RESEARCH ARTICLE

Extracellular Biosynthesis of Silver Nanoparticles from Soil-Isolated *Streptomyces* sp. NBrR7 and its Antibacterial and Anticancer Activities

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Abstract

Silver nanoparticles (AgNPs) have gained considerable attention in the field of medicine owing to their physiochemical properties. Actinomycetes are efficient microbe for metal nanoparticle production extracellularly and intracellularly. In this regard, the extracellular biosynthesis of AgNPs by a *Streptomyces* sp. isolated from the rhizosphere soil in Saudi Arabia was achieved. Eleven actinomycete isolates coded NBrR1 to NBrR11 were isolated and purified on starch nitrate agar medium. Screening the filtrates of these isolates for the biosynthesis of AgNPs revealed that, only 4 isolates exhibited various bio-reduction degrees of Ag⁺ in aqueous solution as suggested by initial changes in the color of the solution. Among them, NBrR7 was selected as the most potent-isolate and identified based on the cultural, morphological as well as the physiological and biochemical characteristics which indicated belonging of this isolate to the genus *Streptomyces* and designated as *Streptomyces* sp. NBrR7. The microbiologically synthesized AgNPs showed promising antibacterial activity, especially against *P.aeruginosa* and *B.subtilis*. Promising cytotoxic activity was recorded for AgNPs against number of human cancer cell lines where it showed strong cytotoxicity against colon carcinoma (HCT-116) with IC₅₀ 21.8 µg/ml, moderate activity against liver carcinoma (HEPG2) with IC₅₀ 39.5 µg/ml while it was weak against breast adenocarcinoma (MCF-7) with IC₅₀ 47.80 µg/ml. The microbiologically synthesized AgNPs obtained from the locally isolated *Streptomyces* sp. NBrR7 has lower toxicity and excellent biomedical applications.

Introduction:

In recent years, silver nanoparticles have got considerable attention due to their use as catalyst, antimicrobial and therapeutic agent marker (Ivrigh *et al.*, 2017) and adsorbent in many applications such as drug delivery, composite synthesis, material and fabric coating (Shrivastava *et al.*, 2007), monitoring of some pesticides (Ivrigh *et al.*, 2017), detoxification of hazardous materials and decolorization of dyes (Adiguzel *et al.*, 2018). Indeed, Nanoparticles are now considered as a viable antimicrobial agent and seem to have tremendous potential to solve the problem of microbial multidrug resistance (Rai *et al.*, 2012; Franci *et al.*, 2015) and microbial biofilm formation (Mallevre *et al.*, 2016). Considering the limitations of antibiotic therapy and the

severe side effects of current antimicrobial drugs, there is an urgent need for a new class of therapeutic agents with better biocompatibility and efficiency (Wypij *et al.*, 2018).

AgNPs are often produced by chemical methods, which were carried out by hazardous reducing agents such as NaBH₄, hydroquinone and gallic acid, and physical methods requiring high temperature, pressure, and energy or through combinations of both chemical and physical methods. Alternatively, biological methods that are cost-effective, safe and eco-friendly, can be applied for AgNPs production. Furthermore, synthesized AgNPs by biological methods is easily purified and recovered from the reaction medium. In biological methods, plant extract or cell lysates of microorganisms work as reducing agents for AgNPs

production. However, the use of plants is not seeming to be more appropriate for industrial-scale production of AgNPs considering the cultivation time, harvesting and transportation costs of plants. Thus, there is a significant concern about the use of microorganisms to produce AgNPs (Adiguzel *et al.*, 2018).

Actinomycetes are considered an important resource for new products of medical and industrial interest such as antimicrobial agents (Bhosale *et al.*, 2015; Mohamedin *et al.*, 2015). They are being used as eco-friendly nano factories (Deepa *et al.*, 2013; Abdeen *et al.*, 2014; Lakshmi *et al.*, 2015). Actinomycetes are efficient microbes for metal nanoparticle production extra and intracellularly. The synthesis of nanoparticles by actinomycetes represents good stability and polydispersity. Actinomycetes possess important biocidal activity against different pathogens (El-Naggar & Abdelwahed, 2014; Golinska *et al.*, 2014; Mohamedin *et al.*, 2015). Moreover, it can be manipulated genetically in order to provide better control oversize (Bhosale *et al.*, 2015).

