University College of Takestan

Available online at **http://UCTjournals.com**

UCT Journal of Research in Science ,Engineering and Technology

UCT . J. Resea. Scien. Engineer.Techno.(UJRSET)

26-32 (2015)

Volume 3 Issue 1

ISSN:2382-977X

Nitrate reductase enzyme in Escherichia coli and its relationship with the synthesis of silver nanoparticles

Bahareh Khodashenas [1]*

[1] *Department of Chemical Engineering, Shahrood Branch, Islamic Azad University, Shahrood ,Iran*

*Corresponding author's E-mail: bahar.khodashenas67@gmail.com

ABSTRACT

Nanostructure materials have attracted a great deal of attention because of their potential for achieving specific processes and selectivity, especially in biological and pharmaceutical applications. Of all kinds of metal nanoparticles, synthesis of silver nanoparticles is of considerable interest due to their wide range of applications in different fields. Chemical, physical and biological methods have been introduced for the synthesis of these nanoparticles. Offering reliable and eco-friendly processes for biological synthesis of metallic nanoparticles (using microorganisms) is an important step in nanobiotechnology. So far, different kinds of bacteria have been reported to be used for synthesis of silver nanoparticles. Escherichia coli bacteria is one of the earliest bacteria for this purpose. Studies showed that generally the presence of nitrate reductase enzyme is essential for the biosynthesis of silver nanoparticles using bacteria. Therefore, this enzyme and its relationship with the synthesis of silver nanoparticles have been studied in the present research work.

Original Article:

Received 25 Sep. 2014
Accepted 20 Nov. 2014
Published 30 Mar. 2015

Keywords:
Silver nanoparticles, Escherichia coli, Synthesis , optimization, Nitrate reductase

1.Introduction

Nanotechnology is predicted to significantly affect science, economics and daily life of the 21st century and become one of the driving forces in the next industrial revolution. In the last century the use of nanoparticles was highly regarded due to their chemical and mechanical characteristics [1,2]. Among various nanoparticles, metallic nanoparticles are the most promising ones , due to their antibacterial properties. Nanoparticles have received much attention because of the high resistance to microbial growth against metallic ions, antibiotics and resistant strains[3]. Today, silver nano particles are highly regarded due to their anti-bacterial properties and also their wide range of applications in different fields by having their electronical, optical and catalytic properties[4, 5]. Generally, silver nano particles are synthesized by physical, chemical and biological methods[6]. Among these methods , biological methods are preferred due to environmental sustainability and cost-effectiveness [7]. Living organisms, such as bacteria, fungus and plants have a great potential in the synthesis of metallic nanoparticles. In fact, the reduction of silver ions (Ag+) is performed by : proteins / enzymes, amino acids, polysaccharides and vitamins found in biomolecules. However, the general mechanism, usually accepted for biosynthesis of silver nanoparticles is the presence of Nitrate reductase enzyme [8]. According to the definitions, enzymes are protein molecules which can increase the rate of chemical reactions to 107 X. Reduction of Nitrate to nitrite is an important step of nitrogen cycle in nature and happens with three objectives : 1) utilization of NO3– as a source of nitrogen , 2) production of metabolic energy during NO3– utilization as the terminal acceptor of electrons and 3) dissipation of excess of reducing energy to maintain oxidation–reduction balance [9,10,11]. Since nitrate is the most significant source of nitrogen in crop plants, understanding the role of Nitrate Reductase enzyme in higher plants , especially in the economic field, is very important. Nitrate reductases, which catalyze the reduction of nitrate to nitrite ,can be produced by various species of eukaryotes (including fungi, algae and protozoa) ,Prokaryotes (including bacteria and blue-green algae) and plants[12]. Eukaryotic Nitrate Reductase could be found in three different forms: 1. NADH-specific , 2. NADPH-specific and 3. NAD(P)H-bispecific [13]. Also, there are three kinds of bacterial nitrate reductases : Assimilatory Nitrate Reductase (Nas) , Respitatory Membrane-Bound Nitrate Reductase (Nar) and Dissimilatory Periplasmic Nitrate Reductase (Nap) [14]. Functional characteristics and physical properties of the enzyme are different according to the organisms .The results of studies clearly show that stress factors in culture medium have a substantial effect on structural and functional characteristics of NRases [12]. In the present article the NR enzyme in Escherichia coli , its relationship with the synthesis of silver nanoparticles, and optimization of the medium culture are investigated to achieve the highest yield.

2. Nitrate reductase of Escherichia coli

Escherichia coli is a gram-negative bacterium from Enterobacteriaceae, discovered in 1855. These bacteria are anaerobic , without spores and usually movable [15]. Escherichia coli is a preferable bacterium in studies of various fields. NR of Escherichia coli is now one of the best-characterized and best-understood membrane proteins.

Nitrate reductase in Eschetichia coli is a membrane-bound enzyme which can be induced in large amounts by growing the organism anaerobically in presence of nitrate. This enzyme is the last enzyme in an electron-transport chain enabling E. coli to utilize nitrate instead of oxygen as the terminal electron acceptor. The carried out process was called Anaerobic respiration [13].

