Chapter 16

Green Nano Actinobacteriology – An Interdisciplinary Study

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Abstract

Green nano actinobacteriology has been considered as a novel field of study in order to develop least expensive and highly qualitative strategies for production of eco-friendly beneficial products with diverse applications. The uniqueness of bioactive actinomycetes has turned the attention of scientists worldwide in order to explore its potentiality as effective "micronanofactories". This chapter provides a brief overview of the synthesis, characterization, and application of actinobacterial nanoparticles with an added note to actinobacterial detoxification.

Keywords: actinomycetes, micronanofactories, synthesis, characterization, application, detoxification

1. Introduction

Nanotechnology is a rapid, upcoming, multidisciplinary promising area that has an influence in medical, agricultural, and industrial fields, where they manufacture materials at the nano scale (one billionth of a meter or 10^{-9} in size). These nanomaterials are synthesized by physical, chemical, and biological methodologies. Physical processes include mechanical smashing, solid phase reaction, laser ablation, melt mixing, high-energy ball milling, physical vapor deposition, freeze drying, spread drying, ion sputtering, solvothermal synthesis, sol-gel technique, and precipitation [16, 17]. Chemical processes include aqueous and nonaqueous chemical reduction, electrochemical reduction, template method, ultrasonic-assisted reduction, photocatalytic reduction, microwave-assisted synthesis, biochemical reduction, irradiation reduction, and micro-emulsion method [32]. Both these physical and chemical processes need high temperature and high pressure to execute. These processes were costlier and produced highly toxic compounds along with consumption of high energy.

Therefore, scientists employ inexpensive, eco-friendly, easier scale-up biological synthesis methodology by utilizing both plant extracts [6, 10, 21, 26, 28] and microbes. The phytochemicals and enzymes present in plants and microbes reduce the metal compounds into corresponding nanoparticles [1]. Initially, among microbes, bacteria was utilized for green synthesis, which was later overcome by the use of yeast, algae [7, 25] and fungi [5, 8, 20, 27]. But the use of actinomycetes (filamentous bacteria) was comparatively less reported, though they act as valuable resource for numerous diverse bioactive metabolites [2]. Compared to bacteria, fungi and actinobacteria secrete more proteins which in turn increase biosynthesis production. Compared to fungi, actinobacteria comes under prokaryotes group. Hence, they can be manipulated genetically in order to achieve better control over size and polydispersity of the nanoparticles [14, 36]. This biogenic process takes place at ambient temperature and pressure conditions and no toxic chemical is involved [37].

In this chapter, green synthesis of nanoparticles by actinobacteria and their application in varied fields were studied under green nano actinobacteriology, which acts as the interface between "nanotechnology" and "actinobacteriology".

2. Biosynthesis of Nanoparticles

Actinomycetes are helpful in the biosynthesis of nanoparticles with good surface and size characteristics showing wide range of bioproperties. Actinobacterial production of metallic nanomaterials was mediated by extracellular and intracellular methodologies. Extracellular synthesis has got more commercial advantages compared to intracellular synthesis since polydispersity plays a significant role. In the case of intracellular synthesis, the accumulated nanoparticles are of particular dimension with less polydispersity [4]. In extracellular synthesis, steps such as fermentation, filtration, followed by enzyme substrate complex formation in dark condition were involved. Whereas in intracellular synthesis, additional steps like ultrasound treatment of enzyme substrate complex or its reaction with suitable detergents were required [4]. Hence due to the above-mentioned drawbacks, unlike bacteria and fungi, only less number of intracellular actinobacterial nanoparticle production was reported.

The basic principle is actinobacteria, when exposed to metal ions, releases enzymes that reduce the metal ions to yield highly stable nanoparticles. Usually, silver ions in the form of silver nitrate ($AgNO_3$) solution [9] or gold ions in the form of chloroauric acid ($AuCl_4$) solution were used as substrate to the enzyme secreted from microbes. Both gold and silver are nonallergic, biocompatible with slow oxidation rate, and effective antimicrobial agents. Several researches are conducted by using other metals like zinc, manganese, and copper for nanoparticle formation.