Actinomycetes are widely distributed in soils and represent a high proportion of the soil microbial biomass. Among actinomycetes, *Streptomyces* is represented by the largest number of species and varieties, which differ greatly in their morphology, physiology and biochemical activities. For several decades, terrestrial actinomycetes have proved to be an important source of biologically active metabolites, including antibiotics, antitumor, immunosuppressant agents and biogenic nanoparticles (Adiguzel *et al.*, 2018). *Streptomyces* are the saprophytic organism that spends the most life cycles as semi-dormant spores. In life cycle, *Streptomyces* spores germinate for producing substrate mycelium, which during maturation fragments into chains of spores (Jayaseelan *et al.*, 2012). Generally, synthesize many different metabolites, antibiotics represent the most known product used in the veterinary and pharmaceutical side. *Streptomyces* spp. positive for gram stain, filament bacteria and produce a different compound with having biologically active compounds such as hydrolytic enzymes, antibiotics and enzyme inhibitors (Sastry *et al.*, 2003).

The extracellular biological synthesis of AgNPs could be an attractive and ecologically friendly alternative method for the preparation of large quantities because it offers the advantage of easy downstream processing. Moreover, these are easy to handle and can be manipulated genetically without much difficulty (Abdeen *et al.*, 2014).

In the present study, we selected actinobacterial strain for the synthesis of biogenic nanoparticles as these microorganisms are known to be important producers of most natural bioactive compounds, mainly antibiotics and antimetabolites (Bérdy, 2005; Newman & Cragg 2007; Olano *et al.*, 2009). The aims of the present study were isolation, screening for silver nanoparticles from *Streptomyces* cultures isolated from soil samples collected from different localities at Rafha governorate in the

Northern Border region, Kingdom of Saudi Arabia. The synthesized AgNPs were tested for antimicrobial activity and then *in vitro* evaluated for cytotoxicity against some human cancer cell lines.

Materials and Methods:

Chemical and reagents: The chemicals used in this study were of analytical grade. Silver nitrate was purchased from Himedia, Mumbai, India. Dulbecco's Modified Eagle's Medium (DMEM), Phosphate Buffered Saline (PBS), Dimethylsulfoxide (DMSO) and 3-(4,5-dimethyl thiazol-2-yl)-5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma Aldrich (St. Louis, MO, USA).

Microbial test strains: antimicrobial activity was assayed against a panel of microorganisms certified by American Type Culture Collection (ATCC) and National Collection of Pathogenic Fungi (NCPF), including two Gram-positive bacteria *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538, and against two Gram-negative *Pseudomonas aeruginosa* ATCC 9027 and *Escherichia coli* ATCC 7839. Cultures were inoculated into specific broths and incubated at 37°C for 24 h.

Cell lines: the human liver carcinoma (HEPG2), the human colon carcinoma (HCT 116) and the human breast adenocarcinoma (MCF-7) cell lines were obtained from American Type Culture Collection (Rockville, MD) then maintained by serial sub-culturing at the National Cancer Institute (NCI), Cairo, Egypt.

Collection of soil samples: a total of fifteen soil samples included; five sandy soils, five rhizospheric soils, and 5 uncultivated soils were collected from different localities of the targeted area. The soil samples were collected in sterile plastic pages and kept in an icebox containing ice packs until they reached the laboratory. Samples treatment: the samples were subjected to physical and chemical pretreatment methods in order to facilitate the isolation of actinomycetes. Firstly, heat treatments were performed by holding soil samples in a water bath (Memmert) at 50°C for 60 min. secondly, the collected samples, were air-dried, pre-treated with CaCO₃ at the ratio (1:10 w/w) and incubated for 4 days at 28°C before plating (Tsao *et al.*, 1960). All samples were diluted with sterile 0.9 % saline prior to inoculation onto isolation plates (Takizawa *et al.*, 1993).

Isolation of actinomycete cultures: isolation of actinomycete was done by serial dilution method from the soil suspension (Hayakawa, 2008). The isolation and enumeration of actinomycete colonies from the collected samples were carried out on a starch nitrate agar medium. The medium was adjusted to the initial pH 7.0 prior to sterilization using 0.1 N NaOH or 0.1 N HCl solution, the medium was supplemented with cycloheximide (50µg/ml) to minimize the fungal contamination. The obtained actinomycete cultures were purified using the dilution plate technique described by Williams & Davis (1965).

Screening for synthesis of silver nanoparticles using *Actinomycetes* spp.: the actinomycete culture was grown in 250 ml Erlenmeyer flasks containing 100 ml of starch casein nitrate medium and incubated at 35°C on a rotary shaker of 120 rpm for 5 days. At the end of the incubation period and under aseptic conditions, the actinomycete cells were separated from the culture broth by using Whatman no. 1 filter paper. 5 ml filtrate was added into 25 ml mM AgNO₃ solution in 100 ml conical flasks at room

temp. in dark for some period. After one hour, the formation of silver particles started. The bio-reduction of Ag^+ in aqueous solution of the most potent isolate was monitored by measuring the UV-Visible spectrum of the reaction medium at different time intervals (12, 24, 48, 72, 96 and 120 h). This UV-Vis spectral analysis has been done by using a Perkin-Elmer Lambda-25 spectrophotometer.