E.coli has become a particular bacterium because: It is the only organism for which two membrane-bound dissimilatory NR have been described. While the synthesis of the major one (98% of the total activity), as all the others membrane bound respiratory NR, requires anaerobiosis and the presence of nitrate, the second enzyme appears to be constitutively expressed, a property reminiscent of the periplasmic nitrate reductase [16]. Apparently the enzyme was first discovered by Taniguchi and and Itagaki. They have expressed that the nitrate reductase is a large molecule that consists of molybdenum and heme-free iron. Electron micrographs, showed that the enzyme had a spherical shape. Also,micrographs showed no holes in the enzyme and the subunits were tightly packed. This structure is very stable [17]. In 1956, Taniguchi, Egami and Sato have found a particulate system in cell-free extracts of E. coli, grown in a peptone broth-agar medium containing nitrate , which reduces nitrate to nitrite under anaerobic conditions [18]. E. coli bacteria consists of 3 kinds of Nitrate Reductases, two of them are membrane bound and biochemically similar:nitrate reductase A (NRA) and nitrate reductase Z (NRZ) [19] . The third kind of nitrate reductase (Nap) is located in the periplasm [20]. Studies demonstrate that the nitrate reductase A enzyme is expressed at the high concentrations of nitrate while the Nap nitrate reductase is expressed at low concentrations of nitrate and expression of the nitrate reductase Z is not related to the amount of nitrate or anaerobic conditions [21]. Nitrate reductase A is a heterotrimer and consists of three subunits, which are, respectively: 1) α subunit =NarG, that is the actual site of Nitrate reduction and Contains molybdenum cofactor [22], 2) β subunit =NarH, is the electron transfer subunit containing the iron-sulfur clusters, one [3Fe-4S] cluster and three [4Fe-4S] clusters [22,23] and 3) γ subunit = NarI, which transfers electrons from the quinone pool to the β subunit [23,24] , and NarJ, which is not part of the final nitrate reductase A enzyme, but is essential for nitrate reductase activity [24,25,22,26].Generally the formation of active membrane-bound nitrate reductase A in Escherichia coli requires the presence of NarG, NarH and NarI subunits, as well as NarJ protein, which is not a part of the active nitrate reductase [26]. Nitrate reductase Z is a heterotrimer like Nitrate reductase A and consists of three subunits : 1) β subunit = NarY is the electron transfer subunit containing the iron-sulfur clusters [22] , 2) α subunit = NarZ is the actual site of nitrate reduction and also contains the molybdenum cofactor [22] and 3) γ subunit = NarV , which transfers electrons from the quinone pool to the β subunit [16]. γ subunit is a membrane-embedded heme-iron subunit resembling cytochrome b, which transfers electrons from the quinone pool to the β subunit [16]. Periplasmic nitrate reductase (Nap) is the third kind of Nitrate Reductase in E.Coli. The physiological role of Nap is acting as a mediated enzyme in anaerobic respiration at the low concentrations of nitrate. Also, its affinity for nitrate is much more than NRA [27]. This enzyme has been divided in to 7 subunits , as shown in Fig.1.

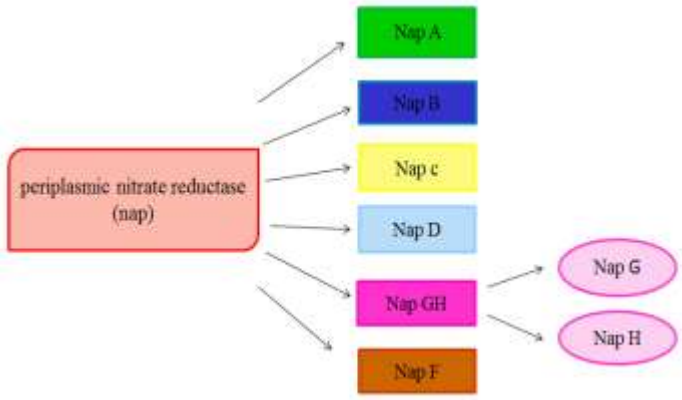

Fig.1 Subunits of Nap nitrate reductase

Periplasmic nitrate reductase (Nap) contains four basic components in many bacteria: napB, napA, napD and napC, while the E.coli bacteria contains three additional genes : napF ,napG and napH which are not required for Nap activity and their function is not known yet [28,29,30]. Cyanide and azide can be mentioned as strong inhibitors of this enzyme and Cyanide's inhibition is competitive . It should be noted that storing the enzyme at 0-1˚C in 60% saturated ammonium sulfate as a precipitate helps it to be more stable[31]. The size of this enzyme varies from 498,000 to 1,000,000 Daltons in different studies [32,17].

