Biological Journal of Microorganism
7th Year, Vol. 7, No. 28, Winter 2019
Received: November 15, 2017/ **Accepted:** December 26, 2017. **Page:** 21- 25

Production of Gold Nanoparticles by Spore Displayed Tyrosinase

Afrouzossadat Hosseini Abari *
Assistant Professor of Microbiology, Faculty of Science, University of Isfahan, Isfahan, Iran, afrouz_hosseini1985@yahoo.com
Byung Gee Kim
Professor, Department of Chemical and Biological Engineering Seoul National University, Seoul, Korea, byungkim@snu.ac.kr
June Hyun Kim
Associate Professor, Department of Chemical Engineering, College of Engineering, Dong-A University, Busan, Korea, june0302@dau.ac.kr

Abstract

Introduction: According to the applications of gold nanoparticle in electronic and medicine, green biological synthesis methods for gold nanoparticle synthesis are considered. Different biological methods cause variant types of metal nanoparticles. Enzymes are one of the powerful tools in this approach. In this study, the role of tyrosinase in gold nanoparticle synthesis was studied by spore displayed tyrosinase.

Materials and methods**:** *Bacillus subtilis* spore displayed tyrosinase developed in our previous work, was used as an enzyme source for synthesis of Au nanoparticles (AuNPs). X-ray diffraction technique and transmission electron microscopy were used to characterize the nanoparticles. To confirm the role of enzyme in AuNPs synthesis, two types of tyrosinases from *Bacillus megaterium* and *Streptomyces* also were studied.

Results: The results revealed that AuNPs were produced due to reducing Au^{3+} to Au^{0} by spore displayed tyrosinase. These biogenic nanoparticles showed mixed structures including spherical, triangular and hexagonal with the approximate size 2.5 to 35 nm. Furthermore, purified *Bacillus megaterium* tyrosinase and *Streptomyces* tyrosinase also produced AuNPs.

Discussion and conclusion: The supposed mechanism of Au nanoparticle synthesis by tyrosinase, is electron transferring from copper ions to Au^{3+}. The results represent a green environmental friendly simple method in synthesis AuNPs by spore displayed tyrosinase.

Key words: Gold Nanoparticles, Spore Displayed Tyrosinase, Tyrosinase

*Corresponding Author

Introduction

In the recent decades, a high level of interests of science and technology is focused on the production of nanoparticles. Integration of nanotechnology and medicine has created significant advances in diagnosis and treatment, molecular biology and biological engineering (1, 2). Among the nanoparticles, metal nanoparticles which have wide applications in molecular engineering, nano medicine and nano electronics are most commonly used (3). Several physical, chemical and biological methods for production of gold nanoparticles were investigated. High levels of pollution due to using toxic chemicals, high costs, and unstable non-uniform particle size after building are some of the problems that encouraged scientists to find green synthesis methods to produce nanoparticles (4). Microbial cells and enzymes are the highly organized factories which can do bio transformation in metals and make metal nanoparticles easily, cheaply, and environmentally friendly (5).

Gold nanoparticle, because of stability in atmospheric conditions, resistance to oxidation, and environmental compatibility is known to have a particular importance in medicine and biotechnology. Also, they are used as biosensors in nanoelectronics and molecular engineering (6, 7). The first biological system in synthesis of gold nanoparticles was recognized in *Pedomicrobium*-like bacterium. Gold nanoparticle synthesis in other microorganism such as iron reducing and metal-like bacteria such as *Cuperiavidous necator and Cuperiavidous metallidorans* also was reported (8). Bacterial deposition of gold and production of gold nanoparticles by microorganisms has created a new way to recycle this valuable metal from the environment.

Regarding to the importance of biological production and the wide applications of gold nanoparticles, attention of scientist was drawn to efficient, easy, low-cost, and effective production of nanoparticles (3).