Antimicrobial activity of AgNPs synthesized from *Streptomyces* sp.: it was tested by the agar disc diffusion method (Chung *et al.*, 2004). Bacterial inoculums (100 μL) were spread evenly onto 20 ml Mueller-Hinton agar set in 90-mm Petri dishes using a sterile cotton swab. Sterilized paper discs (6-mm diameter) were impregnated with 20 μL microliters of AgNPs solution using a micropipette and firmly placed onto the inoculated agar ensuring even distribution to avoid overlapping of zones. After overnight incubation at 37°C , the zone of inhibition around the discs was recorded (Chan *et al.*, 2011).

Cytotoxic activity of the synthesized AgNPs: for maintaining the previously mentioned carcinoma cells, it was cultured in 75 cm_2 cell culture flasks of RPMI-1640 medium containing 1% MEM non-essential amino acid solution, 10% fetal bovine serum and 1% penicillin-streptomycin solution in a humidified atmosphere of 95% air, 5% CO_2 at 35°C . The number of cells was maintained to be between 20 and 25. For studying the anticancer activity, the cells were cultured in plates of 96-well in a concentration 0.2 mL of cell solution/well then the cells were allowed to attach for 24 h before treatment with tested AgNPs. For determination of the cytotoxicity of the tested materials, the cell monolayers were washed with PBS then the serially diluted materials were added and dispensed to the pre-cultured plates (Romero *et al.*, 2003). Anticancer activity was performed at the National Cancer Institute (NCI), Cairo, Egypt.

Cytotoxic activity using MTT assay: the cytotoxic activity of the microbiologically synthesized AgNPs against the selected human cancer cell lines was studied by MTT assay (Mossmann, 1983). Briefly, the tested human cancer cells were incubated for 4 h in serum-free medium containing 0.8 mg of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT). The cells were washed with 1 ml of phosphate buffer saline (PBS) followed by the addition of 1 ml dimethyl sulfoxide (DMSO) then the solution was shaken gently for 10 min until the complete dissolution. 96-well plates were inoculated with aliquots (200 μL) of the prepared solution, the absorbance was recorded using the microplate spectrophotometer system at 560 nm. The obtained results were analyzed using the software of Softmax pro (version 2.2.1) as a percentage of the control value. For obtaining the survival curve, the relationship between the AgNPs concentration and surviving fraction was plotted and IC_{50} (the concentration required for 50% inhibition of cell viability) was calculated.

Conventional characterization of *Streptomyces* sp. NBrR7: the characterization of the most potent *Streptomyces* sp. NBrR7 was done by conventional methods according to the guidelines adopted by the International *Streptomyces* Project (Shirling & Gottlieb, 1966). The cultural characteristics were studied according to the guidelines established by the ISP (Shirling & Gottlieb, 1966), colors were assessed on the scale adopted by Kornerup & Wanscher (1987). Micro-morphological studies were carried out using light and scanning electron microscope (JEOL JSM 5300, JEOL Technics Ltd., Japan) (Bozzola & Russell, 1999) according to the method of Tresner & Davies (Tresner, 1961).

Diaminopimelic acid isomers in the cell-wall and whole-cell sugar pattern were analyzed using the method of Becker *et al.* (1965). The physiological and biochemical characteristics; melanin pigment production, utilization of carbon and nitrogen sources, enzymatic activities and other physiological characters were also studied (Lechevalier & Lechevalier, 1970). All experiments were performed in triplicates.

Statistical analysis: the results were recorded as mean values and the statistical significance was calculated using ANOVA (one-way analysis of variance), the differences were considered significant when $p < 0.05$.

Results:

Isolation and screening for AgNPs of the isolated actinomycete cultures: in the present study eleven actinomycete isolates coded NBrR1 to NBrR11 were isolated and purified on starch nitrate agar medium using dilution plate method. These isolates were arranged as 2 isolates (18.18%) were from sandy soils, 3 isolates (27.27%) were from uncultivated soils as well as 6 isolates (54.54%) were obtained from rhizosphere zone of some cultivated plants in the study area. Data of the obtained actinomycete isolates are tabulated in Table-1.

