Int. J. Nanosci. Nanotechnol., Vol. 10, No. 2, June 2014, pp. 97-102

Assessment of the Antifungal Effect of Silver Nano-particles Produced by Pseudomonas sp1 on Screened Fungus in Meymand Historic Village

P. Parsia[*1], M. Khaleghi[2], M. Madani[3]

1- Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, I. R. Iran
2- Department of Biology, Faculty of science, Shahid Bahonar University, Kerman, I. R. Iran
3- Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, I. R. Iran

(*) Corresponding author: parsia_paria@yahoo.com
(Received: 01 Aug. 2013 and accepted: 12 Jan. 2014)

Abstract:
Today, rocky monuments are being exposed to different physical, chemical and biological factors and therefore going to be ruined. One of physical destructive factors is growing of fungus and lichens on the rocks. This research aims to make use of silver nano-particles produced through microbial method in order to prevent growth and progression of fungus detached from rocky surfaces in Meymand historic village. In the first step, some parts of destructed rocky surfaces and grown lichens on rocky monuments in the village were taken as samples and transferred to lab. After isolation of the fungus from the samples, Identification of all samples were done to the genus level by means of conventional mycological methods, morphology on YGC medium and study of microscopic by means of slide culture. Then the effect of silver nano-particles produced by Pseudomonas sp1 on the fungal isolations was studied. Based on what observed, it was proved that nano-particles produced by Pseudomonas sp1 affected about 68.42% on fungal isolations screened from Meymand rocky and historic village and hindered their growth; therefore growth of fungus on historic rocky surfaces can be prevented by means of such particles in order to protect cultural heritages of the society.
Keywords: *Antifungal effect, Meymand historic village, Silver nano-particles.*

1. INTRODUCTION

The historic, rocky, 6000 year village of Meymand is located in 36 km north eastern part of Shahrbabak city and northwestern part of Kerman province. The village bed made up of silica and lime sediment, and igneous layer around it in the highest level. Beside rough and harsh environments of rocky monuments for microorganisms to grow, many microscopic creatures have this capability. Various studies show that not only different types of bacteria but free fungus and lichens can be fixed on the naked rocks. Rock resident biota, remain two different impacts on lime-rock collections. They intensify erosion process on one side and decrease the effect of environmental erosion factors such as acid rain, moisture, temperature frequencies, etc. by creating a kind of protective cover on their beds on the other side [3,6].

The fungi take part in bio-erosion of rocks and cliffs like those constituting lichens, which live with algae and cyanobacteria symbiotically, and since fungal partner or mycobiont interrelated with minerals due to filamentous structure, thus plays more remarkable role in bio-erosion than its photobiont partner [12].

Among all above, fungi are one of the most important destructive factors of monuments due to the amount of acid they produce. Those who are responsible for restoration of historic rocky monuments especially removing fungi, mostly apply chemical substances [22]. Today, mostly tried to make use of the nature itself in order to prevent harmful impacts by microorganisms. Regarding antibacterial properties of silver nano-particles, decided to remove destructive fungi on monuments by means of such particles as well [39].

Nowadays, nano-science is developing rapidly and nanotechnology evolving rapidly too [16]. In early 20c, antibacterial effect of silver nano-particles was found. Increase in bacteria resistance against antibiotics led to renovation of bio-effects in silver nano-particles because they showed quasi-antibiotic function against some types of bacteria. For instance, Roy *et al.* studied the effect of silver nano-particles on various types of *Aspergillus* in 2013 [30]. Moreover, production of nano-particles biologically (microbial) may have some environmental dangers and should be considered. The mentioned nano-particles have least negative effects due to biological methods of production [1,13].

This research aims to study the effect of silver nano-particles produced by *Pseudomonas* sp1 bacterium on the screened fungi from historic monuments of Meymand village.

2. METHODS

2.1. Microbial Strain

In this case, the microbial strain is *Pseudomonas* sp1 isolated and studied by author from Sirjan Gol-Gohar Mine located in Kerman province[25].

2.2. Isolation of fungi in Meymand historic village

Various fungi were screened from destructing rocky surfaces occupied by lichens in Meymand historic and rocky village. Sampling was done by means of sterile equipments including wide-mouthed utensils and bistoury to take samples from those surfaces potential for fungi and lichens growth. Then the samples were kept in the utensils in 2-5 ^{o}C (icebox) and sent to the lab [25,40]

The rock samples segregated from the monument were put into sterile physiological serum separately and then spread by Glass Spreader after the suspension was prepared in YGC medium, then studied from the viewpoint of fungal growth after keeping in incubator. Identification of all samples were done to the genus level by means of conventional mycological methods, morphology on YGC medium and study of microscopic by means of slide culture.