In our previous study, the role of dipicolinic acid extracted from *Bacillus* spores in silver nanoparticle synthesis was demonstrated and the results revealed that only silver nanoparticles were greatly produced under the experimental conditions (9). In order to optimize synthesis of other metal nanoparticles especially gold nanoparticles by spores, genetically engineered spores were used. In this study, spore displayed tyrosinase is presented a new rapid approach to green synthesis of gold nanoparticles.

Material and method

Spore Preparation and Detection of Enzymatic Activity: *Bacillus subtilis* DB104 (pSDJH-cotE-tyr) which was prepared in our previous research (10), was inoculated into Difco sporulation medium (DSM) containing 0.8 % (w/v) Difco Nutrient Broth, 0.1 % (w/v) KCl, 0.025 % (w/v) $MgSO_4 \cdot 7H_2O$, 1 mM $Ca(NO_3)_2$, 0.01 mM $MnCl_2$, and 0.01 mM $FeSO_4$, pH 7 and incubated at 37 °C for 24 h on a shaker (200rpm) (11). Renografin (sodium diatrizoate, S-4506, Sigma) gradient method was used for spore purification.

Tyrosinase activity was assayed using L-tyrosine as a substrate, and *Bacillus subtilis* DB104 (pSDJH-cotE-tyr) spore solution as the tyrosinase sources (12). Purified tyrosinase from *Bacillus megaterium* DSM319 and *Streptomyces* also were used as confirmatory samples. The reaction mixture without tyrosinase was used as control.

Analysis of Gold Nanoparticles Biosynthesis: Two types of *Bacillus subtilis* spores with displayed tyrosinase and without displayed enzyme were studied. 100 µl of spore solutions containing 1.2 x 10^7 spores was

added into 1 mL of aqueous solution of 1 mM $AuCl_3$. The interaction of the spores and Au ions was completed in room temperature in 2 hours. Also, gold nanoparticle synthesis was studied using the 0.25 mM of purchased standard DPA (2, 6-Pyridinedicarboxylic acid, P63808 Sigma). The biosynthesized AuNps were characterized by XRD and TEM.

XRD Analysis: X-ray diffraction (XRD) analysis of the gold nanoparticles was done by X-ray Diffractometer, D8ADVANCE (Bruker, Germany). X-rays were made by a copper X-ray tube with wavelength 1.5406 Å (Cu Kα) and Ni as a filter. Measurements were performed between 30° and 70° 2θ (9).

TEM (transmission electron microscopy): To prepare the samples for TEM analysis, 5 µL of biosynthesized Au nanoparticle solutions were dropped on carbon-coated copper grids. The grids were observed in a JEM-2100 Electron Microscope (JEOL, Japan) operated at 120 kV.

Results

Production of Gold nanoparticles by Spore Displayed Tyrosinase: After a few hours of adding 1mM $AuCl_3$ in spore suspension, sediments containing AuNPs were demonstrated (Figure1). As it is shown in Figure 1, the brown precipitate was appeared in samples of *Bacillus megaterium* tyrosinase, *Streptomyces* tyrosinase and spore displayed tyrosinase. Production of AuNPs did not demonstrate in the samples with dipicolinic acid and *Bacillus subtilis* spores without tyrosinase during this short period of time.

XRD pattern: The synthesized AuNPs were characterized using XRD technique. The XRD pattern of the nanoparticles produced by *Bacillus subtilis* DB104 (pSDJH-cotE-tyr) spores is presented in Figure 2, where there were three sharp peaks in the whole pattern of 2θ value ranging from 30 to 70. It actually is similar to the spectra took by the previous reports of AuNPs.

TEM analysis: AuNPs also were characterized by TEM. Figure 3 shows TEM images of AuNPs. The size of the biogenic AuNPs was from 2.5 to 35 nm. As shown, the TEM micrographs showed the presence of diverse morphology (cubic, triangular, spherical, and hexagonal structures) of AuNPs.