Screening for AgNPs synthesis: for testing biosynthesis of AgNPs by the obtained actinomycete cultures, it was allowed to grow on starch casein nitrate medium under submerged fermentation at optimum incubation. Among the eleven actinomycete filtrates which were tested for their ability to the bio-reduction of Ag^+ in aqueous solution, only 4 filtrates (36.36%) belonging to 4 different isolates (NBrR2, NBrR3, NBrR7 & NBrR11) exhibited various reduction degrees as suggested by initial changes in the color of the solution (Plate-1) after incubation for 72 h. Among them, NBrR-3 isolate was selected as the most potent strain for the biogenic synthesis of AgNPs where it showed deep brown color in comparing with other isolates. It is well known that AgNPs exhibit brown color in aqueous solution due to the excitation of surface plasmon vibrations in AgNPs. Reduction of the silver ion to silver nanoparticles during exposure to the actinomycete filtrate was followed by a color change and as well as by UV-Vis spectroscopy. The filtrate of NBrR7 isolate was tested for its ability to reduce Ag^+ at different time intervals ranged from 12 to 120 h, it was found that this isolate was able to synthesis AgNPs after 48 h of the reaction (Fig.-1).

Taxonomic characterization of isolate, NBrR7: the cultural characteristics of the isolate NBrR7 was studied on 7-ISP media. It was recorded that, the colony of this organism is of gray color, coccoid, compact raised and rough (Plate-2a). The color of the mature aerial mycelium was grayish to yellowish, therefore, it was related to yellowish-gray color series (Plate-2b). Diffusible pigments were recorded on yeast -malt extract agar media (ISP-2) only also slight melanin pigment was observed on tryptone yeast extract broth (ISP-1) media (Plate-2b,c), the organism was

negative to melanin pigment production except on ISP-1 medium. Chemotaxonomic (Cell wall analysis) of the actinomycete isolate, NBrR7 exhibited that the isolate contained major amounts of LL-diaminopimelic acid (LL-DAP) and glycine indicating that; the cell wall peptidoglycan belonged to type I (Wall-chemo type I), but no characteristic sugars were detected, thus the isolate, had the same composition of symbolic cellular components as the genus *Streptomyces*. The physiological, biochemical properties, C- and N-source utilization, tolerance to NaCl, growth pH, growth temperature, growth inhibitors and sensitivity to antibiotics are given in Table-2.

The cultural, morphological, physiological and biochemical characteristics of actinomycete isolate, NBrR7 indicated belonging of this isolate to the genus *Streptomyces* thus it was designated as *Streptomyces* sp. NBrR7.

Antimicrobial activity of synthesized silver nanoparticles (AgNPs): the biologically synthesized AgNPs from *Streptomyces* sp. NBrR7 showed promising antibacterial activity against all tested bacterial strains (Fig.-4). The highest antibacterial activity was recorded for *P.aeruginosa* (24.5±0.15 mm) followed by *B.subtilis* (18.0±0.25 mm) than *S.aureus* (14.5±0.30 mm) while the lowest antibacterial activity was recorded in case of *E.coli* (13.0±0.10 mm). *In-vitro* anticancer activity of synthesized silver nanoparticles (AgNPs): generally, the results of the cytotoxic activity of AgNPs showed strong cytotoxic activities against the tested cell lines with varying degrees (Fig.-5). The highest cytotoxic activity was against human colon carcinoma (HCT-116) cell line with IC₅₀ 21.8 µg/ml, moderate cytotoxic activity was recorded against human liver carcinoma (HEPG2) cell line with IC₅₀ 39.5 µg/ml while weak cytotoxic activity was recorded against human breast adenocarcinoma (MCF-7) cell line with IC₅₀ 47.80 µg/ml.

Discussion:

Silver nanoparticles have great importance among metal nanomaterials due to their physicochemical properties (Abdeen *et al.*, 2014). Therefore, nanoparticles have huge potential for application in medicine (Firdhouse & Lalitha, 2015). Moreover, the biological synthesis of AgNPs is an easy, efficient and eco-friendly approach as compared to physical or chemical methods (Devi *et al.*, 2012; Rai *et al.*, 2015). Biological synthesis of metal nanoparticles has been well achieved by different microorganisms (Golinska *et al.*, 2014; Zarina & Nanda, 2014). In the present study, a total of eleven actinomycete isolates were isolated from different soil samples and purified on starch nitrate agar medium using dilution plate method. The cell-free supernatant of the obtained pure isolates was tested for extracellular biosynthesis of AgNPs through their ability to the bio-reduction of Ag⁺ in aqueous solution. The synthesis of nanoparticles by actinomycetes has many advantages e.g. they are safe to handle, easily available, and possess variable

Table-1: Distribution of the obtained actinomycetes according to the samples characteristics

