Int. J. Nanosci. Nanotechnol., Vol. 13, No. 4, Dec. 2017, pp. 307-313

Biosynthesis Characteristic of Silver Nanoparticles Produced by Mine Soil Bacteria Isolation, Kerman, Iran

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(Received: 24 October 2016 and Accepted: 20 February 2017)

Abstract

Background: Biosynthesis of is one of the safe methods in nanoparticles (NP) production where local bacteria of every region can be applied for its better functionality. Among these nanoparticles, the silver nanoparticles (SNP) with high applicability due to their antimicrobial characteristic are the most common.

Methods: In the study, the soil samples are collected from metal mines of Kerman province and their bacteria content is screened and purified. Then, the SNP biosynthesis test is run on Biomass and bacterial supernatant. The parameters here consist of two Molarity of silver nitrate (10-2 and 10-3), different temperature effect on biosynthesis of SNP, and light/dark conditions which are assessed here to optimize SNP production rate, and the results are confirmed.

Results: From the bacterial isolations, the Pseudomonas Iranica NCBI: KF742672, was able to produce SNP from silver nitrate salt of 20-80 nm at 30°C, In dark condition and 10-2 Molarity.

Conclusions: Bacteria can be consumed as a safe and clean element to produce NP, which is in wide use due to new characterizations that are revealed through studies, of course with the safety factor is of major concern.

Keywords: *Biosynthesis, Silver nanoparticles, Mine soils, XRD, TEM.*

1. INRODUCTION

Kerman province is located in southeastern region of Iran. It is one of the most important regions endowed with precious ore mines. The oldest and most famous mines are the Sarcheshmeh copper mines which is ranked the 4th (porphyry copper mine) mine with a capacity of 1 billion tons [1]. Golgohar iron mine located in southwest city of Sirjan is another famous rich iron ore mine. These mines are equipped with the latest equipment and facilitaties available in this industry.

Undoubtedly, silver is one of the best substances in antimicrobial field. The surface of silver particles (SP) can be expanded significantly through nanotechnology where the antimicrobial characteristics of SNP are studied for different pathogenic bacteria [2].

Production of materials in Nano size by applying microorganisms like bacteria [3], fungi [4] and alga [5] is of major importance, while still being a challenge in the field of nanotechnology when the appropriate NP size, shape and dispersion is sought. Advances made in biological methods promote Nano-biotechnology application in various fields of sciences [6]. These substances can be monitored through optimizing the cultural environmental conditions. There exist

studies indicating that the morphology of NPs can be modified when exposed to different pH and temperature [7]. Attempt is made here to assess the potential of bacteria isolated from the soil of mines in Kerman province which would yield AgNPs in different concentrations of silver nitrate [8].

For this purpose, the soil from metal mines of Kerman province is selected on a random base. The objective here is to study the capability of natural fluorine existing in mine soils, the iron, copper, zinc and lead in specific to produce NP, especially the SNP.

2. METHOD AND MATERIAL

After collecting the soil samples from the mine vicinity and sorting and refining the available bacteria the SNP production test is run in thermal conditions in presence and non-presence of light on the biomass and supernatant bacteria.

2.1. Soil Sample Collection

The soil samples are collected in aseptic conditions and are transferred to the laboratory for their pH and Eh measurement in deionized water by 1:5 ratio of soil/water respectively[9].

To separate Bacteria from soil, first, the soil sample is poured in a steril physiological serum serial dilution and then three samples of the prepared concentrations are selected and cultured in TSA through glass spread method [9].

2.2. Biosynthesis of Silver Nanoparticles

After bacterial isolation and purification, every bacterial isolate is cultured separately in 250ml Erlenmeyer flask containing 100ml LB medium without NaCl. The Erlenmeyer flasks are kept in a shaker oven at 30°C for 48 hours. The Biomass and supernatant are separated through centrifuge at 4000 r/m for 15 min. The Biomass is rinsed for 3 times by deionized water for any remaining substance to be removed[10].

The considered parameters to be assessed here in providing optimal conditions for SNP biosynthesis consist of light/darkness, two different Molarity of silver nitrate salt (10^{-2} & 10^{-3}), and 4 thermal intervals at the presence of Biomass and Supernatant of bacterial isolates. Equal amounts of the test sample and salt molarity are mixed. The Erlenmeyer flasks are put in both dark and light conditions (sunlight & UV) and studied as to their change color into tarnish and SNP production [10].

To make sure that SNP production is not effected by the medium components appearance of tan color, two negative and positive control samples are tested subject to similar conditions in a synchronous manner [11].

In addition to analytical apparatus of (XRD, TEM) [14, 15] there exist different common methods to diagnose silver nano particle biosynthesis among which one is color change towards tan indicating nano particle production. Another method to determine SNP is the salt test, light absorbent registration and assessing the anti-microbial properties[12] of produced NSP. Polarized Microscopic observation is another method adopted for this purpose [13]. As to SNP biosynthesis tan color consistency through time is an essential point.