2.3. Studying acid-making of fungal isolations from historic and rocky monuments in Meymand village

In this case, at first all the screened fungal isolations were inoculated by Spode in central part of YGC medium which contained Thymol Blue Acid-base reagent. Then they were kept in incubator for 3-5 days in 25 ^{o}C and studied from the viewpoint of acid (red color) production [28,30]

2.4. Studying the effect of silver nano-particles produced by *Pseudomonas* sp1 on fungal isolations

In order to study the effect of silver nano-particles produced by *Pseudomonas* sp1, the isolation was taken from Sirjan Gol-Gohar Mine, after production of silver nano particles by this isolated bacteria, the sample of silver nano particles were sent to KEFA lab in order to confirm and prove the existence of silver nano particles. The implemented tests to prove such existence included photographing by Transmission electron microscope (TEM) and observation of the nano particles and therefore determination of their size.

In addition, XRD was used to determine the elements existed in the produced sample. Then, the effectiveness of the produced silver nano particles on the fungus isolated from Meymand historic village was studied. In this case the serial number of dilutions were prepared from the solution contained the produced silver nano-particles and added to Mueller Hinton Agar medium according to Pour Plate method. Afterward, all isolations were inoculated separately on the medium surface contained silver nano-particles in different serial

dilutions and the inoculation to Muller Hinton Agar medium without the nano-particles was done synchronously. In the next stage, all samples were kept in 25°C for 3-5 days and studied from the viewpoint of growth [39].

3. RESULTS

18 samples were taken from different residential and nonresidential rocks of Meymand historic village and taken to the lab in Kerman city. Based on superficial observations, 4 samples of grown lichens were selected from all obtained and the rest were selected based on possible potential of fungi growth. The following is an image of grown lichens on a monument and the rocky destruction made (Figure 1).

Figure 1: *An image of grown lichens on rocky surfaces in Meymand historic village*

In addition, all obtained fungal isolations in this research whether of lichen samples or of other destruction potential surfaces were 19, which were screened based on appearance and macroscopic properties; some of them are shown in Figure 2.

After all the above, the capability of fungal isolations to produce acid in YGC medium, which contained glucose was studied through observing red color, the reagent of Thymol Blue in the grown colonies. The result showed that all fungal isolations producing acid and the red color of the medium remained intact (Figure 3).

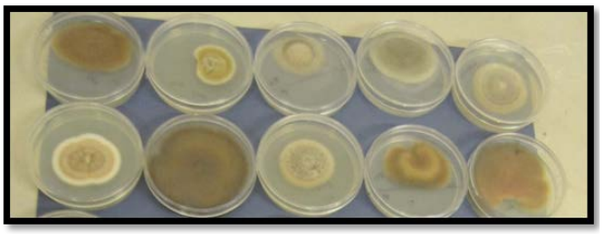

Figure 2: *Some of the fungus screened from rocky surfaces in Meymand historic village*

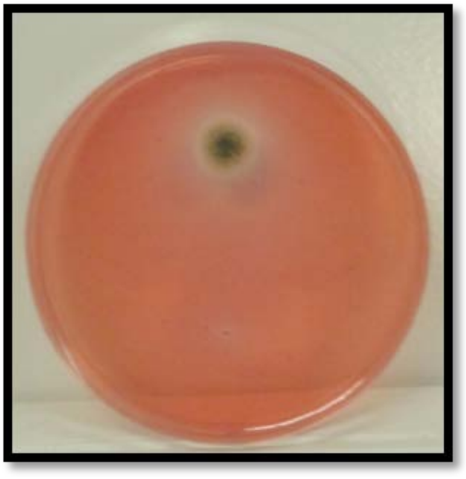

Figure 3: *Acid production by fungal isolation*

The next stage was implemented in order to prove that whether the silver nano-particles produced by *Pseudomonas* sp1 have any effect on isolations taken from rocky and historic monuments of Meymand village.

First of all, the silver nano particles produced by *Pseudomonas* sp1 were observed by Transmission electron microscope and the necessary photographing was done (Figure 4).

The size of produced nano particles was reported as 20-80 nm. In the next step of research, 19 fungal isolations were tested by different dilutions of the produced silver nano-particles. One of the resistant fungi against the produced silver nano-particles was *Alternaria* and one of the sensitive fungi against produced nano-particles was *Aspergillus Terreus*.