No.	ID code	Sample characteristics	Colony Color
1	NBrR-1	Sandy soil	Gray
2	NBrR-2	Uncultivated soil	White
3	NBrR-3	Rhizosphere of wild plants	Yellowish brown
4	NBrR-4	Uncultivated soil	Gray
5	NBrR-5	" "	Gray
6	NBrR-6	Rhizosphere of wild plants	Creamy
7	NBrR-7	" " " "	Gray
8	NBrR-8	" " " "	Creamy
9	NBrR-9	Sandy soil	White yellow
10	NBrR-10	Rhizosphere of wild plants	Gray
11	NBrR-11	" " " "	Gray

Table-2: Physiological and biochemical characteristics of the isolate, NBrR7

Character	Results	Character	Results
Melanin pigment		Hydrolysis of	
Yeast Extract Broth-	- ^a	Protein	+
Tryptone		Starch	+
Yeast extract iron-	-	Lipid	-
agar Peptone		Catalase production	+
Tyrosine agar	-	H ₂ S production	-
Utilisation of N-source		Nitrate reduction	+
L-Asparagine	+ ^b	Tyrosine degradation	
L-Glutamic	++ ^c	Urea test	-
L-lysine	+	Tolerance to NaCl (%)	
L-Ornithine	Wg ^d	1:3	+++
L-Tyrosine	+	4:6	++
L-Valine	++	7	+
L-Histidine	+	8	-
Glycine	+++ ^e	Growth inhibitors	
L-leucine	+	Crystal violet (0.0001%)	+
L-Phenyl alanine	+	Crystal violet (0.0002%)	-
L-Serine	-	Sodium azide (0.02)	+
L-Methionine	+++	Phenol (0.1%)	+
L-Cysteine	-	Growth temperature °C	
Utilisation of C-sources		10	-
D-Mannitol	+	25:37	+
D (+) trehalose	++	Growth pH	
D-Glucose	+++	+	+++
D-Mannose	+	8	+
L-Rhamnose	-	9	-
D-Fructose	+	Sensitivity to antibiotic	
Meso-Inositol	+	Rifampicin (50µg mL ⁻¹)	+
Maltose	-	Erythromycin (15µg mL ⁻¹)	+
Starch	+++	Penicillin (10µg mL ⁻¹)	-
D-Galactose	-		
Cellulose	+		
Sucrose	+		
Salicine	-		
L(+)-Arabinose	++		
Raffinose	-		

^a(-) = negative, ^b(+) = moderate, ^c(++) = good growth, ^d(wg)= weak growth, ^e(+++)= abundant.

Plate-1: Extracellular biosynthesis of AgNPs by cell-free supernatants of the different actinomycete isolates

Plate-2: Culture characteristics of *Streptomyces* sp. NBrR7 grown on different ISP-media; (a) colony morphology, (b) Color of aerial mycelium on ISP-4 and (c) melanin pigment on ISP-1.

Plate-3: Antibacterial activity of the synthesized AgNPs from *Streptomyces* sp. NBrR7 against different bacterial test strains using paper-disc diffusion method

Figure-1: Biosynthesis of AgNPs by cell-free supernatant of *Streptomyces* sp. NBrR7 at different incubation times

Figure-2: Cytotoxicity of the synthesized AgNPs from *Streptomyces* sp. NBrR7 against three different human cancer cell lines using MTT assay method

metabolites that may help in reduction. Moreover, these particles have innumerable applications (Deepa *et al.*, 2013).

It was found that only 4 filtrates (36.36%) exhibited various reduction degrees as suggested by initial changes in the color of the solution. Abd-Elnaby *et al.* (2016) reported that a total of forty-one actinomycetes were checked for their ability to produce silver nanoparticles. The screening revealed that only two isolates showed the ability to synthesis AgNPs. The obtained percentage in this study is higher than that obtained by the previously mentioned study, this is due to most of the obtained isolates in this study were from the rhizosphere zone of some wild plants. Soil rhizosphere contains many microorganisms of which actinomycetes represent the highest proportion which included in the recycling of nutrients, maintaining soil fertility, decomposition of organic residues which enable them to possess different biological applications (Geetha *et al.*, 2014). Among four isolates, NBrR7 isolate was selected as the most potent strain for the biogenic synthesis of AgNPs where it showed deep yellowish-brown color in comparing with other isolates. It is well known that AgNPs exhibit brown color in aqueous solution due to the excitation of surface plasmon vibrations in AgNPs. The yellowish-brown colour that appeared in the flasks mixed with silver nitrate was a clear indication of silver nanoparticle formation (Sastri *et al.*, 2003) due to the

reduction of Ag⁺ ions and formation of surface plasmon resonance in the reaction mixture, while no colour change appeared in culture filtrates without silver nitrate (Narasimha *et al.*, 2013; Zarina & Nanda, 2014). The most potent actinomycete isolate NBrR7 was identified by cultural, morphological, physiological and biochemical characteristics which compared to those reported for actinomycetes as described in Bergy's Manual of Systematic Bacteriology (Lechevalier *et al.*, 1989). The results indicated belonging to this isolate to the genus *Streptomyces* thus it was designated as *Streptomyces* sp. NBrR7. A biologically synthesized AgNPs using *Streptomyces* sp. isolated from soil was recorded (Williams & Cross, 1971; Abd-Elnaby *et al.*, 2016; Wypij *et al.*, 2018).