Most of the nanoparticle biosynthesis work was carried out in *Streptomyces* sp., except the first novel work in *Rhodococcus* sp where Ahmed et al. [38] reported the intracellular synthesis of gold nanoparticle of the dimension 5-15 nm. They were concentrated more on cytoplasmic membrane than on cell wall. Using *Streptomyces* sp., intracellular synthesis of gold nanoparticles [39, 40], silver nanoparticles [41, 42], zinc and manganese nanoparticles [33] were reported till date.

As mentioned above, compared to intracellular synthesis, more number of extracellular silver [2, 11, 14] and gold nanoparticles [49, 50, 51] were reported in *Streptomyces* sp. Rare actinobacteria like *Thermomonospora* [11, 48], *Nocardia farcinica* [47] were used to synthesize gold nanoparticles, whereas *Nocardiopsis* sp. MBRC-1 [23], *Rhodococcus* sp. [45], *Thermoactinomyces* sp. [30], and unnamed actinomycetes [18, 32, 44] were used to synthesize silver nanoparticles. Usha et al. [43] isolated *Streptomyces* sp. that was used for the biosynthesis of copper and zinc nanoparticles.

3. Characterization of Nanoparticles

Techniques used for characterizing nanoparticles and their respective applications are as follows.

a. Colour change test

The formation of nanoparticles was detected by the color change within 72 h. Change of color from pale yellow to brownish color shows formation of silver nanoparticles [19]. Change of color from pale yellow to pinkish color indicates formation of gold nanoparticles. Formation of whitish yellow to yellow color shows formation of manganese and zinc nanoparticles [33].

b. UV--Visible Spectroscopy

The reduction of metal ions was estimated by measuring the absorption level using UV-visible spectroscopy. Light wavelengths of 200-800 nm are considered for nanoparticle characterization. Absorption measurements in the wavelength range of 400-450 nm and 500-550 nm are used in characterizing silver and gold nanoparticles, respectively [4].

c. X-ray Diffraction (XRD)

The phase identification and characterization of nanoparticle crystal structure were studied using XRD. X-rays penetrate into the freeze-dried and powdered nanoparticle at the scan speed of 0.02/min and the resultant diffraction is compared with the standard in order to derive its structural information [2, 4].

d. Fourier Transform Infrared Spectrometer (FT-IR) Analysis

Dried nanoparticle sample was mixed with KBr in 1:100 ratio and scanned using IR rays of around 4000-400 cm^{-1} with reflectance mode at 4 cm^{-1} resolution. The chemistry and the variation in functional group attached to the nanoparticle surface are analyzed using FT-IR [4, 12, 30].

e. Dynamic Light Scattering (DLS)

DLS was performed to determine the size, surface charge, and distribution of nanoparticles suspended in a liquid [17].

f. Energy Dispersive X-ray (EDX)

EDX was performed to determine the elemental composition of metal nanoparticles [17].

g. Atomic Force Microscopy (AFM)

AFM was performed to determine the size and topological appearance of metal nanomaterials. Porosity, roughness, and fractal dimension are also analyzed with the help of AFM images. For AFM study, the sonicated metal nanoparticles were formed as a thin film on slide [29, 32].

h. Scanning Eelectron Microscope (SEM)

SEM was performed to determine the size and surface morphology of biosynthesized metal nanomaterials. For SEM study at an operating voltage of 15 kV, the nanoparticle solution was sonicated, centrifuged, and the resultant dried powdered nanoparticles were used as sample [29].

i. Transmission Electron Microscope (TEM)

Compared to SEM, TEM gives 1,000 fold higher morphological resolution including both size and shape. The ultrasonicated nanomaterial sample was placed on copper grid coated with 300 mesh palladium and carbon for TEM study at 80 kV [33].

4. Applications

Nowadays, biosynthesized nanoparticles, especially gold and silver, were used in diverse applications especially in diagnostic field due to its antibacterial, antifungal, larvicidal, antifouling, anticancerous, antioxidant properties. Green synthesis of nanoparticles using dextran as ligand was explored since dextran was considered as cheaper, nontoxic and biocompatible agent [31].