Generally, the result of studying the effect of silver nano-particles produced through microbial method on the obtained fungal isolations was that 68.42% of funguses were sensitive and the rest 31.54% were

resistant against the produced silver nano-particles (Figure 5).

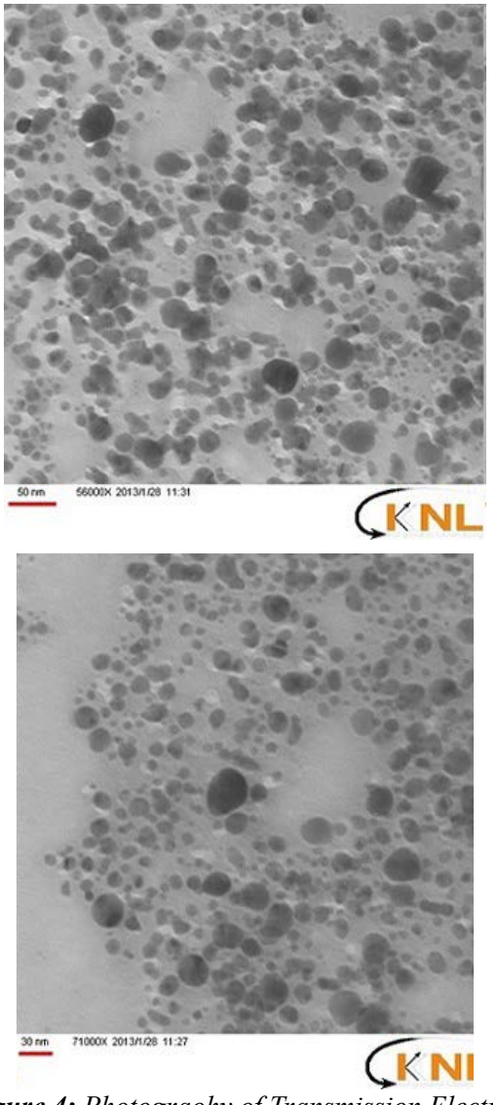

Figure 4: *Photograohy of Transmission Electron Microscope to show Silver nano particles*

Rocks are always destructed because of various physical, chemical and environmental factors; in this regard, growth and reproduction of microorganisms on this rocky environments were proved based on what Geylard et al studied in 2002[6].

One of such microorganisms grown on the rocky environments is fungi. Growth sources of fungi are air pollutant particles and bio-pollutants in villages that are placed on rocks by wind and other physical factors; therefore, fungi are capable of growth on such rocky and tough-grown environments.

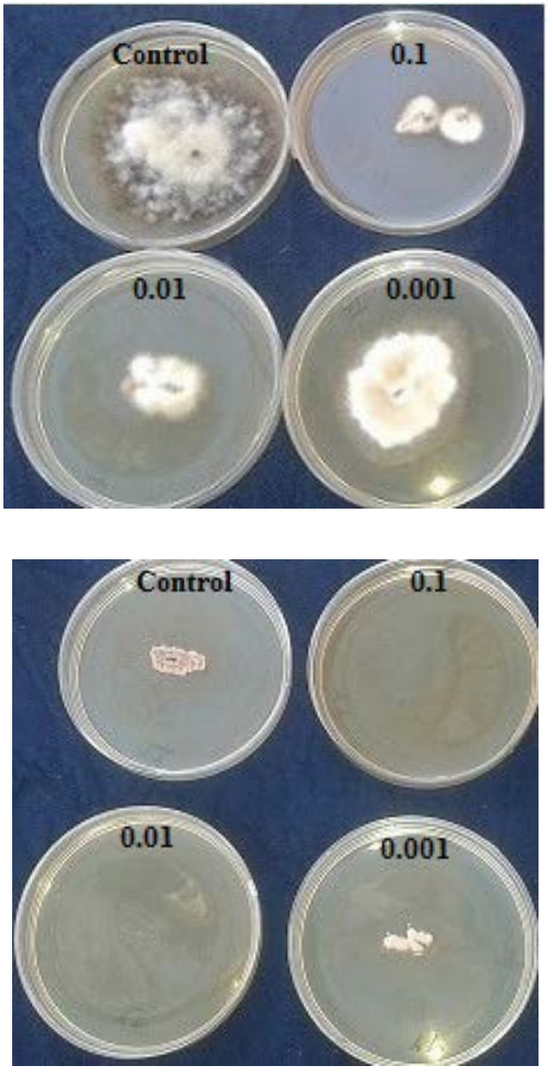

Figure 5: *Positive/negative effect of the silver nano-particles on two fungal samples; right and then left figure respectively.*

4. DISCUSSION

Remarkable point is that fungi are capable to produce acidic environments on rocks through producing several metabolites such as acids, which is one of the factors destructing rocks; a research was implemented in this case by Liscia et al in 2008 [12]. Moreover, fungi growth causes penetration

of their misleum into rocks and destructs them by which destruction of rocky monuments are appeared as time passes.

