Malaysian Journal of Microbiology, Vol 12(4) 2016, pp. 327-331
http://dx.doi.org/10.21161/mjm.88516

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Antimicrobial effects of biosynthesized silver nanoparticles produced by *Actinomyces* spp. based on their sizes and shapes

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Received 27 June 2016; Received in revised form 14 July 2016; Accepted 22 July 2016

ABSTRACT

Aims: *Actinomyces* are dominant soil microflora with potent activity for production of several enzymes and metabolites. In order to increase their survival in the environment these bacteria detoxify the metal ions and consequently produce the nanoparticles. The present study was undertaken to isolate *Actinomyces* strains from soil samples and their evaluation for the production of silver nanoparticles with antimicrobial property.
Methodology and results: Two hundred soil samples were collected and subjected to isolation and identification (based on16SrRNA gene sequencing) of silver nanoparticles producing *Actinomyces*. The silver nanoparticles produced by *Actinomyces* were confirmed by UV-visible spectral analysis, scanning electron microscope (SEM) and Inductively Coupled Plasma (ICP). Furthermore, antimicrobial property of silver nanoparticles was assessed against pathogenic microorganisms viz., *Staphylococcus aureus* (PTCC 1431), *Acinetobacter baumannii* (PTCC 19606), *Bacillus cereus* (PTCC 1816), *Escherichia coli* (PTCC 1397), *Salmonella typhi* (PTCC 1609), *Pseudomonas aeruginosa* (PTCC 1707), *Aspergillus niger* (PTCC 5010) and *Candida albicans* (PTCC 5072). Of 48 *Actinomyces* isolated, 26 strains could produce silver nanoparticles and three of which showed potent activity for production of silver nanoparticles. Molecular identification of these strains exhibited detection of *Actinomyces amycolicicoccus subflavus*, *Streptomyces flavoviridis* and *Streptomyces lateritius*. The results obtained from characterization of the biosynthesis silver nanoparticles illustrated that their shapes and sizes were spindle and spherical and 47-103 nm respectively. However, the antimicrobial effect of silver nanoparticles against the pathogenic microorganisms was varied. Yet *S. typhi* followed by *P. aeruginosa*, were more sensitive and *A. baumannii* was relatively less sensitive. In addition, spherical shape with small average size relatively showed more antimicrobial property.
Conclusion, significance and impact of study: Soil *Actinomyces* could produce silver nanoparticles and these particles have antimicrobial effect. In addition, the antimicrobial effect of silver nanoparticles, not only because of their chemical property (such as formation of free radical) but also depended on their shapes and sizes.

Keywords: Silver nanoparticle, *Actinomyces,* antimicrobial effect**,** pathogenic microorganisms

INTRODUCTION

The prefix nano describes one billionth (10 m) in size. In contrast, to bulk material, nanoparticles show different physical, chemical and biological properties (Basavaraj *et al.*, 2012).Two main methods are depicted for production of nanoparticles: top-down and bottom-up (Wong *et al.*, 2009). In top-down method nanoparticles are constructed by breaking down the mass and in bottom–up method nanoparticles are produced from atoms or molecules via chemical reactions (Lavan *et al.*, 2003; Senapati and Ahmad, 2005).

However, physical and chemical methods are more popular, but biosynthesis of nanoparticles widely recommended because of more reliable, nontoxic, and eco-friendly properties (Kumar *et al.*, 2007). In this regards two microorganisms; fungi and bacteria have shown their potent activity for biosynthesizing nanoparticles (Kalimuthu *et al.*, 2008).

Nanoparticles are produced when an exoenzymes are secreted by the microorganisms into the environment and the target ions reduced to synthesis the nanoparticles (Pourali *et al.*, 2013a; Lakshmi *et al.*, 2014). The biosynthesized nanoparticles have variety of applications including drug carriers for targeted delivery, cancer treatment, gene therapy, DNA (deoxyribonucleic acid) analysis and antimicrobial activity. Nowadays antimicrobial property of nanoparticles could provide several advantages such as low frequency of occurrence of antibiotic resistant microorganisms and production of

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 ISSN (print): 1823-8262, ISSN (online): 2231-7538

anti-odor textile. However, mechanism of the antimicrobial effect of nanoparticles is not clearly, but it might be influenced by their physical characters such as shapes and sizes. In fact, the size and the shapes dependent antimicrobial activity of some nanoparticles have already been investigated (Elechiguerra *et al.*, 2005) but to our knowledge the effect of physical characters (shape and size) of silver nanoparticles produced by *Actinomyces* have not been reported previously. Hence the present study was undertaken to isolate *Actinomyces* from soil and the antimicrobial effect of silver nanoparticles produced by them was assessed based on their physical characters.