Fig .2 structure of nitrate reductase A from E. coli [33]

3. Using bacteria for the synthesis of silver nanoparticles

Generally green synthesis of Ag nanoparticles involves three main steps, which must be evaluated based on green chemistry perspectives, including: 1) selection of solvent medium , 2) selection of environmentally benign reducing agent, and 3) selection of nontoxic substances for the Ag NPs stability [34,35]. Joerger et al (2000) reported the first synthesis of Ag nanoparticles using P. stutzeri AG259

bacteria [36]. Also, various species like : B.licheniformis and Escherichia coli were used for the same purpose [8,37]. The results showed that the culture supernatants of Klebsiella pneumonia, Escherichia coli [38,39], and Enterobacter cloacae have the ability of producing silver nanoparticles in a short time (5 minutes) [37]. Anil Kumar et al , used α -NADPH-dependent nitrate reductase for the first time using and synthesized stable silver nanoparticles with 10-25 nm diameter [40].

4. Synthesis of Silver Nanoparticles by E.coli

Shahverdi et al (2007) reported the rapid synthesis of silver nanoparticles using the culture supernatant of Escherichia coli. The size of synthesized nanoparticles were in the range of 28.2–122 nm [37]. Gurunathan et al (2009) could synthesize silver nanoparticles by the culture supernatant of Escherichia coli. They characterized the produced nanoparticles after their purification by using sucrose density gradient centrifugation. The average size of nanoparticles was 50 nm [53]. Safekordi et al (2011) could synthesize silver nanoparticles by E.coli bacterium (DH5α). They also investigated the effect of silver nitrate concentration , mixing ratio of filtrate of bacterium culture to silver nitrate, temperature and pH on size and production efficiency [54]. El-Shanshoury et al (2011) synthesized silver nanoparticles by using culture supernatants of E. coli ATCC 8739. Transmission electron microscopy (TEM) images showed that the particle size ranges between 5–25 nm [55]. Muthukkumarasamy et al (2012) could synthesize polygonal silver nanoparticles in the range of 10–50nm using extract of Escherichia Coli ATCC 25922 [56]. Ghorbani (2012) reviewed the synthesis of silver nanoparticles using the cell extract of E. coli [57]. Ghorbani (2013) investigated the synthesis of silver nanoparticles by reduction of aqueous Ag^+ ions with the culture supernatant of Escherichia coli (DH5a). The synthesized silver nanoparticles were in the range of 10-100 nm [58].

5. The relationship between NR enzyme in E.coli with the synthesis of silver nanoparticles

Studies have shown that in all the organisms that can synthesize silver nanoparticles, nitrate reductase might be an integral part [59] and presence of this enzyme in bacterial supernatant is one of the reasons that makes synthesis of silver nanoparticles using E.coli specific . Nitrate can be used as the main source of nitrogen or as an alternative electron acceptor in energy generation [17,60]. For example, in Bacillus licheniformis bacteria Nitrate Reductase is found at the membrane [61]. The probable mechanism for the formation of silver nanoparticles involves the NADH-dependent nitrate reductase enzyme that may convert Ag^+ to Ag^0 through electron shuttle enzymatic metal reduction process, shown in Fig.3 [8].

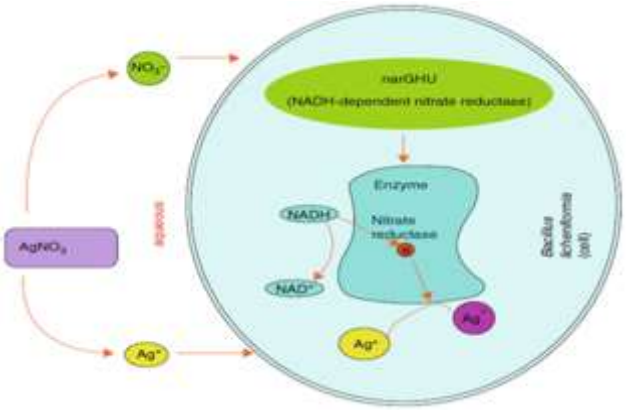

Fig .3 Possible mechanism for silver nanoparticles synthesis in B. licheniformis [8]

Generally, the reduction of silver ions (Ag^+) in aqueous solution yields the formation of silver nanoparticles [36]. At first, the formation of silver atoms (Ag^0) happens by reduction of various complexes with Ag^+ ions, which is followed by agglomeration into oligomeric clusters and finally, the colloidal silver particles are formed by these clusters [59]. As mentioned before, the Nitrate Reductase enzyme, released by microorganisms, is a major factor in the synthesis of silver nanoparticles. Studies have shown that NADH and NADH-dependent enzymes, especially nitrate reductase, are important factors in the biosynthesis of metal nanoparticles [8,62]. One of the advantages of using this enzyme in the in vitro synthesis of silver nanoparticles is that the presence of (α-nicotinamide adenin dinucleotide) in bacteria, would do away with the downstream processing required for the use of these nanoparticles in homogeneous catalysis and other applications. During the reduction process , nitrate is converted to nitrite and an electron will be shuttled to the silver ions. As a result, silver ions are converted to silver [10,41].

5. Enhanced silver nanoparticle synthesis by optimization of the medium components:

In order to achieve the highest yield of silver nanoparticles synthesis , methods for providing the best conditions for the growth of E.coli bacteria and maximal expression of the nitrate reductase enzyme as the important factors were investigated.