The microbiologically synthesized AgNPs from *Streptomyces* sp. NBrR7 showed promising antibacterial activity against a number of both Gram-positive and Gram-negative bacterial test strains. The formation of the inhibition zone around the discs was an indication of the antibacterial activity of silver nanoparticles (Narasimha *et al.*, 2013). Silver nanoparticles exhibited a broad bactericidal activity against Gram-positive and Gram-negative bacteria (Shrivastava *et al.*, 2007; Gajjar *et al.*, 2009).

The results of the cytotoxic activity of AgNPs was the highest against human colon carcinoma (HCT-116) cell line, moderate cytotoxicity against human liver carcinoma (HEPG2) cell line and weak activity were recorded against human breast adenocarcinoma (MCF-7) cell line. Owing to their small size, SNPs impair the sulfur and phosphorus-containing essential macromolecules such as proteins and DNA (Wei *et al.*, 2009). Thus, the action of SNPs appears to be a consequence of adherence to and penetration inside the cell of the target cells. The anti-proliferative effect of SNPs and Ag⁺ was reported (Ahamed *et al.*, 2008; Rahman *et al.*, 2009). Furthermore, the interaction of SNPs and Ag⁺ with certain proteins has been the subject of extensive studies in recent years. The results of cytotoxic activity obtained in the present study are similar to the results obtained by Shawkey *et al.* (2013) and Kaler *et al.* (2013).

Conclusively, due to the extensive applications of nanomaterials in different fields, their global demand is growing significantly. This demand led to the development of a reliable and eco-friendly process for the biosynthesis of nanoparticles, especially from microorganisms. *Streptomyces* sp. NBrR7 has been effectively used as a living nano factory of silver nanoparticles. On the basis of the obtained results, I suggest that biogenic AgNPs from *Streptomyces* sp. NBrR7 has much potential for application as an antimicrobial and anticancer agent.

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References:

- Abdeen, S., Geo, S., Sukanya, S., Praseetha, P.K. & Dhanya, R.P. (2014): Biosynthesis of Silver nanoparticles from Actinomycetes for therapeutic applications. *Int. J. Nano Dimension*, 5(2):155-162.
- Abd-Elnaby, H.M., Abo-Elala, G.M., Abdel-Raouf, U.M. & Hamed, M.M. (2016): Antibacterial and anticancer activity of extracellular synthesized silver nanoparticles from marine *Streptomyces rochei* MHM13. *Egypt. J. Aquat. Res.*, 42(1):301-312.
- Adiguzel, A.O., Adiguzel, S.K., Mazmanci, B., Tunçer, M. & Mazmanci, M.A. (2018): Silver nanoparticle biosynthesis from newly isolated *Streptomyces* genus from soil. *Mat. Res. Exp.*, 5(4):045402.
- Ahamed, M., Karns, M., Goodson, M., Rowe, J., Hussain, S.M., Schlager, J.J. & Hong, Y. (2008): DNA damage response to different surface chemistry of silver nanoparticles in mammalian cells. *Toxicol. Appl. Pharm.*, 233(3):404-410.
- Becker, B., Lechevalier, M.P., Gordon, R. & Lechevalier, H.A. (1965): Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole cell hydrolysates. *Appl. Microbiol.*, 12:421-423.
- Bérdy, J. (2005): Bioactive microbial metabolites. *J. Antibiot.*, 58(1):1-26.
- Bhosale, R.S., Hajare, K.Y., Mulay, B., Mujumdar, S. & Kothawade, M. (2015): Biosynthesis, characterization and study of antimicrobial effect of silver nanoparticles by actinomycetes spp. *Int. J. Curr. Microbiol. Appl. Sci.*, 2(2):144-151.
- Bozzola, J.J. & Russell, L.D. (1999): Electron Microscopy Principles and Techniques for Biologists. Pub. by: Jones and Bartlett Publishers, Sudbury, MA.
- Chan, W., Ng, P., Tan, V. & Low, Y. (2011): Antioxidant and antibacterial properties of *Alpinia galanga*, *Curcuma longa* and *Etlingera elatior* (Zingiberaceae). *Phcog. J.*, 3(1):54-61.
- Chung, Y., Chung, Y., Ngeow, F., Goh, H. & Imiyabir, Z. (2004): Antimicrobial activities of Malaysian plant species. *Pharm. Biol.*, 42(1):292-300.
- Deepa, S., Kanimozhi, K. & Panneerselvam, A. (2013): Antimicrobial activity of extracellularly synthesized silver nanoparticles from marine derived actinomycetes. *Int. J. Curr. Microbiol. Appl. Sci.*, 2(9):223-230.
- Devi, J.S., Bhimba, V. & Ratnam, K. (2012): *In vitro* anticancer activity of silver nanoparticles synthesized using the extract of *Gelidiella* sp. *Int. J. Pharm. Pharm. Sci.*, 4(4):710-715.
- El-Naggar, N.A. & Abdelwahed, N.A. (2014): Application of statistical experimental design for optimization of silver nanoparticles biosynthesis by a nanofactory *Streptomyces viridochromogenes*. *J. Microbiol.*, 52(1):53-63.
- Firdhouse, M.J. & Lalitha, P. (2015): Biosynthesis of silver nanoparticles and its applications. *J. Nanotechnol.*, 2015:1-18.
- Franci, G., Falanga, A., Galdiero, S., Palomba, L. & Rai, M., Morelli, G. & Galdiero, M. (2015): Silver nanoparticles as potential antibacterial agents. *Molecules*, 20(5):8856-8874.
- Gajjar, P., Pettee, B., Britt, D.W., Huang, W., Johnson, W.P. & Anderson, A.J. (2009): Antimicrobial activities of commercial nanoparticles against an environmental soil microbe, *Pseudomonas putida* KT 2440. *J. Boil. Eng.*, 3:9.