a. Antibacterial activity

Using *Streptomyces viridogens*, gold nanoparticles of spherical shape with 18-20 nm was synthesized through intracellular mode [39]. A considerable number of silver nanoparticles using *Streptomyces* sp. were reported to show antibacterial activity against varied human pathogens through extracellular mode [14, 22, 24, 29, 51, 53-57]. Unnamed actinomycetes mediated silver nanoparticles were also reported to produce antibacterial activity [13, 18, 44]. Rare actinobacteria such as *Thermoactinomyces* sp. produce spherical shaped 20-40 nm sized silver nanoparticles were reported by Deepa et al. [30]. Similarly, Usha et al. [43] reported the production of copper and zinc nanoparticles sized 100-150 nm using *Streptomyces* sp. showing antimicrobial activity. The mechanism behind this bactericidal effect is silver nanoparticles attach to the bacterial cell membrane and disturb both respiration and permeability. Then by penetrating deep into the DNA part, it causes further damage by disrupting DNA replication due to release of silver ions, which leads to target cell destruction [32, 34].

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b. Antifungal activity

Biosynthesis of gold nanoparticles using *Streptomyces* sp. VITDDK3 showed antifungal activity against *M. gypseum* and *T. rubrum* by changing the membrane potential and inhibiting ATP synthase activity of the target cell [49].

c. Anti-biofouling property

Anti-biofouling is defined as the process of eliminating the microbes that aggregate on wetted surface forming biofilms leading to foul smell production. Shanmugasundaram et al. [42] reported the antibiofouling property of 5-50 nm spherical-shaped silver nanoparticles using *Streptomyces naganishii* MA7.

d. Antioxidant property

An antioxidant prevents the oxidation of other molecules by interfering with defense mechanism leading to ROS generation [46]. Antioxidant property of silver nanoparticles mediated by *Streptomyces naganishii* MA7 was reported by Shanmugasundaram et al. [42], showing positive DPPH scavenging activity.

e. Larvicidal property

Silver nanoparticles synthesized by using *Streptomyces* sp. GRD showed effective larvicidal activity against *Culex quinquefasciatus* and *Aedes aegypti*, which would be an effective bioprocess for mosquito control [35]. Nanoparticles penetrate through larval membrane into the intracellular space leading to denaturation of organelles and enzymes [35]. Karthik et al. reported the biosynthesis of gold nanoparticles with antimalarial activity using *Streptomyces* sp. LK3 [50].

f. Anticancerous property

Varied researches are going on in order to treat cancer and reduce its side effects worldwide. Manivasagan et al. reported the cytotoxic effect of silver nanoparticles mediated by *Nocardiopsis* sp. MBRC-1 [23]. Same way, an actinomycete PSBVIT-13 [18] and *Streptomyces naganishii* MA7 [42] were also reported to mediate silver nanoparticles with cytotoxic effect.

g. Others

Torres-Chavolla et al. [52] reported the production of gold nanoparticles using *Thermomonospora* sp. which can be utilized as biosensing enhancement analytical device meant for detection purpose in military field and pollution control field.

5. Drawbacks (Nanoparticle toxicity)

Exposure of cells to actinobacterial nanoparticles like silver nanoparticles alters mitochondrial functioning by collapsing proton-motive force across the cell membrane, which leads to increased membrane permeability. As a result, ROS (reactive oxygen species) will be generated, which seems to be the initiator for toxicity. Factors like breakdown of its unique surface plasmon resonance, magnetic, chemical, and optical properties are responsible for toxicity.

6. Actinobacterial detoxification

Actinomycetes are resistant to toxic heavy metals due to their chemical detoxification, solubility alteration, as well as energy-dependent ion efflux from the cell by membrane proteins that function either as ATPase or chemiosmotic cation or proton anti-transporters. Detoxification can be made by reduction, precipitation, biosorption, biomineralization, and bioaccumulation [4].

7. Future directions

Compared to physical and chemical approaches, biosynthesis of nanoparticles consumes extra time. Actinobacterial-mediated nanoparticle biosynthesis has got more advantages such as the possibility of scaling up the process, economic viability, and the possibility of covering a large surface area due to mycelial growth. [11]. Hence, several criteria like synthesis time, particle size, solubility, stability, monodispersity should be given utmost priority in order to obtain highly efficient actinobacterial nanoparticles. By optimizing parameters like type of microbe, growth stage, growth medium, synthesis conditions, source compound of target nanoparticle, etc., it is possible to obtain clean, nontoxic, and eco-friendly nanoparticles [15].