5-1. Affecting Factors on the activity of Nitrate reductase enzyme in E.coli :

E.coli cells may grow on a solid or in a liquid growth medium under a laboratory condition. Solid and liquid media may have exactly the same composition except that the solid medium contains an extra 1.5% agar .LB media (Luria broth, Luria-Bertani medium, or lysogeny broth) are the most common medium used for E.coli cultures [63,64,65] .The two main components of LB media are Tryptone and Yeast Extract. Other medium cultures like : SOB (Super Optimal Broth or Hanahan's broth), SOC (Super Optimal broth with Catabolic repressor) , TB (Terrific Broth) and SB (Super Broth) [66] can be used too. Studies showed that Peptone, Yeast extract, Glucose (as carbon source) and KNO_3 are essential Nutrients that are

commonly used in growth media of Nitrate reductase [67,68,69]. Wainwright in 1995 found that the activity of Nitrate reductase enzyme in E.coli increased in anaerobiosis by vitamin K3 [70]. Heredia et al (1960), claimed that, the activity of Nitrate reductase enzyme in E.coli was not affected by nitrate, while it increased remarkably by vitamin K3, which confirmed the results of Wainwright & Nason (1954). This group also studied the effect of PH on the activity of the nitrate reductase enzyme and the results showed the maximum activity in the range of 6-7 [71].

6. Enzymatic Assay of Nitrate Reductase

Determination of the best medium, in which the enzyme nitrate reductase has the maximum activity, requires measuring the enzyme activity in each medium . Various researchers have introduced different methods for this purpose [72, 73]. Enzymatic Assay of Nitrate Reductase (EC 1.6.6.1) by spectrophotometric Stop Rate Determination from sigmaaldrich that is based on Redinbaugh [74] and Smarrelli [75] papers and another method for Enzymatic Assay of NR (Cytochrome EC 1.9.6.1) by Colorimetric are methods for measuring NR activity that has been discussed by Worthington [76] and Low [72]. Vaidyanathan et al (2010) optimized nitrate reductase activity in order to increase produced silver nanoparticles. They showed that the optimized medium contained: 1.5% Glucose, 1 % Peptone, 0.35% Yeast extract and 0.35 % KNO3[59].

7. Conclusion

In this paper, NR enzyme in E.coli, it's relationship with the synthesis of silver nanoparticles and also optimization of the medium components were studied to reach the maximum activity of the enzyme and thereby increase the production efficiency of silver nanoparticles.

It is known that, NR in E.coli , catalyzes the reaction in which Nitrate is converted into Nitrite . The reaction that takes place is as follows [77] :

$$NO_3^- + NAD(P)H + 2e^- + H^+ \rightarrow NO_2^- + NAD(P)^+ + H_2O \quad (1)$$

On the other hand, when $AgNO_3$ dissolved in deionized water gets split into Ag^+ and NO_3^- .

$$AgNO_3 \longrightarrow Ag^+ + NO_3^- \quad (2)$$

So , using the free electrone of the first reaction (1) , Ag^+ ions are reduced into Ag. As was expressed the presence of vitamin K_3 (KNO_3) , Glucose, Yeast extract and Peptone in culture media leads into the maximum expression of enzyme and results in a decrease in enzyme activity. Other factors to be considered are the enzyme's optimal temperature and PH. Also, it should be noted that, in addition to culture media, the enzyme's optimal temperature and PH, there are other factors such as : the concentration of the precursor [54,78], the temperature [50,79] and PH [50, 79,80,81,2] of the solution used for the synthesis of silver nanoparticles , a solution with a certain molarity that includes a precursor ($AgNO_3$) and NR enzyme, are the effective factors which influence the production of these particles. In order to reach the final culture media and to determine the exact substance ratios, for the synthesis of silver nanoparticles , doing further experiments are required.