- Geetha, K., Venkatesham, E., Hindumathi, A. & Bhadraiah, B. (2014): Isolation, screening and characterization of plant growth promoting bacteria and their effect on *Vigna radita* (L.) R. Wilczek. *Int. J. Curr. Microbiol. App. Sci.*, 3(6):799-809.
- Golinska, P., Wypij, M., Ingle, A.P., Gupta, I., Dahm, H. & Rai, M. (2014): Biogenic synthesis of metal nanoparticles from actinomycetes: biomedical applications and cytotoxicity. *Appl. Microbiol. Biotechnol.*, 98(19):8083-8097.
- Hayakawa, M. (2008): Studies on the isolation and distribution of rare actinomycetes in soil. *Actinomycetologica*, 22:12-19.
- Ivrih, Z.J., Fahimi-Kashani, N. & Hormozi-Nezhad, M.R. (2017): Aggregation-based colorimetric sensor for determination of prothioconazole fungicide using colloidal silver nanoparticles (AgNPs). *Spectrochim. Acta A: Molecul. Biomol. Spect.*, 187(1):143-148.
- Jayaseelan, C., Rahuman, A.A., Kirthi, A.V., Marimuthu, S. & Santhoshkumar T., Bagavan, A., Gaurav, K., Karthik, L. & Rao, K.V. (2012): Novel microbial route to synthesize ZnO nanoparticles using *Aeromonas hydrophila* and their activity against pathogenic bacteria and fungi. *Spectrochim. Acta A: Molecul. Biomol. Spect.*, 90(1):78-84.
- Kaler, A., Jain, S. & Banerjee, U.C. (2013): Green and rapid synthesis of anticancerous silver nanoparticles by *Saccharomyces boulardii* and insight into mechanism of nanoparticle synthesis. *Biomed. Res. Int.*, 2013:Article ID 872940.
- Kornerup, A. & Wanscher J.H. (1978): **Methuen Handbook of colouredition**. Pub. by: Methuen, London, UK).
- Lakhsmi, S.S., Lakhsmi, H. & Sharmila, S. (2015): Isolation, screening, identification, characterization and applications of green synthesized silver nanoparticle from marine Actinomycetes-*Streptomyces althioticus*. *World J. Pharm. Res.*, 4(7):1592-1611.
- Lechevalier, H.A., Williams, S.T., Sharpe, M.E. & Holt, J.G. (1989): The Actinomycetes: A practical guide to genetic identification of actinomycetes. In S T Williams; D H Bergey (eds.): **Bergey's Manual of Systematic Bacteriology**, Pub. by: Williams & Wilkins.
- Lechevalier HA (1989). The Actinomycetes III, A Practical Guide to Generic Identification of Actinomycetes. In S T Williams; D H Bergey (eds.): **Bergey's Manual of Systematic Bacteriology**, Pub. by: Williams & Wilkins.
- Lechevalier, M.P. & Lechevalier, H. (1970): Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int. J. Sys. Evol. Microbiol.*, 20(4):435-443.
- Malleve, F., Fernandes, T.F. & Aspray, T.J. (2016): Pseudomonas putida biofilm dynamics following a single pulse of silver nanoparticles. *Chemosph.*, 153:356-364.
- Mohamedin, A., El-Naggar, N.E., Hamza, S.S. & Sherief, A.A. (2015): Green synthesis, characterization and antimicrobial activities of silver nanoparticles by *Streptomyces viridodistaticus* SSHH-1 as a living nanofactory: statistical optimization of process variables. *Curr. Nanosci.*, 11(5):640-654.
- Mosmann, T. (1983): Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, 65(1-2):55-63.