8. Conclusion

As discussed earlier, due to increased cognizance towards green chemistry approach, the need of the hour is to develop eco-friendly strategies for the synthesis of nontoxic nanoparticles. To serve that purpose, the least explored actinomycetes have to be further explored so that they can emerge as effective "micronanofactories" in future. Still, the exact mechanism of nanoparticle biosynthesis is not clear. By decrypting the never ending clues involved in its synthesis, actinomycetes would be considered as the efficient microbial group for harnessing nanoparticles.

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References

[1] Udaya Prakash NK, et al. Green synthesis of silver nanoparticles using airborne actinomycetes. Int J ChemTech Res 2014;6(9):4123-7.

[2] Vidhya A, Balagurunathan R. Isolation and screening of alkalophilic actinobacteria, Streptomyces for biosynthesis and characterization of silver nanoparticles. Int J Novel Trends Pharm Sci 2013;3(1):7-14.

[3] Khalil MMH, et al. Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity. Arab J Chem 2014;7:1131-9.

[4] Manivasagan P, et al. Actinobacteria mediated synthesis of nanoparticles and their biological properties. Crit Rev Microbiol 2014; Nov 28: 1-13.

[5] Rai M, Yadav A, Gade A. Mycofabrication, mechanistic aspect and Multifunctionality of Metal Nanoparticles - where are we? And where should we go? In: Mendez-Vilas A. (ed.) Current Research, Technology and Education topics in Applied Microbiology and Microbial Biotechnology. 2010. pp. 1343-1354.

[6] Narayanan P, et al. Antibacterial effect of green synthesized silver nanoparticles against Vibrio sp. isolated from broiler chicken. J Adv Sci Res 2012;3(4):51-4.

[7] Yason Tsibakhashvili N, et al. Microbial synthesis of silver nanoparticles by *Streptomyces glaucus* and *Spirulina platensis*. Nanomater: Applic Prop (NAP-2011). 2011;2(2): 306-10.

[8] Gupta S, Sharma K, Sharma R. Myconanotechnology and application of nanoparticles in biology. Rec Res Sci Technol 2012;4(8):36-8.

[9] Renganathan S, et al. A review on green synthesis of silver nanoparticles. Asian J Pharm Clin Res 2013;6(1):8-12.

[10] Kulkarni AP, et al. Phytofabrication of silver nanoparticles from a novel plant source and its application. Int J Biol Pharm Res 2012;3(3):417-21.

[11] Sastry M, Ahmad A, Islam Khan M, Kumar R. Biosynthesis of metal nanoparticles using fungi and actinomycetes. Curr Sci 2003;85(2):162-70.

[12] Sanghi R and Verma P. pH dependant fungal proteins in the ‘green’ synthesis of gold nanoparticles. Adv Mat Lett 2010;1(3):193-9.

[13] Sunitha A, et al. Evaluation of antimicrobial activity of biosynthesized iron and silver nanoparticles using the fungi *Fusarium oxysporum* and *Actinomycetes* sp. on human pathogens. Nano Biomed Eng 2013;5(1):39-45. DOI: 10.5101/nbe.v5i1.

[14] Singh D, et al. Biologically reduced silver nanoparticles from *Streptomyces* sp. VDP-5 and its antibacterial efficacy. Int J Pharm Pharm Sci Res 2014;4(2):31-6.

[15] Li X, Xu H, Chen Z-S, Chen G. Biosynthesis of nanoparticles by microorganisms and their applications. J Nanomater 2011;Article ID 270974:1-16.

[16] Lavanya M, et al. Synthesis, characterization and evaluation of antimicrobial efficacy of silver nanoparticles using *Paederia foetida* L. leaf extract. Int Res J Biol Sci 2013;2(3): 28-34.

[17] Panda SS, et al. Synthesis of silver nano particles from plant extract and its application in cancer treatment: a review. Int J Plant Animal Environ Sci 2014;4(3):137-45.

[18] Rao B, et al. Biosynthesis of silver nanoparticles using actinobacteria and evaluating its antimicrobial and cytotoxicity activity. Int. J Pharm Pharm Sci 2013;5(2):709-12.

[19] Singh P, Balaji Raja R. Biological synthesis of nanoparticle from *Trichoderma hHarzianum*. Asian J Exp Biol Sci 2011;2(4):600-5.