References

1. Faramarzi, M., and Forootanfar, H., 2011 ,"Biosynthesis Characterization and of Gold Nanoparticles Produced by Laccase from Paraconiothyrium variabile", Colloids and Surfaces B: Biointerfaces, 87(1), ,23-27. doi: 10.1016 /j.cols urfb.2011. 0 4.022.
2. Klaus, T., Joerger, R., Olsson, E., and Granqvist, C.G., 2001,Bacteria as Workers in the Living Factory: Metal Accumulating Bacteria and their Potential for Materials Science, Trends in Biotechnology,19(1), 15-20. DOI: http://dx.doi.org/10.1016/S0167-7799(00)01514-6
3. Kouvaris, P., Delimitis, A., Zaspalis, V., Papadopoulos, D., Tsipas, S., and Michailidis, N., 2012 ,"Green synthesis and characterization of silver nanoparticles produced using Arbutus Unedo leaf extract", Materials Letters,76, 18–20.
4. Jeon, H.J., Yi, S.C., and Oh, S.G., 2003, "Preparation and antibacterial effects of Ag-SiO2 thin films by sol-gel- method, Biomaterials", 24(27), 4921-4928.
5. Lue, J.T., 2001, "A review characterization and physical property studies of metallic nanoparticles", Journal of Physics and Chemistry of Solids,62, 1599-1612. http://dx.doi.org/10.1016/S0022-3697(01)00099-3
6. Ghorbani, H. R., Safekordi,A.A., Attar ,H., and Rezayat Sorkhabadi, S. M., 2011, "Biological and Non-biological Methods for Silver Nanoparticles Synthesis", Chemical and Biochemical Engineering Quarterly,25 (3) , 317–326.
7. Li ,S., Shen ,Y., Xie, A., Yu, X., Qui ,L., Zhang ,L., and Zhang, Q., 2007 ,"Green synthesis of silver nanoparticles using Capsicum annuum L. extract", Green Chemistry , 9, 852-858. DOI: 10.1039/ B615357 G
8. Kalimuthu, K., Babu ,R.S., Venkataraman, D., Mohd, B., and Gurunathan, S., 2008, "Biosynthesis of silver nanocrystals by Bacillus licheniformis", Colloids and Surfaces B: Biointerfaces, 65, 150–153.
9. Berks, B. C., Ferguson, S. J., Moir,J. W. B., and Richardson ,D. J., 1995, "Enzymes and associated electron transport systems that catalyse the respiratory reduction of nitrogen oxides and oxyanions", Biochimica et Biophysica Acta (BBA),1232(3), 97–173.
10. Unden, G., and Bongaerts, J., 1997, "Alternative respiratory pathways of Escherichia coli: energetics and transcriptional regulation in response to electron acceptors", Biochim. Biophys. Acta ,1320, 217–234
11. Moreno-Vivián, C., and Ferguson, S. J., 1998, "Definition and distinction between assimilatory, dissimilatory and respiratory pathways". Mol. Microbiol. 29, 664–666.
12. Morozkina, E. V., and Zvyagilskaya, R. A., 2007 ,"Nitrate Reductases: Structure, Functions, and Effect of Stress Factors", Biochemistry (Moscow), 72(10), 1151-1160. DOI: 10.1134 /S000 6297 907100124.
13. Fischer ,K., Barbier, G .G., Hecht, H .J., Mendel, R. R., Campbell, W. H. and Schwarz, G., 2005, "Structural