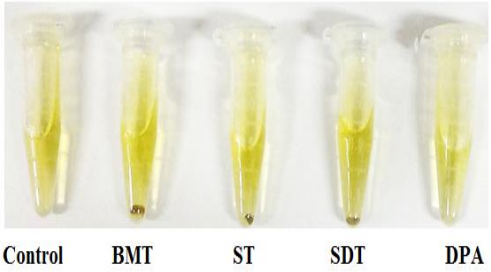

Fig. 1- The formation of AuNPs precipitate. Control: $AuCl_3$ solution. BMT: *Bacillus megaterium* tyrosinase, ST: *Streptomyces* tyrosinase, SDT: Spore displayed tyrosinase, DPA: dipicolinic acid.

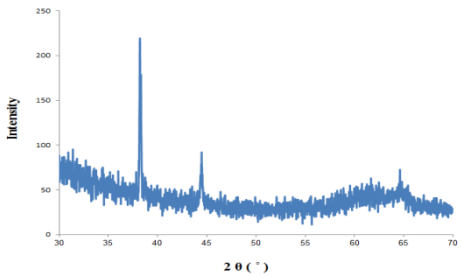

Fig. 2- XRD pattern of synthesized AuNPs.

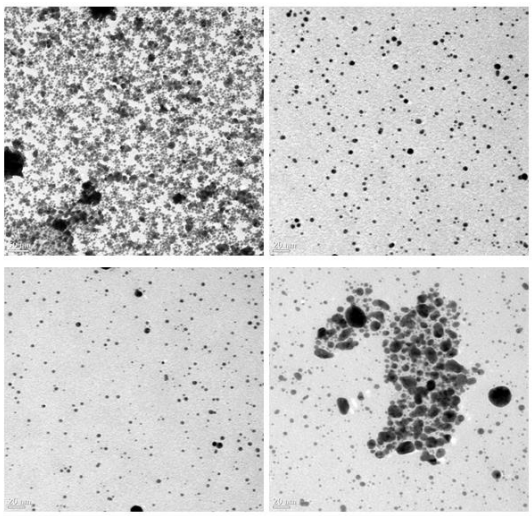

Fig. 3- TEM images of biogenic AuNPs.

Discussion and Conclusion

Our previous research revealed that the extracted dipicolinic acid from *Bacillus* spores can quickly synthesis Ag nanoparticles (9). However a question about the role of dipicolinic acid in the production of other metal nanoparticles had remained. Our results showed that the production of AuNPs did not occur obviously by dipicolonic acid in a short period of time. Previous researches revealed that different enzymes have various patterns for synthesis nanoparticles so finding suitable enzymes for synthesis each metal nanoparticles is interesting. In order to improve spore properties to change trivalent metal ions to nanoparticles, the role of enzymes was studied. According to this phenomenon, in this research, the role of tyrosinase in making AuNPs by using spore displayed tyrosinase was considered.

The results revealed that spore displayed tyrosinase changes Au^{3+} to Au^{0}. Furthermore, purified *Bacillus megaterium* tyrosinase and *Streptomyces* tyrosinase also produced AuNPs. The role of enzymes in the production of nanoparticles has been proven. Das *et al.*, showed the role of (NADPH) oxidoreductases in the production of AuNPs in *Rhizopus oryzae* (13). Also, other reductases, hydrogenases, hydrolases, desulfhydrase and syntases were reported before (14). Li *et al*. used laccase as a reducing agent for green synthesis of gold nanoparticles (15).

Sanghi *et al*. also presented the role of laccase in extracellular synthesis of AuNPs and the role of ligninase in intracellular production of AuNPs in *Phanerochaete Chrysosporium* (16).

