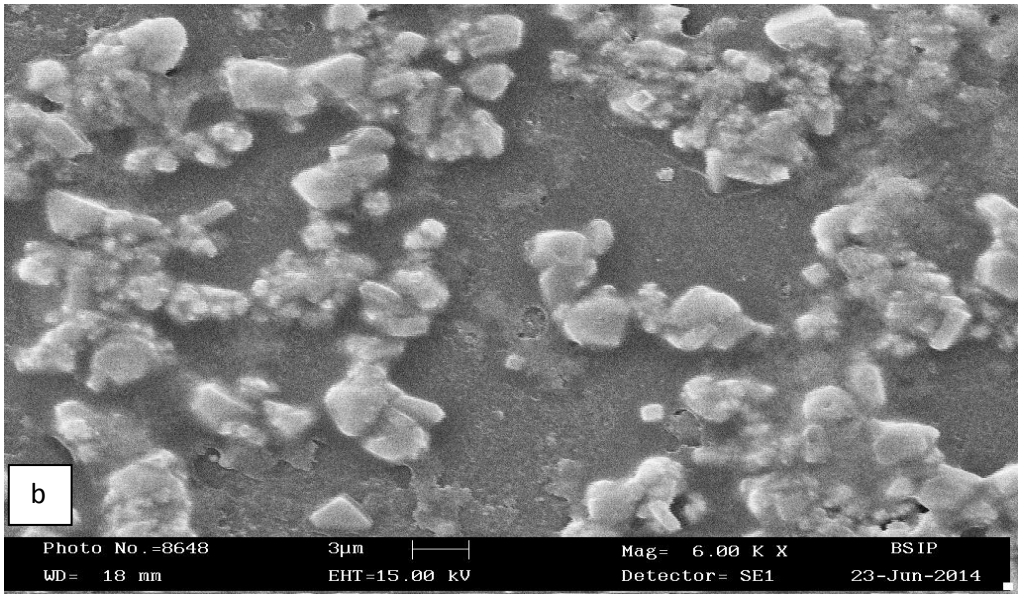
Mag= 6.00 K X
BSIP
WD= 16 mm
EHT=15.00 kV
Detector= SE1
23-Jun-2014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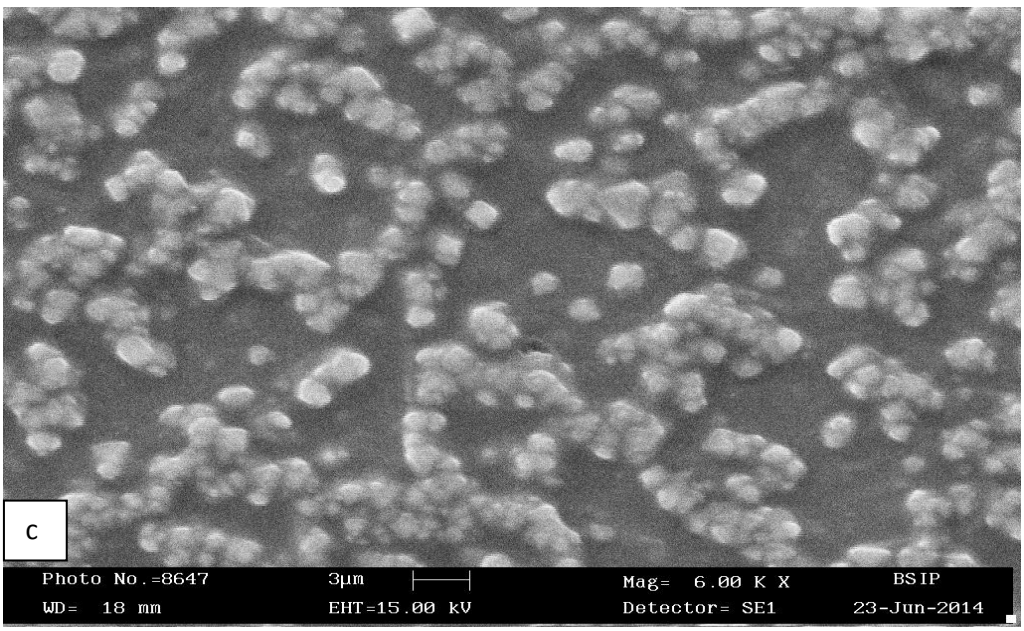
23-Jun-2014