[20] onary S, et al. Green synthesis of copper oxide nanoparticles using *Penicillium aurantiogriseum, Penicillium citrinum* and *Penicillium waksmanii*. Digest J Nanomater Biostruct 2012;7(3):999-1005.

[21] Hiremath S, et al. Green synthesis of Tio2 nanoparticles by using neem leaf extract. Int Rev Appl Biotechnol Biochem 2014;2(1):11-7.

[22] Shanmuga Priya T, Balasubramanian V. Enzyme mediated synthesis of silver nanoparticles using marine actinomycetes and their characterization. Biosci Biotechnol Res Asia 2014;11(1):159-65.

[23] Manivasagan P, Venkatesan J, Senthilkumar K, Sivakumar K, Kim S-K. Biosynthesis, antimicrobial and cytotoxic effect of silver nanoparticles using a novel *Nocardiopsis* sp. MBRC-1. BioMed Res Int 2013;Article ID 287638:1-9. DOI: http://dx.doi.org/10.1155/2013/287638.

[24] Kannabiran K, et al. Antimicrobial activity of Streptomyces sp. VITBT7 and its

[25] synthesized silvernanoparticles against medically important fungal and bacterial pathogens. Der Pharmacia Lett 2013;5(3):192-200.

[26] Roychoudhury P, Pal R. Synthesis and characterization of nanosilver-A blue green approach. Ind J Appl Res 2014;4(1):54-6.

[27] Ganesan V, et al. Bio-inspired synthesis of silver nanoparticles using leaves of *Millingtonia hortensis* L.F. Int J Adv Biotechnol Res 2014;5(2):93-100.

[28] Chauhan A, Zubair S, et al. Fungus-mediated biological synthesis of gold nanoparticles: potential in detection of liver cancer. Int J Nanomed 2011;6:2305-19.

[29] Dwivedi R. Green synthesis of silver nanoparticle using *Terminalia chebula* and assessment of its antimicrobial activity. Int J Pure App Biosci 2013;1(6):1-6.

[30] Zarina A, et al. Green approach for synthesis of silver nanoparticles from marine Streptomyces- MS 26 and their antibiotic efficacy. J Pharm Sci Res 2014;6(10):321-7.

[31] Deepa S, Kanimozhi K, Panneerselvam A. Antimicrobial activity of extracellularly synthesized silver nanoparticles from marine derived actinomycetes. Int J Curr Microbiol App Sci 2013;2(9):223-30.

[32] Hussain MA, et al.. One pot light assisted green synthesis, storage and antimicrobial activity of dextran stabilized silver nanoparticles. J Nanobiotechnol 2014;12(53):1-6.

[33] Chauhan R, Kumar A, Abraham J. A biological approach to the synthesis of silver nanoparticles with Streptomyces sp. JAR1 and its antimicrobial activity. Sci Pharm 2013;81:607-21.

[34] Waghmare SS, et al. Biosynthesis and characterization of manganese and zinc nanoparticles. Univ J Environ Res Technol 2011;1:64-9.

[35] Kaler A, Patel N, Banerjee UC. Green synthesis of silver nanoparticles. CRIPS 2010;11(4):68-71.

[36] Rajesh K, Padmavathi KC, et al. Green synthesis, characterization and larvicidal activity of AgNps against *Culex quinquefasciatus* and *Aedes aegypti* larvae. Am J Drug Disc Develop 2013;3(4):245-53.

[37] Singh D, Rathod V, Shivaraj N, Jyothi H, Singh AK, Jasmine M. Optimization and characterization of silver nanoparticles by endophytic fungi *Penicillium* sp. isolated from *Curcuma longa* (turmeric) and application studies against MDR *E. coli* and *S. aureus*. Bioinorg Chem Applic 2014; Article ID 408021,1-8.

[38] Gade AK, Bonde P, Ingle AP, Marcato PD, Duran N, Rai MK. Exploitation of *Aspergillus niger* for synthesis of silver nanoparticles. J Biobased Mater Bioenergy 2008;2:243-7.

[39] Ahmad A, Senapati S, Khan MI, et al. Intracellular synthesis of gold nanoparticles by a novel alkalotolerant actinomycete, *Rhodococcus* species. Nanotechnology 2003b; 14:824- 8.