The supposed mechanism for the production of gold nanoparticles by Tyrosinase is electron transferring from copper ions (the cofactors of tyrosinase) to Au^{3+}. Transformation of Au^{3+} to Au^{0} is a kind of reducing reaction. As it is mentioned before, several reductase enzymes reduce ions and make nanoparticles. Regarding the reduction of potential elements in aqueous solution, Cu give electron to Au^{3+}. According to the mechanism of tyrosinase activity, during the production of L-DOPA from L-tyrosine a reducing agent is produced that generates AuNPs (Figure 4) (17).

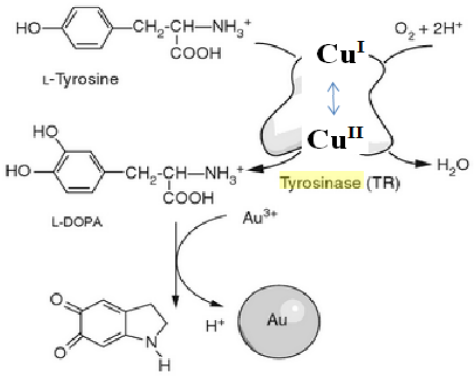

Fig. 4- Mechanism of AuNPs synthesis by tyrosinase

References

(1) Li X., Xu H., Chen ZS., Chen G. Biosynthesis of nanoparticles by microorganisms and their applications. *Journal of Nanomaterials* 2011; doi.org/10.1155/2011/270974.

(2) Mandal D., Bolander ME., Mukhopadaya D., Sarkar G., Mukherjee P. The use of microorganisms for the formation of metal nanoparticles and their application. *Appled Microbiology and Biotechnology* 2006; 485-492.

(3) Katz E., Willner I., Wang J. Electroanalytical and bioelectro-analytical systems based on metal and semiconductor nanopar-ticles. *Electroanalysis* 2004; 16: 19–44.

(4) Palomo JM., Filico M. Biosynthesis of metal nanoparticles: Novel efficient heterogeneous nanocatalysts. *Nanomaterials* 2016; 6, 84.

(5) Tyagi PK. Production of metal nanoparticles from biological resources. *International Journal of Current Microbiology and Applied Science* 2016; 5 (3): 548-558.

(6) Zayats M., Baron R., Popov I., Willner I. Biocatalytic growth of Au nanoparticles: from mechanistic aspects to biosensors design. *Nano Letters* 2005; 5: 21–25.

(7) Kulkarni N., Muddapur U. Biosynthesis of metal nanoparticles: A Review. *Journal of Nanotechnology* 2014*;* [Article ID 510246].

(8) Iravani S. Bacteria in nanoparticle synthesis: current status and future prospects. International scholarly research notices. *Journal of Nanomaterials* 20ll; [Article ID 359316].

(9) Hosseini-Abari A., Emtiazi G., Lee SH., Kim BG., Kim JH. Biosynthesis of Silver Nanoparticles by *Bacillus stratosphericus* Spores and the Role of Dipicolinic Acid in This Process. *Applied Biochemistry and Biotechnology* 2014; DOI 10.1007/s12010-014-1055-3

(10) Hosseini-Abari A., Kim BG., Lee SH., Emtiazi G., Kim W., Kim JH. Surface display of bacterial tyrosinase on spores of *Bacillus subtilis* using CotE as an anchor protein. *Journal of Basic Microbiology* 2016; 56, 1–7.

(11) Harwood C., Cutting S. *Molecular biological methods for Bacillus*. England: John Wiley & Sons Ltd, Chichester. (pp.16-30); 1990.

(12) Kohashi PY., Kumagai T., Matoba Y., Yamamoto A., Maruyama M., Sugiyama M. An efficient method for the overexpression and purification of active tyrosinase from *Streptomyces castaneoglobisporus*. *Protein Expression and Purification* 2004; 34, 202–210.

(13) Das SK., Dickinson C., Lafir F., Brougham DF., Marsili E. Synthesis, characterization and catalytic activity of gold nanoparticles biosynthesized with *Rhizopus oryzae* protein extract. *Green Chemistry* 2012**;** 14**,** 1322-1334.

