جامعة الإسكندرية
ALEXANDRIA
UNIVERSITY
Faculty of Science

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Large-scale biosynthesis of antimicrobial silver nanoparticles by a local *Streptomyces* isolate

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

In

Microbiology

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B.Sc. in Microbiology / Chemistry, Faculty of Science, Alexandria 2013

2018

6. SUMMARY

Recently, biosynthesis of AgNPs and its applications considered as the most important metal nanoparticles that attracted the biotechnologist. Although there are several fabrication methods but the biological method is more preferred as it considered clean, nontoxic, simple to handle and environmental friendly method. *Actinomycetes* considered as the most economically valuable microorganisms, which produced about half of the discovered bioactive metabolites. Since, many of the important antibiotics and secondary metabolites produced industrially by *Streptomyces* species. Recently, *Streptomyces* sp., have been reported as a potential bio-factory for synthesis of AgNPs which having a good stability and significant antimicrobial activities against various human pathogens. As, the overuse of antibiotics against pathogenic bacteria cause a harmful side effect that resulted in emergence of resistance to antibiotics leading to increase patients posed by antibiotic-resistance. Applying antimicrobial activity of the nanoparticles to eradicate bacterial and fungal infection could be considered as one of these valuable health issues. This work focused on statistical bioprocess strategies for bio-fabrication of AgNPs from local *Streptomyces* and applied as a novel antimicrobial agent against hospital-acquired infectious pathogens.

The obtained results can be summarized as follows:

1. The present work was carried out by collecting the soil samples from different localities in Egypt; Salt march sediments, Nile sediment and cultivated soil samples at El-Sharkia, Alexandria and Kafr El-Sheikh Governorates.

2. Sixteen Actinomyces were isolated and checked for their ability to produce AgNPs. Among these isolates, only E3.2 isolate showed ability to fabricate more affective AgNPs that used as antimicrobial agent against human pathogenic bacteria) ***Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhimurium, Shigella flexneri, Staphylococcus aureus, Vibrio cholera, and Escherichia coli***) and fungi (Fusarium **Sp.**, ***Aspergillus niger, Aspergillus fumigatus, and Candida albicans.)***.

3. This isolate was identified by using molecular phylogenetic analysis. Comparisons of the partial 16S rDNA sequence of this isolate with other 16S rRNA sequences available in GenBank using BLAST searches were used to select related sequences for constructing a multiple alignment. The 16S rDNA gene sequence showed that the *actinomycete* isolate was similar to *Streptomyces rectiviolaceus* with an identity of 99 %. Neighbor-joining method with the software package MEGA6 was used to construct the phylogenetic tree based on 16S rDNA gene sequences of members of the genus *Streptomyces*. The sequence of nucleotide was deposited into the database of GenBank as *Streptomyces rectiviolaceus* strain SMWN3.2 with accession number KX077914. (https://www.ncbi.nlm.nih.gov/nuccore/1036392264).

4. As a general the success of industrial production for biological control agents depending not only the isolation, characterization and pathogenicity, but also on the successful mass production of the microbial cells in laboratory. So, there are several media were tested in this work to cultivate the *Streptomyces rectiviolaceus* strain SMWN3.2. Among these media, medium no. 3 produced significantly higher 4.2 g/l of biomass production and 900 mg/l of AgNPs dry weight.

5. The chemical structural and morphological properties of the biosynthesized AgNPs were evaluated by using UV, SEM, TEM, XRD, EDX, and FTIR analysis.

6. Biosynthesized AgNPs was confirmed by UV- visible spectra (at a range of 300 nm- 600 nm) of the reaction mixture after 24 hr. The UV-vis spectrum showed SPR peak of AgNPs at 420 nm.

7. The electron microscopy micrographs (SEM and TEM) of the bio-fabricated AgNPs revealed the formation of extracellular spherical and elongated nanoparticles with a size range of 8.9–11.4 nm.

8. EDX analytical technique used for analysis the elemental composition of metal nanoparticles and confirmed the presence of the silver ions as the major constituent element. The spectrum that showed at 3 keV indicates a strong signal for silver.

9. The obtained XRD spectrum was matched with JCPDS card No.010893722 that exhibits the silver peaks observed at 2θ, values of 22.71°, 32.16°, 38.28°, 52.70°, and 64.64°.

10. Fourier transformed infrared spectroscopy (FTIR) used for determining the chemical composition of the nanoparticles surface which used as a stabilizing agent that prevent the reduced silver particles agglomeration and identifying the possible biomolecules which responsible for the reduction of the Ag^{+} ions into Ag°. In this work, the functional groups such as -C-O-C-, -C-O-, and -C=C- are derived from heterocyclic compounds like proteins which may present as the capping ligands of the AgNPs.

11. To optimize the biomass production, the sequential optimization approaches based on statistical-mathematical experimental designs were applied. By using placket Burman design, the optimized medium components were (g/l): 15 Yeast extract, 15 Peptone, 25 Glucose, 8 NaCl, 0.2 K_2HPO_4, 1 $MgSO_4$ and 10 $CaCO_3$, that formed the larger biomass production (10g/l) than that produced by using the basal conditions (2.38-fold).

12. The second multi-factorial experimental design was applied according to the Box-Behnken design to find out the optimum level of the most significant variables. The optimal levels of the biomass production were studied and the larger biomass production was recorded as 16 g/l that increased by 3.8 fold than the basal condition.

13. By applying the statistical experimental design (Box-Behnken design) for optimizing the AgNPs biosynthesis reaction, the optimal reaction conditions were 0.5M precursor concentration that added to 5 v/v reductant concentration at 45 °C. The optimal levels of the process variables AgNPs production recorded the larger yield production (20.4 g/l) than that in the basal conditions (0.9 g/l) by 22.7 folds' increase.

14. For industrial applications, *Streptomyces rectiviolaceus* strain SMWN3.2 should have certain properties, which include high production of specific metabolite, high growth rate, easy handling in large-scale production and low-cost requirement for line production. So in this study the batch fermentation mode was applied for scaling up the biomass production hence AgNPs yield via 7 L bioreactor. By calculating the growth kinetics measurements, the maximum biomass production (X_{max}) was recorded as 67.5 g/l, maximum specific growth rate, μ_{max} (0.85 hr^{-1}), maximum AgNPs yield (P_{max}) was 85.5 g/l, yield coefficient for biomass ($Y_{X/S}$) was 30.5, and yield coefficient for AgNPs ($Y_{P/X}$) was 42.6.

15. Generally, by bio-application of AgNPs as antimicrobial agent strategy, AgNPs showed higher activities against the tested pathogenic bacteria rather than tested pathogenic fungi. The maximum zones of inhibition that produced against all tested pathogenic bacteria was 42–65 mm by using 30 and 60 µg/ml of AgNPs, whereas, the maximum zones of inhibition that produced against tested human pathogenic fungi was 35–54 mm by using 30 and 60 µg/ml of AgNPs.

16. The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were measured and the best results for MIC/ MBC and MIC/MFC were recorded against *S. pneumonia* and *A. fumigates* as 30 µg/ml / 50 µg/ml and 50 µg/ml/ 60 µg/ml respectively. Finally, this work is the first report, which focuses on large-scale production of AgNPs as a novel antimicrobial agent against hospital-acquired infectious pathogens by using local *Streptomyces rectiviolaceus*.