MATERIALS AND METHODS

Sample collection and microbiological analysis

Two hundred soil samples were collected from different areas of Ahvaz, Shiraz and Kazeroon cities in Iran. All the samples were taken to the laboratory within two hour and subjected to microbiological analysis. Each sample was serially diluted (10-1- 10-7) by sterile distillated water and 0.1 mL of last three dilutions was streaked on the Mueller Hinton agar and the plates were incubated at 30 °C for five days. The suspected colonies were picked up and identified by phenotypic method Api coryne kit (Biomerieux) (Singh *et al.*, 2015).

Biosynthesis of silver nanoparticles

A loop full of each isolate was inoculated into the flask (100 mL) containing 50 mL sterile nutrient broth (Merck) and incubated in the shaker incubator at 150 rpm at 30 °C for 24 h. The cells were harvested by centrifugation (at 6000 rpm for 10 min) and the supernatant (25 mL) was challenged with 25 µL of 1 mM silver nitrate solution (Sigma Aldrich, USA) (Faghrizonooz and Salouti, 2011). The flask was incubated in shaker incubator (at 200 rpm) at 45 °C. During 48 h, the biosynthesis of silver nanoparticles was assessed (in time interval of 12 h) by changing the color suspension from yellow to brown (Al Juraifani and Ghazwani, 2015).

Molecular Identification of silver nanoparticles producing *Actinomyces* isolates

Authentication of the isolates was performed by 16SrRNA gene sequencing. To perform the test, DNA of the isolates was extracted (DNA extraction Kit, Roche-Germany) then, 16SrRNA gene of each isolates was amplified by Polymerase Chain Reactions (PCR). The primers were 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT). Purity of the PCR products was evaluated by gel electrophoresis and finally the PCR products were sent to Macrogen in South Korea (http://www.macrogen.com/) for DNA sequencing.

Characterization of silver nanoparticles produced by *Actinomyces* isolates

UV–visible spectral analysis

Bioreduction of the silver ion in the bacterial supernatant as a test and silver nitrate as control was monitored by UV-visible spectrometry (Shimadzu UV-1700) adjusted to 400 - 600 nm (Dan *et al.*, 2015).

Scanning electron microscope (SEM)

The average size and morphology of silver nanoparticles were evaluated by SEM. To perform the test, a drop of each bacterial culture supernatant was placed on carbon coated copper grids. Excess of the solution was removed using a blotting paper and the grid was dried under Infrared lamp. Each grid was placed into the Microscope and the nanoparticles were observed and recorded as well (Islam and Miyazaki, 2009).

Inductively Coupled Plasma-Mass Spectrometry

In the present study Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) method was used for detection and verification of the silver nanoparticles. This method detects different wavelengths of each suspension hence, its efficiency relatively was superior (Bhattacharya and Gupta, 2005). To perform the test the bacterial culture supernatant was submitted to the ICP-MS instrument and outputs were recorded (Gschwind *et al.*, 2013).

Antibacterial activity of silver nanoparticles against pathogenic microorganisms

Antibacterial property of biosynthesized silver nanoparticles was assessed against Gram positive and negative pathogenic bacteria by well diffusion method. Bacterial strains used in this study were obtained from the Persian Type Culture Collection (PTCC). These strains were *S. aureus* (PTCC 1431), *A. baumannii* (PTCC 19606), *B. cereus* (PTCC 1816), *E.coli* (PTCC 1397), *S. typhi* (PTCC 1609), *P. aeruginosa* (PTCC 1707), *A. niger* (PTCC 5010) and *C. albicans* (PTCC 5072).

Each microorganism was inoculated onto Muller-Hinton broth (Merck, Germany) and incubated at 37 °C in a shaker incubator at 200 rpm for 18 h. The fresh cultures were streaked onto the Muller Hinton agar and wells (6 mm in diameter) were made in the medium using sterile sharp borer. Then 100 µL of each silver nanoparticles was added into the wells. All plates were incubated at 37 °C for 24 to 96 h. Afterward, the clear zone of the growth inhibition for each nanoparticles was measured and recorded (Pourali *et al.*, 2013b).

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

RESULTS AND DISCUSSION

Isolation and identification of *Actinomyces* from soil samples

A total two hundred soil samples collected from different areas of Ahvaz, Shiraz and Kazeroon cities in Iran. Microbiological analysis of the samples based on phenotypic character resulted isolation of 48 *Actinomyces* strains (Figure 1).