2.3. DNA Extraction

To identify the molecular strains that produce SNP the DNA is extracted through a kit made by to Sinagen Company, نام کشو. After spectrophotometer reads the absorption of extracted DNA, the DNA and Gelred are put on 1% Agaruz gel and are shocked electricity and then the image of the gel is taken by Geldoc for assessment [16].

To reproduce the 16SrRNA gene the universal primers (Table 1) are applied. These primers do multiplicate the total sequence of 16SrRNA gene by 1500 open pairs.

The reaction materials in PCR is 50µl composed of PCR buffer 5 µl, $MgCl_2$ 2 µl,

Table 1. *Universal primers.*

8F	5´-AGAGTTTGATCCTGGCTCAG-3´
1541R	5´-AAGGAGGTGATCCAGCCGCA-3´

dNTPs 1 µl, F-primer 1 µl, Taq-polymerase 0.6 µl, ddwater 37.4 µl and Template DNA 2 µl.

In the next stage, the PCR product is assessed by Agarz gel 1%, 80 volts shock and TBE buffer. Finally, the PCR product is sent to South Korea for its sequence to be determined.

3.RESULTS

The pH and Eh of soil samples transferred to the lab are measured by pH-meter (Table 2). Moreover, the obtained isolates are purified and cultured for 24h for SNP evaluation.

Table 2. *The pH and Eh contact in mine soils.*

Sample	pH	Eh /mV
1	7.65	-55
2	7.51	-50
3	7.66	-54
4	7.73	-59
5	7.78	-69
6	7.33	-57
7	7.78	-64
8	8.8	-83
9	7.49	-51
10	8.17	-81
11	7.3	-49

3.1. Results Obtained from Assessing SNP Production Through Bacterial Isolates Biomass

On this basis, after isolation of supernatant from Biomass, the Biomass of each one of bacteria is rinsed by deionized water in order to remove any residue components of the medium. The SNP production test is run by equal amounts of Biomass and Molarity of silver nitrate and the samples are expressed to (sunlight, UV) and darkness at 30, 40, 60 and 100°C. One of the bacterial isolates showed a faded tan color after 48 hours exposure to sunlight and is named *GA17*. In this case, the initial criterion in SNP production is to reduce nitrate to nitrite and creating tan color. To make sure about the results, silver nitrate solution (10^{-2} & 10^{-3} Molar) each one put as negative control separately in different conditions of the test.

3.2. Results Obtained from Assessing SNP Production Through Bacterial Isolates Supernatant

The process here is similar to that of Biomass of bacteria . By exposing the samples to sunlight they all changed to tan and by exposing the samples to UV, they all changed to dark violet. By putting negative control which contains medium components and silver nitrate salt without bacteria it is determined that each one of the compounds of LB mixtures combined with silver nitrate when exposed to sunlight, would turn tan due to nitrate reduction and when exposed to UV, would turn to dark violet (Figure1).

Figure 1*. change in color of all samples exposed to sunlight (right); UV (left).*

As to the effect of temperature on SNP production , in darkness and several thermal conditions (30, 40, 60 and 100°C), supernatant of a bacterial isolate change to tan after 24 hours at 30°C and 10^{-2}

Molarity, which becomes darker as time passes (Figure3).

The test for the obtained isolate known as *GH10* test is run in 3 iterations and according to the obtained results, the best conditions for SNP production in this case is in darkness, at 30°C, for 48 hours, with 10^{-2} molar concentration of silver nitrate solution in deionized water[20] (Figure 2).

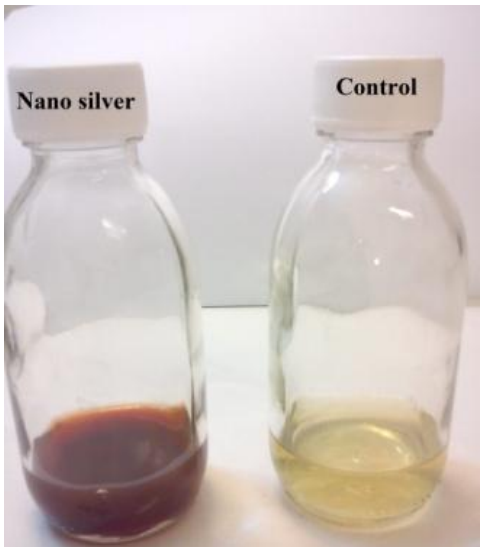

Figure 2. *Control sample next to the bacterium sample producing SNP.*

Screening tests are run on both Biomass and Supernatant to select the two positive samples. A slight sedimentation is observed at salt test run on Biomass sample something not observed while mixing salt with SNP solution in supernatant. Stability of SNP produced by both samples are assessed in 15 days, after in which at room conditions in presence of oxygen existence no sediment and change in color is observedon the sample *GH10* The sample *GA17* turned gray after 7 days, consequently, the process continued on isolate *GH10* and the *GA17 was* removed during screening.