Basis of Eukaryotic Nitrate Reduction: Crystal Structures of the Nitrate Reductase Active Site", The Plant Cell,17, ,1167-1179.
14. Viva´n ,C M., Cabello, P., Luque, M M., Blasco, R., and Castillo, F., 1999, "Prokaryotic Nitrate Reduction: Molecular Properties and Functional Distinction among Bacterial Nitrate Reductases", J Bacteriol,181, 6573-6584,.
15. Maier, M., and Pepper, I ., 2000 ,"Environmental Microbiology Arizona State", Academic Press., 2, 491-493.
16. Bonnefoy ,V., Demoss ,J.A., 1994, "Nitrate reductases in Escherichia coli", Antonie Van Leeuwenhoek,66(1-3), 47-56.
17. Taniguchi, S., and Itagaki, E., 1960 , "Nitrate reductase of nitrate respiration type from E. coli. I. Solubilization and purification from the particulate system with molecular characterization as a metalloprotein", Biochim. Biophys. Acta, 4(44), 263-279.
18. Taniguchi, S., Sato, R., and Egami, F., In A Symposium on Inorganic Nitrogen Metabolism, p. 87. Ed. by McElroy, W. D. & Glass, B. Baltimore: Johns Hopkins Press(1956).
19. Blasco ,F., Iobbi, C., Ratouchniak ,J., Bonnefoy ,V., and Chippaux ,M., 1990, "Nitrate reductases of Escherichia coli: sequence of the second nitrate reductase and comparison with that encoded by the narGHJI operon", Mol Gen Genet,222(1), 104-111,.
20. Nilavongse, A., Brondijk ,T.H., Overton ,T.W., Richardson, D.J., Leach ,E.R., and Cole, J.A., 2006 "The NapF protein of the Escherichia coli periplasmic nitrate reductase system: demonstration of a cytoplasmic location and interaction with the catalytic subunit, NapA", Microbiology,152(11), 3227-3237. doi: 10.1099/ mic .0. 29 157-0
21. Chang, L., Wei ,L.I., Audia ,J.P., Morton ,R.A., and Schellhorn, H.E., 1999 "Expression of the Escherichia coli NRZ nitrate reductase is highly growth phase dependent and is controlled by RpoS, the alternative vegetative sigma factor", Mol Microbiol., 34(4), 756-766.
22. Blasco ,F., Pommier, J., Augier ,V., Chippaux M., and Giordano, G., 1992 "Involvement of the narJ or narW gene product in the formation of active nitrate reductase in Escherichia coli"., Mol Microbiol., 6(2), 221-230.
23. Rothery, R.A., Blasco, F., Magalon, A., Asso, M., and Weiner ,J.H., 1999 "The hemes of Escherichia coli nitrate reductase A (NarGHI): potentiometric effects of inhibitor binding to narI"., Biochemistry, 38(39), 12747-12757.
24. Dubourdieu ,M., and DeMoss, J.A., 1992 "The narJ gene product is required for biogenesis of respiratory nitrate reductase in Escherichia coli"., J Bacteriol.,174(3), 867-872.
25. Stewart V., 1982 "Requirement of Fnr and NarL functions for nitrate reductase expression in Escherichia coli K-12"., J Bacteriol. ,151(3), 1320-1325.
26. Blasco ,F., Dos Santos ,J.P., Magalon ,A., Frixon, C., Guigliarelli, B., Santini, C.L., and Giordano ,G., 1998 "NarJ is a specific chaperone required for molybdenum cofactor assembly in nitrate reductase A of Escherichia coli"., Mol Microbiol. ,28(3), 435-447.
27. Potter, L.C., Millington ,P., Griffiths, L., Thomas ,G.H., and Cole, J.A., "Competition between Escherichia coli strains expressing either a periplasmic or a membrane-bound nitrate reductase: does Nap confer a selective advantage during nitrate-limited growth?", Biochem J.,344 (1), 77-84(1999).
28. Brondijk, T.H., Nilavongse ,A., Filenko, N., Richardson ,D.J., Cole, J.A., 2004 "NapGH components of the periplasmic nitrate reductase of Escherichia coli K-12: location, topology and physiological roles in quinol oxidation and redox balancing", Biochem J. ,379(1), 47-55.
29. Brondijk, T.H., Fiegen, D., Richardson, D.J., and Cole ,J.A., 2002 "Roles of NapF, NapG and NapH, subunits of the Escherichia coli periplasmic nitrate reductase in ubiquinol oxidation", Mol Microbiol, 44(1), 245-255.
30. Potter, L.C., and Cole ,J.A., 1999 "Essential roles for the products of the napABCD genes, but not napFGH, in periplasmic nitrate reduction by Escherichia coli K-12", Biochem J. , 344 (1), 69-76.
31. Forget, P., 1974 "The Bacterial Nitrate Reductases. Solubilization, Purification and Properties of the Enzyme A of Escherichia coli K12", Eur J Biochem ,42(2), ,325-332.
32. Enoch, H.G., and Lester, R.L., 1975 "The purification and properties of formate dehydrogenase and nitrate reductase from Escherichia coli", J. Biol. Chem., 250, 6693 -6705.
33. Bertero ,M.G., Rothery ,R.A., Palak, M., Hou, C., Lim ,D., Blasco , F., Weiner ,J.H., and Strynadka ,N.C., 2003 "Insights into the respiratory electron transfer pathway from the structure of nitrate reductase A", Nat. Struct. Biol. ,10 (9), 681–687. doi:10.1038/nsb969.
34. Raveendran,P. Fu,J., and Wallen,S.L., 2003 "Completely "green" synthesis and stabilization of metal nanoparticles", J. Am. Chem. Soc., 125(46) , 13940–13941.
35. Sharma, V.K., Ria, A., and Lin,Y.Y., 2009 "Silver nanoparticles: green synthesis and their antimicrobial activities", Adv. Colloid Interface Sci. 145(1-2) , 83–96. doi: 10.1016/j.cis.2008.09.002.
36. Joerger, R., Klaus, T., and Granqvist, C. G., 2000, "Biologically produced silver-carbon composite materials for optically functional thin-film coatings", Adv. Mater. ,12, 407-409.
37. Shahverdi, A. R., Minaeian, S., Shahverdi, H. R., Jamalifar, H., and Nohi, A., 2007 "Rapid synthesis of silver nanoparticles using culture supernatants of Enterobacteria: A novel biological approach",Process Biochem. ,42 , 919-923.
38. Lee, S. Y., 1996 "High cell-density culture of Escherichia coli"., Trends Biotechnol., 14 (3), 98-105.
39. Shehata, T. E., and Marr, A. G., 1971 "Effect of nutrient concentration on the growth of Escherichia coli" , J. Bacteriol, 107(1) , 210-216.
40. Kumar, S.A., Abyaneh,M.K. Gosavi, S.W., Kulkarni,S.K. ,Pasricha, R., Ahmad, A., and Khan ,M.I., 2007 "Nitrate reductase-mediated synthesis of silver nanoparticles from $AgNO_3$", Biotechnol. Lett., 29 , 439–445.
41. Fu ,J.K., Zhang, W.D., Liu, Y.Y., Lin ,Z.Y., Yao, B.X. and Weng, S.Z., 1999 "Characterization of adsorption and reduction of noble metal ions by bacteria", Chin J Chem Univ ,20, 1452–1454.
42. Kalishwaralal, K., Deepak, V., Ramkumarpandian, S., Nellaiah, H., and Sangiliyandi, G., 2008 "Extracellular

biosynthesis of silver nanoparticles by the culture supernatant of Bacillus licheniformisMater", Lett.,62 , 4411-4413.

43. Zhang, H., Li, Q., Lu, Y., Sun, D., Lin, X., and Deng, X., 2005 "Biosorption and bioreduction of diamine silver complex by Corynebacterium", J Chem Technol Biotechnol, 80, ,285–290.