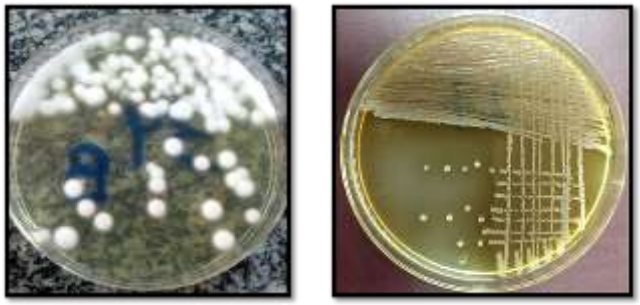

Figure 1: Morphology of *Actinomyces* isolates.

Biosynthesis of silver nanoparticles

The results obtained from biosynthesis of silver nanoparticles indicated that of 48 *Actinomyces* isolates, 26 isolates could change the colour of cultural supernatant from yellow to dark brown. It means they were able to produce silver nanoparticles. The time average for bio synthesis of silver nanoparticles was 12 h.

Authentication of the *Actinomyces* isolates

The results obtained from 16SrRNA gene sequencing indicated that 91, 99 and 99% of gene sequence was similar to *A. subflavus* strain DQS39A1, *S. flavoviridis* strain NBRC 12772 and *S. lateritius* strain NBRC 12788 respectively (Table 1).

Table 1: Identification of silver nanoparticles producing *Actinomyces* based on 16SrRNA gene sequencing.

Similar strain	Identical	Accession
A. subflavus strain DQS39A1 16S ribosomal RNA gene, partial sequence	91 %	NR_116057.1
S. flavoviridis strain NBRC 12772 16S ribosomal RNA gene, partial sequence	99 %	NR_041218.1
S. lateritius strain NBRC 12788 16S ribosomal RNA gene, partial sequence	99 %	NR_112277.1

Characterization of silver nanoparticles produced by *Actinomyces* isolates

To confirm the production of silver nanoparticles light absorbance of the suspension was measured by spectrometry at different wavelengths. The results obtained indicated that maximum light absorbance of silver nanoparticles produced by *Actinomyces* isolates was 420 nm. In addition, the scanning electron microscope showed the size of silver nanoparticles was 20-200 nm. The particles were single and cluster and the shapes were spherical to spindle (Figure 2).

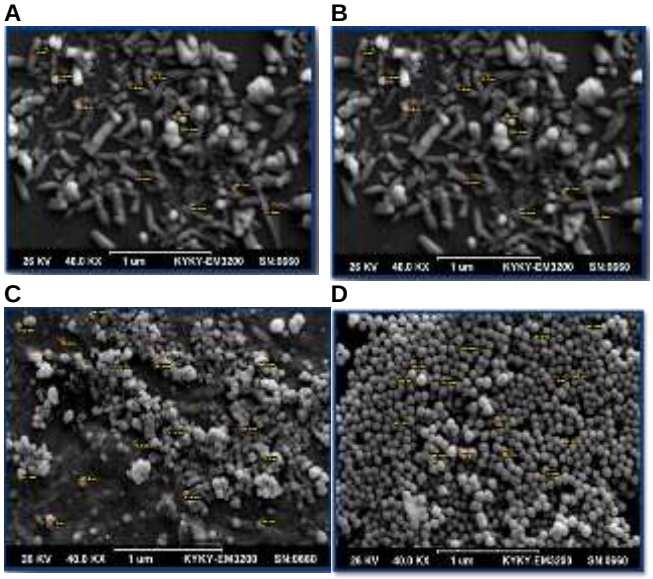

Figure 2: Physical characters of nanosilver particles produced by *Actinomyces* isolates. A, spindle shape; B & C, spherical shape; D, cluster.

The results obtained from Inductively Coupled Plasma (ICP-MS) have shown in Figure 3. As seen in this figure, the maximum light absorbance was at 328 nm. Hence the suspension was purely containing silver nanoparticles.

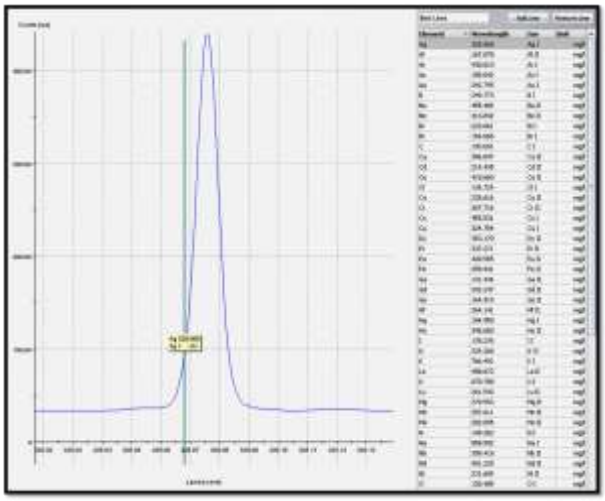

Figure 3: Inductively Coupled Plasma-Mass Spectrometry diagram of nanosilver particles produced by *Actinomyces* isolates.