Light absorbed synthesized SNP are shown by measuring the UV-Vis spectrum of the sample. Absorbance is recorded at 300-500 nm. Optical absorption results of tanned solution produced by isolate *Pseudomonas iranica*.

The registered UV-Vis spectra show an increase in vibrations of surface Plasmon in 440 nm wavelength and existing of SNP in different sizes (Figure 3) [14].

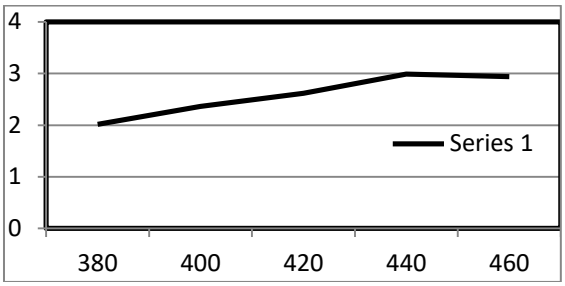

Figure 3. *Optical absorption of isolate Pseudomonas iranica*

To assess the anti-microbial effect of produced SNP, its anti-microbial properties are assessed against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans are applied*. The well diffusion method is adopted here to evaluate the antimicrobial effect of the produced SNP [20]. The antibiogram results of the produced SNP from the subject sample are tabulated in table 3.

Table 3. *Zone of inhibition results of the SNP reduced by isolate Pseudomonas iranica.*

Bacteria	IZ(mm)
Pseudomonas. aueroginosa	13
Staphylococus. aureus	17
E. coli	15
Candida. albicans	21

In addition, results of measuring pH of SNP solution produced of isolate *GH10* indicate that pH of SNP solution in the four designated time intervals in relation to the initial sample have the tendency towards alkaline pH(table 4).

3.3. Assessing the Presence of SNP Through XRD Analysis

X-Ray Diffraction (XRD) is run in

***Table 4**. pH results of SNP produced by isolate Pseudomonas iranica after several times incubation.*

Time (24 hours)	pH
Before inoculation	7.3
24 hours	8.77
48 hours	8.8
72 hours	8.87
96 hours	9.03

KEFA laboratory, where is under supervision of Nanotechnology Initiative Council of Iran (Figure4). The result obtained by XRD proved SNP content in the solution produced by isolate *GH10*, Table (5). The size average of SNP is calculable by the means of data obtained from XRD analysis, (Figure. 4) and is 50 nm size average[21].

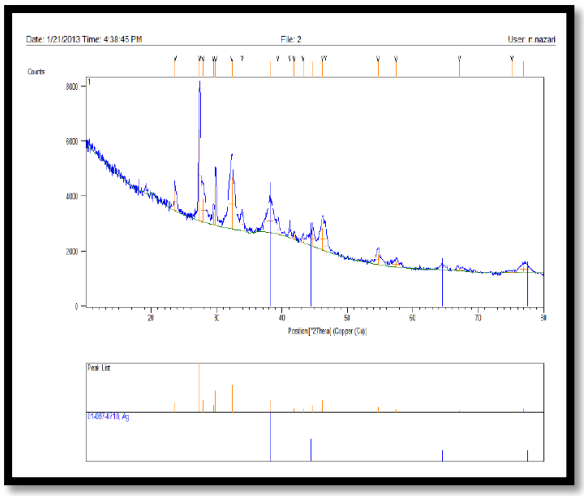

***Figure 4**. output of XRD, confirm Pseudomonas iranica reduced Ag^{+} to Ag^{0}*

3.4. Photographing SNP by TEM

After transferring and preparing SNP in KEFA Lab, the container content are photographed by TEM where SNP of size 20nm 30, 50 and 80 nanometers are observed (Figure5).

***Table 5**. SNP content in isolate Pseudomonas iranica.*

Ref.Code	01-087-0718
Score	54
Compound Name	Silver
Displ.[°2Th]	0.000
Scale Fac.	0.225
Chem. Formula	Ag

As observed, there exists no direct connection among SNP, indicating their being surrounded by protein [21].