[40] Balagurunathan R, Radhakrishnan M, Rajendran RB, Velmurugan D. Biosynthesis of gold nanoparticles by actinomycetes *Streptomyces viridogens* strain HM10. Indian J Biochem Biophys 2011;48:331-5.

[41] Sapkal M, Deshmukh A. Biosynthesis of gold nanoparticles by Streptomyces species. Res J Biotechnol 2008;3:36-9.

[42] Alani F, Moo-Young M, Anderson W. Biosynthesis of silver nanoparticles by a new strain of *Streptomyces* sp. compared with *Aspergillus fumigatus*. World J Microbiol Biotechnol 2012;28:1081-6.

[43] Shanmugasundaram T, Radhakrishnan M, Gopikrishnan V, et al. A study of the bactericidal, anti-biofouling, cytotoxic and antioxidant properties of actinobacterially synthesised silver nanoparticles. Colloids Surf B 2013;111:680-7.

[44] Usha R, Prabu E, Palaniswamy M, et al. Synthesis of metal oxide nano particles by *Streptomyces* sp for development of antimicrobial textiles. Global J Biotechnol Biochem 2010;5:153-60.

[45] Sukanya M, Saju K, Praseetha P, Sakthivel G. Therapeutic potential of biologically

[46] uced silver nanoparticles from actinomycetes cultures. J Nanosci 2013;2013:1-8.

[47] Otari S, Patil R, Nadaf N, et al. Green biosynthesis of silver nanoparticles from an actinobacteria *Rhodococcus* sp. Mater Lett 2012;72:92-4.

[48] Oyaizu M. Studies on products of browning reaction - antioxidative activities of products of browning reaction prepared from glucosamine. Eiyogaku zasshi Jpn J Nut 1986;44:307- 15.

[49] Oza G, Pandey S, Gupta A, et al. Biosynthetic reduction of gold ions to gold nanoparticles by *Nocardia farcinica*. J Microbiol Biotechnol Res 2012;2:511-15.

[50] Ahmad A, Senapati S, Islam Khan M, et al. Extracellular biosynthesis of monodisperse gold nanoparticles by a novel extremophilic actinomycete, *Thermomonospora* sp. Langmuir 2003a;19:3550-3.

[51] Vinay Gopal J, Thenmozhi M, Kannabiran K, et al. Actinobacteria mediated synthesis of gold nanoparticles using *Streptomyces* sp. VITDDK3 and its antifungal activity. Mater Lett 2013;93:360-2.

[52] Karthik L, Kumar G, Bhattacharyya A, et al. Marine actinobacterial mediated gold nanoparticles synthesis and their antimalarial activity. Nanomed: Nanotechnol Biol Med 2013;9:951-60.

[53] Sadhasivam S, Shanmugam P, Veerapandian M, et al. Biogenic synthesis of multidimensional gold nanoparticles assisted by *Streptomyces hygroscopicus* and its electrochemical and antibacterial properties. BioMetals 2012;25:351-60.

[54] Torres-Chavolla E, Ranasinghe RJ, Alocilja EC. Characterization and functionalization of biogenic gold nanoparticles for biosensing enhancement. Trans Nanotechnol IEEE 2010; 9:533-8.

[55] Manikprabhu D, Lingappa K. Antibacterial activity of silver nanoparticles against methicillin-resistant *Staphylococcus aureus* synthesized using model *Streptomyces* sp. pigment by photo-irradiation method. J Pharm Res 2013;6:255-60.

[56] Sivalingam P, Antony JJ, Siva D, et al. Mangrove *Streptomyces* sp. BDUKAS10 as nanofactory for fabrication of bactericidal silver nanoparticles. Colloids Surf B 2012; 98:12-17.

[57] Samundeeswari A, Dhas SP, Nirmala J, et al. Biosynthesis of silver nanoparticles using actinobacterium *Streptomyces albogriseolus* and its antibacterial activity. Biotechnol Appl Biochem 2012;59:503-7.

[58] Shetty PR, Kumar YS. Characterization of silver nanoparticles synthesized by using marine isolate *Streptomyces albidoflavus*. J Microbiol Biotechnol 2012;22:614-21.

[59] Subashini J, Khanna VG, Kannabiran K. Anti-ESBL activity of silver nanoparticles biosynthesized using soil *Streptomyces* species. Biopro Biosys Eng 2014;37(6):999-1006. DOI:10.1007/s00449-01.