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Antimicrobial activity of silver nanoparticles produced by *Actinomyces* isolates

As mentioned above, 26 strains of *Actinomyces* could produce silver nanoparticles. Among all, 3 strains showed potent activity for production of silver nanoparticles. The results obtained from antimicrobial property of silver nanoparticles produced by these strains indicated that (Table 2) pathogenic microorganisms approximately were sensitive to nanoparticles. However, spindle nanoparticles produced by *A. subflavus* showed less antimicrobial effect and spherical nanoparticles produced by *S. flavoviridis* exhibited more antimicrobial effect. Among all microorganisms *S. typhi* followed by *P. aeruginosa* were most sensitive and *A. baumannii* was relatively more resistant microorganism.

Nowadays nanotechnology as an interdisciplinary science that related to medical, engineering, pharmacological and different sciences (Morones *et al.*, 2005; Mohanpuria *et al.*, 2008). Regarding medical science, the occurrence of high frequency of antibiotic resistant microorganisms has strongly demanded for introducing a new source of remedy (Panacek *et al.*, 2006; Sharma *et al.*, 2009). Among all methods for production of silver nanoparticles, the biological method

Table 2: Antimicrobial effect of silver nanoparticles against pathogenic microorganisms.

Nanoparticle produced by	Shapes	Average size (nm)	Antimicrobial effect of silver nanoparticles against (mm)							
			S. typhi	*P. aeruginosa*	*A. baumannii*	*S. aureus*	*E. coli*	*B. cereus*	*C. albicans*	*A. niger*
A. subflavus	Spindle	200	8	9	R*	R	R	9	9	10
S. flavoviridis	Spherical	65	21	16	9	15	15	12	15	16
S. lateritius	Spherical	75	25	17	8	11	15	11	15	15

relatively prefers. It is because this method provides the silver nanoparticles with relatively with regular in shapes and sizes (Mandal *et al.*, 2006; Priyaragini *et al.*, 2013; Abdeen *et al.*, 2014).

Our finding showed that soil *Actinomyces* could produce silver nanoparticles. Similar to our finding, Saminathan (2015) isolated fourteen strains of silver nanoparticles producing *Actinomyces* from soil samples. In addition, Abdeen *et al.* (2014) and Saminathan (2015) exhibited production of silver nanoparticles with size of 65 - 80 nm by *Streptomyces* isolated from soil.

Recently, several reports have been published on antimicrobial activity of silver nanoparticles. However, the exact mechanism of antimicrobial action of silver nanoparticles is not clearly and known it is a disputed topic, but Sondi and Salopek-Sondi (2004) believe that the silver nanoparticles have the ability to change permeability of the cell membrane and might be produce free radical and damage the cell membrane and lead to cell death. However the results obtained from the present study opined that antimicrobial effect of silver nanoparticles produced by each soil *Actinomyces* was depended on the shape and average size. Parallel with our finding, Manivasagan *et al.* (2013) demonstrated the size-dependent interaction of silver nanoparticles with seven gram-negative bacteria. This study had verified that spherical shape of silver nanoparticles with the small size have relatively more antimicrobial effect. To interpret, these nanoparticles could penetrate and interact quickly with the target molecule in the cells and therefore eliminate growth of the bacteria. It must be noted that electron transport chain, RNA and DNA of bacteria are targets of silver nanoparticles and therefore, interaction of silver nanoparticles with these molecules could be induced antimicrobial property. Regarding the antimicrobial effect of silver nanoparticles produced by the isolated, the present study illustrated that all microorganisms were sensitive to silver nanoparticles with various responses. However, *S. typhi* followed by *P. aeruginosa* were more sensitive and *A. baunannii* was relatively more resistant microorganism.

Shanmugaiah *et al.* (2015) reported sensitivity of *S. aureus, E.coli, K. pneumonia* and *P. aeruginosa* to biosynthesized silver nanoparticles (Shamugaiah *et al.*, 2015). In conclusion, soil *Actinomyces* could produce silver nanoparticles. In addition, antimicrobial property of silver nanoparticles might be depended on their physical characters viz., their shapes and sizes. It means that the antimicrobial effect of silver nanoparticles, not only because of their chemical property such as formation of free radical but also depended on their shapes and sizes.

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 ISSN (print): 1823-8262, ISSN (online): 2231-7538