44. Saifuddin ,N., Wong ,C.W., and Nur yasumira ,A.A., 2009 "Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation"., Eur J Chem , 6, ,61–67.

45. Parikh, R.Y., Singh, S., Prasad ,B.L., Patole ,M.S., Sastry ,M., and Shouche, Y.S., 2008 "Extracellular synthesis of crystalline silver nanoparticles and molecular evidence of silver resistance from Morganella. sp.: towards understanding biochemical synthesis mechanism"., Chembiochem, 9, 1415–1422.

46. Nanda ,A. and Saravanan ,M., 2009 "Biosynthesis of silver nanoparticles from Staphylococcus aureus and its antimicrobial activity against MRSA and MRSE", Nanomedicine , 5(4), 452–456.

47. Ghorbani ,H .R., 2013Biosynthesis of nanosilver particles using extract of Salmonella typhirium, Arabian Journal of Chemistry. http://dx.doi.org/10.1016/j.arabjc.2013.06.017

48. Ranganath, E., Vandana, R., and Banu ,A., 2012 "Biosynthesis of Silver Nanoparticles By Lactobacillus Sps & Its Activity Against Pseudomonas Auerogenosa", Asian Journal of Biochemical and Pharmaceutical Research , **2**, 49-55.

49. Arun, P., Shanmugaraju, V., Renga Ramanujam, J., Senthil Prabhu, S., and Kumaran ,E., 2013 "Biosynthesis of Silver Nanoparticles from Corynebacterium sp. and its antimicrobial activity", Int. J. Curr. Microbiol. App.Sci, , 2(3) , 57-64.

50. Oza,G., Pandey,S., Shah,R., and Sharon, M., 2012 "Extracellular Fabrication of Silver Nanoparticles using Pseudomonas aeruginosa and its Antimicrobial Assay", Advances in Applied Science Research, 3(3), 1776-1783.

51. Mahdieha,M., Zolanvari, A., Azimeea,A.S., and Mahdieh,M., 2012 "Green biosynthesis of silver nanoparticles by Spirulina platensis" Scientia Iranica F , 19 (3), 926–929.

52. Lakshmi Das, V., Thomas, R., Varghese, R. T., Soniya, E. V., Mathew ,J. and Radhakrishnan, E. K., 2013 "Extracellular synthesis of silver nanoparticles by the Bacillus strain CS 11 isolated from industrialized area", 3 Biotech. DOI 10.1007/s13205-013-0130-8.

53. Gurunathan, S., Kalishwaralal, K., Vaidyanathan, R., Venkataraman, D., Pandian, S.R.K., Muniyandi, J., Hariharan, N., and Eom, S.H., 2009 "Biosynthesis, purification and characterization of silver nanoparticles using Escherichia coli", Colloids Surf B., 74(1), 328–335.

54. Safekordi,A. A., Attar H., and Ghorbani H. R., 2011 "Optimization of Silver Nanoparticles Production by E.coli and the study of reaction kinetics", International Conference on Chemical, Ecology and Environmental Sciences (ICCEES'2011) Pattaya Dec.

55. El-Shanshoury,A.E.R.R. ,ElSilk, S.E., and Ebeid, M. E., "Extracellular Biosynthesis of Silver Nanoparticles Using Escherichia coli ATCC 8739 Bacillus subtilis ATCC 6633, and Streptococcus thermophilus ESh1 and Their Antimicrobial Activities", , International Scholarly Research Network ISRN Nanotechnology, Article ID 385480, 7 pages(2011). doi:10.5402/2011/385480.

56. Muthukkumarasamy,S., SHaradha,A., Vignesh,S., Dhanabalan, K., and Gurunathan, K., 2012 "EXTRACELLULAR SYNTHESIS OF POLYGONAL SILVER NANOPARTICLES USING EXTRACT OF ESCHERICHIA COLI ATCC 25922 AND ITS ANTIBACTERIAL ACTIVITIES", 7(4), 1419-1426.

57. Ghorbani ,H. R., 2012 "Silver nanoparticles biologically synthesized using extract of E. coli", MINERVA BIOTECNOLOGICA, 24(2), 58-61.

58. Ghorbani, H. R., 2013 "Biosynthesis of Silver Nanoparticles by Escherichia coli", Asian Journal of Chemistry, 25(3), 1247 -1249.. DOI: 10. 14233 /ajchem. 2013.12805

59. Vaidyanathan, R., Gopalram, S., Kalishwaralal, K., Deepak, V., Pandian,S.R.K., and Gurunathan,S.(2010). "Enhanced silver nanoparticle synthesis by optimization of nitrate reductase activity", Colloids and Surfaces B: Biointerfaces ,75 , 335–341.

60. Torres-Cisneros, M., Velásquez-Ordónez, C., Sánchez-Mondragón, J., Campero, A., Ibarra-Manzano, O. G., May-Arrioja, D. A., Plascencia –Mora , H., Espinoza-Calderón, A ., and Sukhoivanov, I., 2009 "Synthesis and Optical Characterization of Ag0 Nanoparticles", Microelectr. J., 40 , 618-620.