- Narasimha, G., Janardhan, A., Alzohairy, M., Khadri, H. & Mallikarjuna, K. (2013): Extracellular synthesis, characterization and antibacterial activity of silver nanoparticles by actinomycetes isolative. *Int. J. Nano Dimensions*, 4(1):77-83.
- Olano, C., Méndez, C. & Salas, J.A. (2009): Antitumor compounds from marine actinomycetes. *Mar. Drugs*, 7(2):210-248.
- Rahman, M.F., Wang, J., Patterson, T.A., Saini, U.T., Robinson, B.L., Newport, G.D., Murdock, R.C., Schlager, J.J., Hussain, S.M. & Ali, S.F. (2009): Expression of genes related to oxidative stress in the mouse brain after exposure to silver-25 nanoparticles. *Toxicol. Letters*, 187(1):15-21.
- Rai, M., Ingle, A.P., Gade, A. & Duran, N. (2015): Synthesis of silver nanoparticles by *Phoma gardeniae* and *in vitro* evaluation of their efficacy against human disease-causing bacteria and fungi. *IET Nanobiotechnol.*, 9(2):7175.
- Romero, D., Gomez-Zapata, M., Luna, A. & Garcia-Fernandez, A.J. (2003): Morphological characterisation of Bgm (buffalo green monkey) cell line exposed to low doses of cadmium chloride. *Toxicol. In vitro*, 17(3):293-299.
- Sastry, M., Ahmad, A., Khan, M.I. & Kumar, R. (2003): Biosynthesis of metal nanoparticles using fungi and actinomycetes. *Curr. Sci.*, 85(2):162170.
- Shawkey, A.M., Rabeh, M.A., Abdulall, A.K. & Abdellatif, A.O. (2013): Green nanotechnology: anticancer activity of silver nanoparticles using *Citrullus colocynthis* aqueous extracts. *Adv. Life Sci. Technol.*, 13(2013):6070.
- Shirling, E.B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bacteriol.*, 16(3):313-340.
- Takizawa, M., Colwell, R.R. & Hill, R.T. (1993): Isolation and diversity of actinomycetes in the Chesapeake Bay. *Appl. Environ. Microbiol.*, 59(4):997-1002
- Tresner, H.D., Davies, M.C. & Backus, E.J. (1961): Electron microscopy of *Streptomyces* spore morphology and its role in species differentiation. *J. Bacteriol.*, 81(1):70-80.
- Tsao, P., Leben, C. & Keitt, G. (1960): Medium constitution for isolation of actinomycetes. *Phytopath.*, 50(1):88-89.
- Wei, D., Sun, W., Qian, W., Ye, Y. & Ma, X. (2009): The synthesis of chitosan-based silver nanoparticles and their antibacterial activity. *Carbohydr. Res.*, 344(17):2375-2382.
- Williams, S.T. & Davis, F.L. (1965): Use of antibiotics for selective isolation and enumeration of actinomycetes in soil. *J. Gen. Microbiol.*, 38(2):251-261
- Williams, S.T. & Cross, T. (1971): Isolation, purification, cultivation and preservation of actinomycetes. *Methods Microbiol.*, 4(1):295-334.
- Wypij, M., Czarnecka, J., Ćwiecimska, M., Dahm, H., Rai, M. & Golinska P. (2018): Synthesis, characterization and evaluation of antimicrobial and cytotoxic activities of biogenic silver nanoparticles synthesized from *Streptomyces xinghaiensis* OF1 strain. *W. J. Microbiol. Biotechnol.*, 34(2):23.
- Zarina, A. & Nanda, A. (2014): Combined efficacy of antibiotics and biosynthesised silver nanoparticles from *Streptomyces albaduncus*. *Int. J. Pharm. Technol. Res.*, 6(6):1862-1869.