Thus, silver nano-particles produced by microbial and bio-method were used to study the effect of silver nano-particles on 19 screened fungal isolations taken from Meymand rocky and historic village. Production of silver nano-particles through microbial method was done same as Kalishwaralal *et al.* did in 2008 [9,10] and then the effect of silver nano-particles on fungal isolations was studied after producing and proving the presence of the particles by means of Transmission electron microscope.

Considering the obtained in vitro scale results, the produced silver nano-particles were capable of impacting on 68.42% of fungal isolations and therefore it is recommended to use the silver nano-particles produced through microbial method in rocky environments in order to prevent growth of fungi which results in destruction of rocky monuments.

5. ACKNOWLEDGMENT

The current investigations are jointly supported by Mr. Javid Amini, M.s,department of Microbiology, Academy for Scientific Researchers, Kerman, Iran

REFERENCES

1. A.I., W. and L. S.K. “Use of nano silver as an antimicrobial agent for cotton.” Autex Research Journal., Vol. 9, (2009).
2. Ben-Nissan, C. *et al.* “Crystallographic and spectroscopic characterization and morphology of biogenic and synthetic apatites.” Tech.biotechnol., (1999), pp. 191-221.
3. Dandridge, D. E. ”LICHENS: The challenge for rock art CONSERVATION.” European Journal of Experimental Biology., (2006), pp. 2:1-30.
4. El-Badawy, A., D. Feldhake, *et al.* ”State of the Science Literature Review: Everything nanosilver and more.” United States Environmental Protection Agency(EPA)., (2010). pp. 1-355.
5. Fernandez, I. J., L. E. Rustad, *et al.* ”Experimental acidification causes soil base-cation depletion at the Bear Brook Watershed in maine.” Soil Sci. Soc. Am. J., (2003), pp. 67: 1909–1919.
6. Gaylarde, C. and P. Gaylarde. ”Biodeterioration of historic buildings in Latin America.” European Journal of Experimental Biology., (2002), pp. 9: 171-180.
7. Ismail, A. B. ”Isolation, characterization and identification of microorganisms from soil contaminated with pesticide.” Indian Journal of Science and Technology., (2008), pp. 3: 27-36.
8. Kalimuthu, K., D. Venkataraman, *et al.* ”Biosynthesis of silver and gold nanoparticles using Brevibacterium casei.” Colloids and Surfaces B: Biointerfaces., (2010), pp. 77: 257–262.
9. Kalishwaralal, K., V. Deepak, *et al.* ”Extracellular biosynthesis of silver nanoparticles by the culture supernatant of Bacillus licheniformis.” Elsevier Materials Letters., (2008), pp. 62: 4411–4413.
10. Kalishwaralal, K., S. B. Ramkumarpandian, *et al.* ”Biosynthesis of silver nanocrystals by Bacillus licheniformis.” Colloids and Surfaces B: Biointerfaces., (2008), pp. 65: 150–153.
11. Kumar, M. and L. Philip. ”Enrichment and isolation of a mixed bacterial culture for Complete Mineralization of endosulfan.” Journal of Environmental Science and Health., (2006), pp. 41: 81–96.
12. Liscia, M., M. Monteb, *et al.* Lichens and higher plants on stone. Elsevier Science Ltd., (2002).
13. Maliszewska, I. and M. Puzio ”Extracellular biosynthesis and antimicrobial activity of silver nanoparticles.” Proceedings of the III National Conference on Nanotechnology_NANO., (2009)., p. 116.
14. Manoudis, P., S. Papadopoulou, *et al.* ”Polymer-Silica nanoparticles composite films as protective coatings for stone-based monuments.” Journal of Physics., (2007), pp. 1361–1365.
15. Minaeian, S., A. R. Shahverdi, *et al.* ”Extracellular biosynthesis of silver nanoparticles by some bacteria.“ J. Sci. I . A . U (JSIAU)., (2008), pp. 1-4.
16. Moharrer, S., B. Mohammadi, *et al.* ”Biological synthesis of silver nanoparticles by Aspergillus flavus, isolated from soil of Ahar copper mine.” Indian Journal of Science and Technology., (2012), pp. 5.