(14) Mirkin CA., Niemeyer CM. *Nanobiotechnology II, more concepts and applications.* England: John Wiley & Sons Ltd (pp. 210- 250); 2007.

(15) Li F, Li Z, Zeng C, Hu Y. Laccase-assisted rapid synthesis of colloidal gold nanoparticles for the catalytic reduction of 4-Nitrophenol. *Journal of Brazilian Chemical Socity* 2017; 960-966.

(16) Sanghi R., Verma P., Puri S. Enzymatic formation of gold nanoparticles using *Phanerochaete Chrysosporium*. *Advances in Chemical Engineering and Science* 2011; 1, 154-162.

(17) Baron R., Zayats M., Willner I. Dopamine-, L-DOPA-, adrenaline-, and noradrenaline-induced growth of Au nanoparticles: assays for the detection of neurotransmitters and of tyrosinase activity. *Analytical Chemistry* 2005; 77 (6):1566-1571.

تولید نانوذرات طلا توسط تیروزیناز تثبیت شده در سطح اسپور

افروز السادات حسینی *: استادیار میکروبیولوژی، دانشگاه اصفهان، اصفهان، ایران، afrouz_hosseini1985@yahoo.com
بیانگی کیم: استاد، گروه مهندسی شیمی زیستی دانشگاه ملی سئول، سئول ، کره، byungkim@snu.ac.kr
جونیان کیم: دانشیار، گروه مهندسی شیمی، دانشکده مهندسی، دانشگاه دانگ آ، بوسان، کره، june0302@dau.ac.kr

چکیده

مقدمه: با توجه به کاربردهای گسترده نانوذرات طلا در پزشکی و الکترونیک، روش‌های سنتز زیستی این نانوذرات قابل ملاحظه است. روش‌های زیستی متفاوت به ایجاد انواع متنوعی از نانوذرات فلزی منجر می‌شوند. آنزیم‌ها ابزارهایی قدرتمند در این مسیر هستند. در پژوهش حاضر، نقش تیروزیناز در سنتز نانوذرات طلا توسط تیروزیناز تثبیت شده در سطح اسپور مطالعه شده است.

مواد و روش‌ها: تیروزیناز بیان شده در سطح اسپور *باسیلوس سابتلیس* به عنوان منبع آنزیمی برای تولید نانوذرات طلا استفاده شد. روش‌های انکسار اشعه **X** و میکروسکوپ الکترونی برای بررسی ویژگی‌های نانوذرات استفاده شد. برای تائید نقش آنزیم در سنتز نانوذرات طلا، دو نوع تیروزیناز از *باسیلوس مگاتریوم* و *استرپتومایسز* نیز مطالعه شدند.

نتایج: نتایج نشان داد که تیروزیناز تثبیت شده در سطح اسپور Au^{3+} را به Au^{0} تبدیل می‌کند. نانوذرات تولید شده مخلوطی از ساختارهای کروی، مثلثی و شش ضلعی با اندازه حدود ۲/۵ تا ۳۵ نانومتر را نشان دادند. همچنین، تیروزیناز خالص شده *باسیلوس مگاتریوم* و *استرپتومایسز* نانوذرات طلا تولید کردند.

بحث و نتیجه‌گیری: مکانیسم احتمالی تولید نانوذرات طلا به وسیله تیروزیناز، انتقال الکترون از یون‌های مس به کاتیون طلاست. این نتایج روشی ساده و سازگار با محیط زیست را در سنتز نانوذرات طلا با استفاده از تیروزیناز بیان شده در سطح اسپور نشان می‌دهد.

واژه‌های کلیدی: نانوذرات طلا، تیروزیناز بیان شده در سطح اسپور، تیروزیناز

* نویسنده مسؤول مکاتبات

تاریخ دریافت: ۱۳۹۶/۰۸/۲۴- تاریخ پذیرش: ۱۳۹۶/۱۰/۰۵