c
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EHT=15.00 kV
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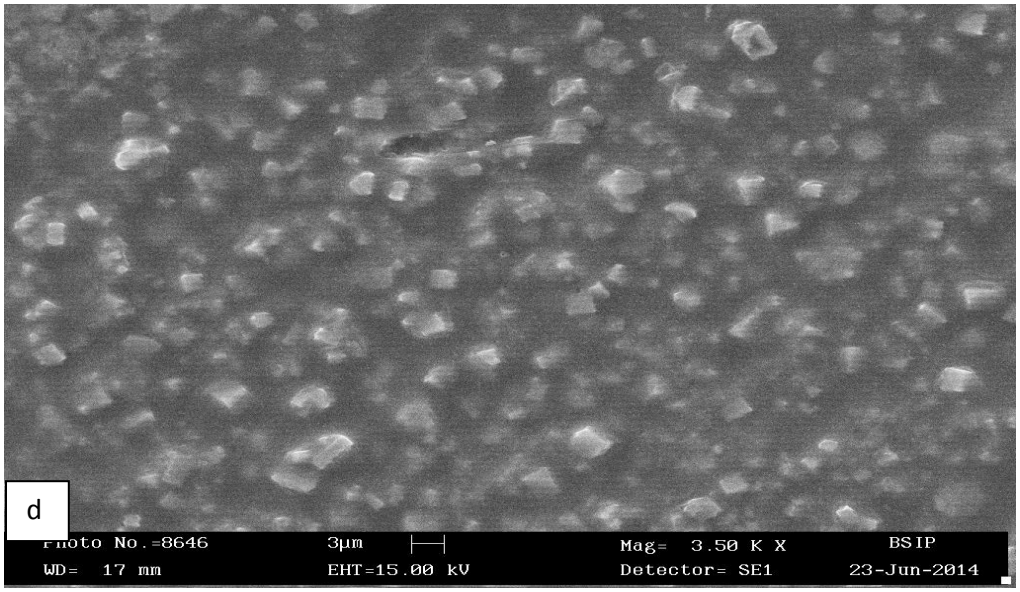
23-Jun-2014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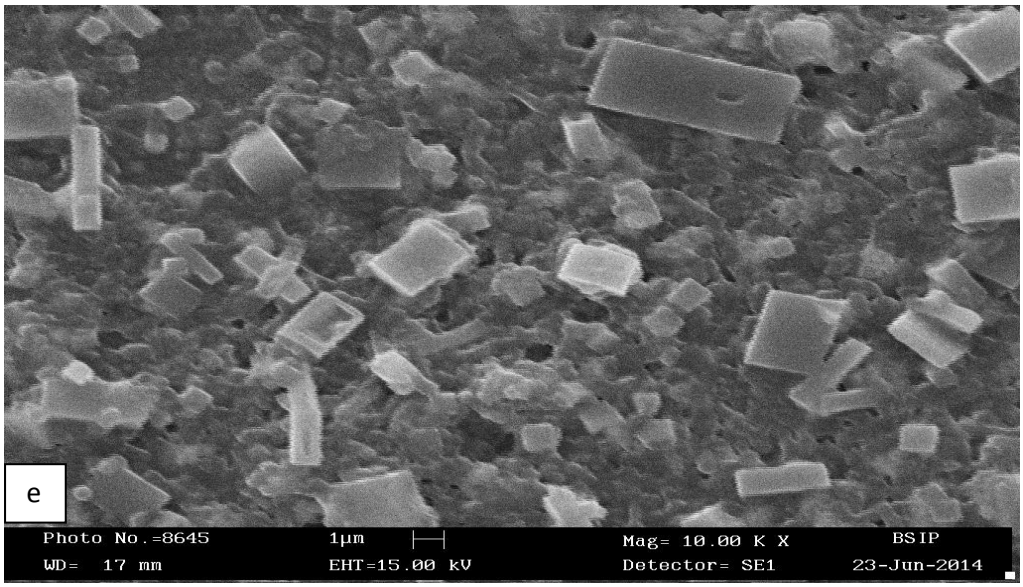
23-Jun-2014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23-Jun-2014