17. Mohseniazar, M., M. Barin, *et al.* "Potential of microalgae and Lactobacilli in biosynthesis of silver Nanoparticles." BioImpacts., (2011), pp. 149-152.

18. N, A. and R. D. "Review of biotechnology application to nuclear waste treatment." J chem. Tech Biotechnol., (1999), pp. 381-394.

19. Nascimento, G. G. F., J. Locatelli, *et al.* "Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria." Brazilian Journal of Microbiology., (2000), pp. 247-256.

20. Natarajan, K., S. Selvaraj, *et al.* "Microbial production of silver nanoparticles." Digest_Journal of Nanomaterials and Biostructures., (2010), pp. 135 – 140.

21. Oza, G., S. Pandey, *et al.* "Extracellular fabrication of silver nanoparticles using Pseudomonas aeruginosa and its antimicrobial assay." Advances in Applied Science Research., (2012), pp. 1776-1783.

22. Pandey, A. K., A. Shrivastav, *et al.* "Diversity of monument deterioration-causing fungi at gwalior fort (M.P.) India." Annals of Environmental Science., (2011), pp. 35-40.

23. Prucek, Kvit.L, *et al.* "Silver collids-methods of prepration and utilization." Chemica., (2004), pp. 57-69.

24. Pugazhenthiran, N., S. Anandan, *et al.* "Microbial synthesis of silver nanoparticles by Bacillus sp." J Nanopart Res., (2009), pp. 1811–1815.

25. Rabenhorst, M. C., W. D. Hively, *et al.* "Measurements of soil redox potential." Soil Science Society of America., (2009), pp. 668-674.

26. Ramanathan, R., A. P. O'Mullane, *et al.* "Bacterial kinetics-controlled shape-directed biosynthesis of silver nanoplates using Morganella psychrotolerans." Lungmuir Article., (2010), pp. 714–719.

27. Ramya, M. and M. S. Subapriya. "Green synthesis of silver nanoparticles." International Journal of Pharma Medicine and Biological Science., (2012), p. 1.

28. Reddy, A. S., C.-Y. Chen, *et al.* "Biological synthesis of gold and silver nanoparticles mediated by the bacteria Bacillus Subtilis." Journal of Nanoscience and Nanotechnology., (2010), pp. 1–8.

29. Remya, M. and R. Vijayakumar. "Isolation and characterization of marine antagonistic Actinomycetes from westcoast of India." Medicine and Biology., (2008), pp. 13 - 19.

30. Roy, N. and R. R. "Synthesis of silver nanoparticle using pleurotus Sajor Caju and its antimicrobial study." Digest Journal of Nanomaterials and Biostructures., (2009), pp. 623 - 629.

31. S.Y.Liau, D.C.Read, *et al.* "Interaction of silver nitrarte with readily identifiable groups:relationship to the antibacterial action of silver ions." Letters in Applied Microbiology., (1997), pp. 279-283.

32. Sadeghi, B., M. Jamali, *et al.* "Synthesis and characterization of silver nanoparticles for antibacterial activity." International Journal of Nano Dimension., (2010), pp. 1: 119-124.

33. Sadowski, Z. "Biosynthesis and application of silver and gold nanoparticles." Journal of_Applied Biosciences., (2009), pp.531-540.

34. Saifuddinn, N., C. W. Wong, *et al.* "Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation." E- Journal of Chemistry., (2009), pp. 61-70.

35. Sangiliyandi, G., K. Kalimuthu, *et al.* "Biosynthesis, purification and characterization of silver nanoparticles using Escherichia coli." Colloids and Surfaces B: Biointerfaces., (2009), pp. 328–335.

36. Sinha, S., I. Pan, et al. "Nanoparticles fabrication using ambient biological resources." Journal of Applied Biosciences., (2009), pp. 1113 - 1130.

37. Song, H. Y., K. K. Ko, *et al.* "Fabrication of silver nanoparticles and their antimicrobial mechanisms." European Cells and Materials., (2006), pp. 11- 58.

38. Starowicz, Stypola, *et al.* "Electronical synthesis of silver nanoparticles." Electrochemical Communications., (2006), pp. 227-230.

39. Sterflinger, K. and H. Sert. "Microbial degradation of stone in monuments – its impact on conservation practice." Geophysical Research Abstracts., (2005), pp. 1738.

40. Steubing, P. M. "Isolation of an unknown bacterium from Soil." Association For Biology Laboratory Education (ABLE) Chapter., (1993), pp. 82-113.