61. Rey, M.W., Ramaiya, P., Nelson,B.A, Karpin,S.D, Zaretsky, E.J, Tang,M., Leon,A., Xiang, H., Gusti,V., Clausen, I.G, Olsen,P.B, Rasmussen,M.D, Andersen, J.T, Jorgensen,P.L, Larsen,T.S, Sorokin,A., Bolotin, A., Lapidus, A., Galleron,N., Ehrlich,S.D., and Berka,R.M., Complete genome sequence of the industrial bacterium Bacillus "licheniformis and comparisons with closely related Bacillus species, Genome Biol", 5(10) , R77(2004). doi: 10.1186/gb-2004-5-10-r77

62. Jepson ,B. J. N., Mohan,S., Clarke, T. A., Gates ,A, J., Cole ,J, A., Butler C. S., Butt , J .N., Hemmings, A. M ., and Richardson, D. J., 2007 "Spectropotentiometric and Structural Analysis of the Periplasmic Nitrate Reductase from Escherichia coli", JOURNAL OF BIOLOGICAL CHEMISTRY, 282, 6425–6437.

63. Luria, S. E. and Burrou J. W. , 1955 "Hybridization between Escherichia coli and Shigella", J. Bacteriol., 74, 461-476.

64. Lennox, E. S., 1955 "Transduction of linked genetic characters of the host by bacteriophage P1", Virology,1(2), 190-206.

65. Luria, S. E., Adams, J. N., and Ting ,R. C., 1960 "Transduction of lactose-utilizing ability among strain of E. coli and S. dysenteriae and the properties of the transducing phage particles", Virology, 12, 348-390.

66. Botstein, D., Lew, K. K., ,Jarvik, V. , and Swanson, C. A., 1975 "Role of antirepressor in the bipartite control of repression and immunity by bacteriophage P22" , J. Mol. Biol. ,91(4), 439-462.

67. MacGregor, C. H., 1975 "Synthesis of nitrate reductase components in chlorate-resistant mutants of Escherichia coli.", J. Bacteriol., 121, pp.,1117-1121.

68. MACGREGOR, C. H., 1976 "Biosynthesis of Membrane-Bound Nitrate Reductase in Escherichia coli: Evidence for a Soluble Precursor", JOURNAL OF BACTERIOLOGY, 126(1) , pp.,122-131.
69. Hackett, C. S., and MacGregor ,C. H., 1981 "Synthesis and degradation of nitrate reductase in Escherichia coli.", J. Bacteriol., 146, 352-359
70. Wainwright, S. D., 1955 "Menadione derivatives and ferrous iron as cofactors of the nitrate reductase system of a coliform organism", Biochim. biophys. Acta, 18(4), 583-585.
71. HEREDIA, C. F. & MEDINA,A.(1960). Nitrate Reductase and Related Enzymes in Escherichia coli, Biochem. J. , 77(1), pp.,24-30.
72. Lowe, R. and Evans, H., 1964 "Preparation and Some Properties of a Soluble Nitrate Reductase from Rhozobium japonicum", Biochim Biophys Acta, 85, 377-389.
73. Skerman, V.B.D., 1967 "A guide to the ident ification of the genera of bacteria", The Williams & Wilkins Co., Baltimore, MD. ,218 - 220.
74. Redinbaugh, M.G. and Campbell, W.H.,1985," Quaternary structure and composition of squash NADH:nitrate reductase" , Journal of Biological Chemistry ,260,pp.,3380-3385.
75. Smarrelli, J.Jr. and Campbell, W.H. , 1983 "Heavy metal inactivation and chelator stimulation of higher plant nitrate reductase", Biochimica et. Biophysica Acta, 742, ,435-445.
76. Worthington, C. E.(1988). in Worthington Enzyme Manual ,pp.,240-241, Worthington Biochemical Corporation, Freehold, NJ.
77. Ingledew, W. J. and Poole, R.K., 1984 "The Respiratory Chains of Escherichia coli" ,MICROBIOLOGICAL REVIEWS,pp., 48(3), 222-271.
78. Ajitha ,B., Divya ,A., Harish ,G. S. and Sreedhara Reddy ,P., 2013 ,"The Influence of Silver Precursor Concentration on Size of Silver Nanoparticles Grown by Soft Chemical Route" , Res. J. Physical Sci., 1(7), 11-14.
79. Iravani ,S. and Zolfaghari , B., 2013 ,"Green Synthesis of Silver Nanoparticles Using Pinus eldarica Bark Extract" , BioMed Research Int. , doi: 10.1155/2013/639725.
80. Kotthaus,S., G¨unther,B. Haug,H. R. and Sch¨afer, H., 1997, Study of isotropically conductive bondings filled with aggregates of nano-sized Ag-particles." , IEEE Transactions on Components Packaging and Manufacturing Technology A. 20(1) : 15–20.
81. Korbekandi,H., Iravani, S. and Abbasi, S., 2009 ,"Production of nanoparticles using organisms production of nanoparticles using organisms" ,Critical Reviews in Biotechnology, 29(4): 279–306.