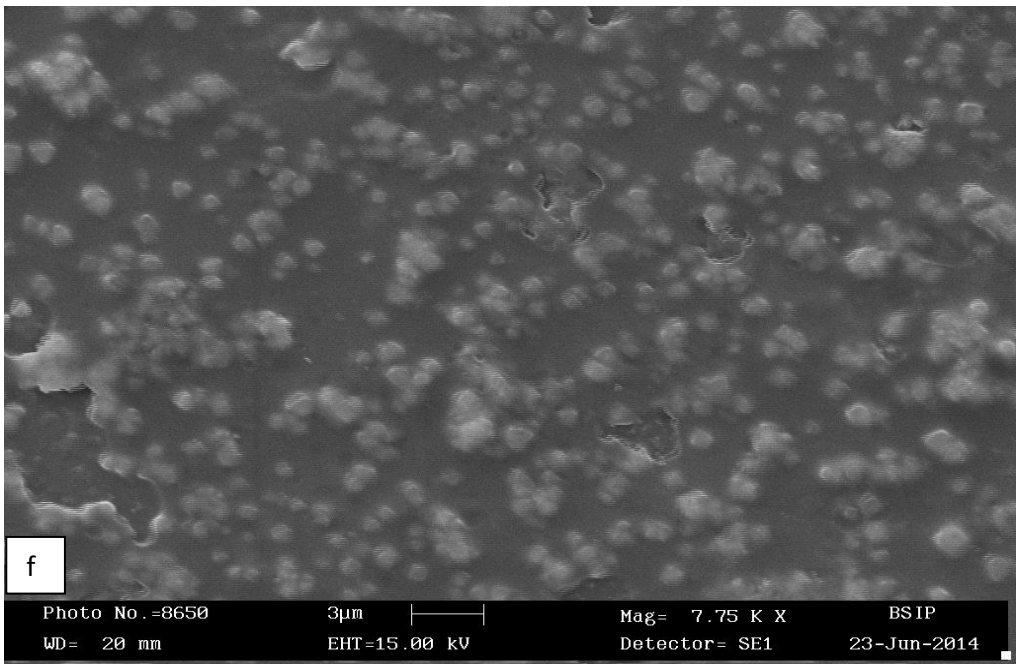
f
Photo No.=8650
3µm
Mag= 7.75 K X
BSIP
WD= 20 mm
EHT=15.00 kV
Detector= SE1
23-Jun-2014

Fig. 6: Scanning Electron Microscope (SEM) Images of Intracellular and Extracellular Broths of Culture Ra3 for all the three different concentrations of AgNO3.(a) Ra3 intracellular (25mM); (b) Ra3 intracellular (15mM); (c) Ra3 intracellular (10mM); (d) Ra3 extracellular (25mM); (e) Ra3 extracellular (15mM); (f) Ra3 extracellular (10mM)

DISCUSSION

Today modern science is inclining toward the study of nanoparticles, their production and their application in various spheres of life. Nanoparticle technology with its immense projected scope in science is attracting a lot of research. Specially the metal nanoparticles are becoming a favorite topic of discussion among the scientists relating to various fields. Conventional methods of silver nanoparticle production include physical and chemical methods, which are of course costly and also carry some environmental and health hazards. Thus it becomes necessary to find out an alternative to the conventional methods. Biological production of silver nanoparticles is a new approach which is cheaper and least in hazards.

In the current study the aim was to identify such bacteria which could bear extreme conditions like pH or salt tolerance and then they were desired to be capable of producing silver nanoparticles. The study has given very positive results with regard to the nanoparticle production. This study has also shown the potential of the bacteria to carry out such job, which is a new approach as compared to the conventional methods. Both the intracellular and extracellular components of all the three bacteria were able to produce precipitates. Ra3 culture was the best although other cultures also showed significant results. As all the three bacterial strains are non pathogenic and showing excellent results, so they can be further used in pharmaceutical and cosmetics etc. This preliminary study have shown very optimistic results which can be further used to discover the potential of bacteria new dimensions in the production of nanoparticles.

ACKNOWLEDGEMENT

We are thankful to the Management of Amity University, Lucknow for providing infrastructure and financial support. We are also thankful to Birbal Sahani Institute of Paleobotany, Lucknow, India for providing Scanning Electron Microscopy facility.

CONFLICT OF INTEREST

The authors declare that we have no conflict of interest in the publication.

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