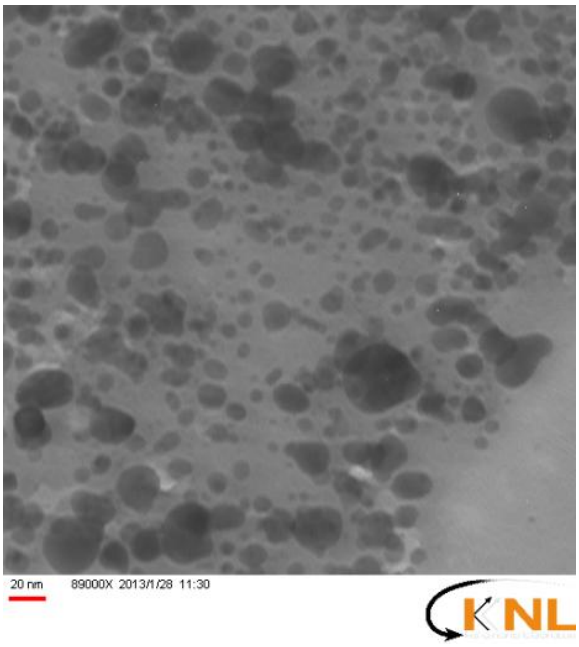

***Figure 5**. TEM image of Pseudomonas iranica GH10 reduced Ag nanoparticles.*

3.5. Identifying the Bacterium Producing SNP Through Molecular Method

This identification is run through molecular method in addition to biochemical tests like gram staining, cathalase test, oxidase test, gelatinase test etc.. The sequence is obtained in NCBI processed. The Neighbor-joining method

and 1000 repetitions of bootstrap are applied in drawing the phylogenetic tree in MEGA5 software environment. The sequence in phylogenic tree is determined by *pl.* The phylogenic tree obtained from the studied bacterium sequence is shown in Figure6. On this basis, the obtained isolate is homologous with *Pseudomonas by* 75% and the newly obtained strain named *Pseudomonas iranica GH10* is registered in NCBI by the KF742672 code.

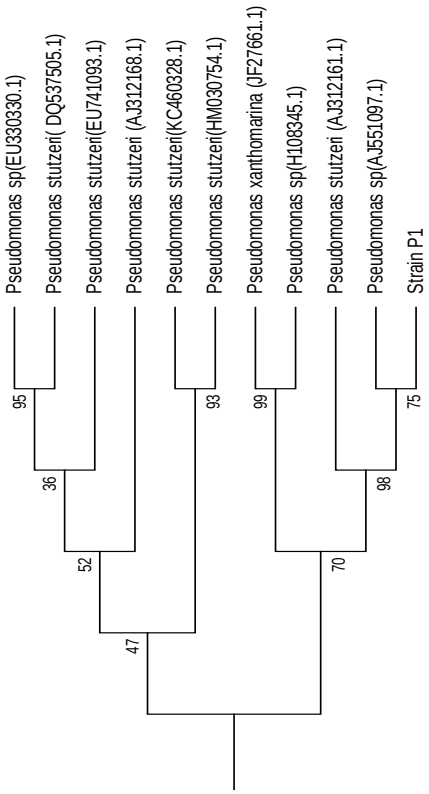

Figure 6*. Phylogenic tree obtained from isolate Pseudomonas iranica.*

4.DISCUSSION

Nanotechnology is a general term referred to all advanced technologies involved in Nano size [17]. There exist several physical and chemical processes for SNP synthesis. For this purpose, various microorganisms like bacteria can be applied in SNP biosynthesize.

In this study, soils from metal mines are selected for their richness in bacteria capable of survival environments rich in metals. Assessing pH and Eh of soil samples reveals that soil pH tending towards alkalinity and oxidation-reduction potential tends toward negative. This fact indicates the reduction capability of these soil types to become reduced, thus, promoting the possibility of isolating the reducible bacteria thereof.

One of the parameters studied here is the effect of sunlight and UV which lead to weak result on Biomass of bacteria while supernatants of the isolate changed to tan and then purple which due to reduction capability of existing nitrate in silver nitrate salt along with compounds of the medium like the yeast extract, peptone or glucose and the samples change color into the ambient color. The color change in all samples is indicative of a fake positive result. Note that according to a study run by[18] on *Pseudomonas aeruginosa*, this bacterium is capable of producing SNP while exposed to sunlight.

By optimizing the conditions, the best result of producing SNP has to do with related to the isolate *Pseudomonas Iranica GH10 NCBI:* KF742672, at 30°C, without light and 10^{-2} Molarity of silver nitrate salt that is capable of producing SNP of 30, 50 and 80 nanometers. In addition, The pH of SNP produced in different time intervals is measured and it is determined that pH tends towards alkalinity on daily bases, thus providing conditions for sample reduction [19].

Since bacteria are of the creatures sustainable in different environmental conditions, they can produce Nano size materials in a biosynthetic manner.

ACKNOWLEDGEMENT

The author wish to thank Dr. Javid Amini.

Abbreviations SP: silver particles

SNP: Silver nanoparticles
XRD: X-Ray Diffraction
Np: Nanopartichles
TEM:Transmission Electron Microscope
TSA: Tripticase Soy Agar

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