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Microbial Production of Silver Nanoparticles by *Pseudomonas aeruginosa* Cell Free Extract

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Abstract: Microbial production of silver nanoparticles (AgNPs) synthesis by microbial isolated bacterial strains have been investigated. Four bacterial isolates isolated from soil samples contaminated with petroleum crude oil were used in the present study. Bacterial cell free supernatants extracts were used for AgNPs synthesis. The most efficient bacterial isolate MAM-42 was identified by 16S- rRNA as *Pseudomonas aeruginosa with* accession No. 3NP0614. *P. aeruginosa* MAM-42 formed AgNPs with rounded shape in range of 15-35 nm as indicated by Atomic Force Microscope (AFM). This results revealed that maximum AgNPs production was recorded at 85.0C° after 30min. incubation with 3.0 mM $AgNo_3$ at pH value of 7.0. The synthesized AgNPs was produced by extracellular crude extract have a great biomedical application and represent a future for more therapeutic and pharmacological applications.

Keywords: AgNPs, *Pseudomonas aeruginosa*. Cell free supernatant (extract), production conditions.

1 Introduction

Metal nanoparticles, which have a high precise surface area and a high fraction of surface atoms, have been attracted considerable attention and studied extensively because of their unique physicochemical characteristics, including catalytic activity, optical properties, electronic properties, anti-microbial activity,and magnetic properties [38].

New strategies are therefore needed to identify and develop the next generation of drugs or agents to control bacterial infections. The interaction of nanoparticles with microorganisms and biomolecules is an expanding field of research, which as yet is largely unexplored [13].

Silver nanoparticles are definitely the most widely used nanomaterials among all, which are used in antimicrobial agents, textile industries, water treatment, sunscreen lotions etc. Due to the outbreak of the infectious diseases caused by different pathogenic bacteria and the development of antibiotic resistance the pharmaceutical companies and the researchers are searching for new antibacterial agents [20].

Regarding that silver is a nontoxic, safe inorganic antibacterial agent used for centuries and is capable of killing about 650 types of diseases causing microorganisms [27]. Processes used for nanoparticles synthesis are chemical, physical, and a recently developed biological method. The latter is an advance over chemical and physical methods of nanoparticle synthesis, as it is cost-effective and environmentally friendly [33]. Besides, the physical methods have low yields and the chemical methods cause contamination due to precursor chemicals,use of toxic solvents and the generation of hazardous by-products [37].

Biological synthesis process provides a wide range of environmentally acceptable methodology, low cost production and minimum time required [12]. Hence, there is a great need to develop high yield, safe, reliable, clean and eco-friendly methods for the preparation of nanoparticles. Biosynthesis methods, employing microorganisms, have emerged as a simple, clean and viable alternative to chemical and physical methods. A vast array of biological resources available in nature, including bacteria, fungi, yeasts, algae and plants, can be used for the synthesis of nanoparticles. Prokaryotic bacteria have received the most attention in this area. One advantage of using bacteria for synthesis of nanoparticles is ease of handling and their genetic manipulation without much difficulty [36].

Microbial source to produce the silver nanoparticles shows the great interest towards the precipitation of nanoparticles due to its metabolic activity. Extracellular synthesis of nanoparticles using cell filtrate could be

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beneficial over intracellular synthesis [19].

The use of bacterial strain in the bio-manufacturing process has the advantage that ease of handling than the fungal sources [5].

Cell-free culture supernatants (extract) of five psychrophilic bacteria *Pseudomonas antarctica*, *Pseudomonas proteolytica*, *Pseudomonas meridiana*, *Arthrobacter kerguelensis* and *Arthrobacter gangotriensis* and two mesophilic bacteria *Bacillus indicus* and *Bacillus cecembensis* have been used to synthesize silver nanoparticles [31].

The studies have indicated that culture supernatants of some bacterial genera, like Bacillus, Arthrobacter, Pseudomonas and Escherichia, could induce the synthesis of silver nanoparticles [32].

The antibacterial properties of AgNPs have been attributed to the ability of the AgNPs to anchor and penetrate the bacterial cell wall and to modulate cellular signaling [14].

While various hypothesis exist regarding the mechanism of antimicrobial activity of silver nanoparticles, one of the principal mechanisms is incorporation of silver nanoparticles in the cell membrane, resulting in leakage of intracellular substances which eventually causes cell death [11].

Also responsible for the reduction of metals are enzymes like reductases in various microbes [21]. Extracellularly produced nanoparticles were stabilized by the proteins and reducing agents secreted by the fungus. A minimum of four high molecular weight proteins released by the fungal biomass have been found in association with nanoparticles. One of these was strain specific NADH-dependent reductase.However, emission band produced by fluorescence spectra indicate the native form of these proteins present in the solution as well as bound to the surfaces of nanoparticles [3].

The rate of intracellular particle formation and therefore the size of the nanoparticles could, to an extent, be manipulated by controlling parameters such as pH, temperature, substrate concentration and exposure time to substrate [15]. So the aim of the present work is to produce silver nanoparticles (AgNPs) extracellularly by a most efficient bacterial strain isolated from soil contaminated with petroleum oil, and optimize AgNps production to be used in medical application.

2 Materials and Methods

2.1 Chemicals

AgNO3 was purchased from Sigma Aldrich (St. Louis, USA) and media from Oxoid (UK).

2.2 Microorganism

Four bacterial isolated strains were used in the present work. The bacterial strains (MAM-24, MAM-29, MAM-68 and MAM-42) were isolated from soil samples of Cairo Oil Refining Company. Mostorod. Al Qalyubia, Egypt and bacterial strain MAM -42 from soil sample contaminated with crude petroleum oil from Suze canal, Egypt.

Isolates MAM-24 and MAM-29 were identified by 16S- rRNA as Bacillus mucaligenosis accession No. HQ 013329 [18] and Achromobacter xylosoxidans accession NO.JN 038055[17].

2.3 Extracellular synthesis of AgNPs

In order to screen the most efficient bacterial isolated strain (s) for the synthesis of AgNPs, bacterial strains were inoculated in 50 ml L.B broth medium (tryptone, 10.0,yeast extract ,5.0 g/l) with ommition of NaCl [24] in 250 ml Erlenmeyer flask . The flasks were incubated at 37 c° for 48 h. in shaking incubator (150 rpm).

After incubation, cultures supernatants were obtained by centrifugation at 8000 rpm for 10 min. The final concentration of 1mM $AgNo_3$ in deionized water was added into 2ml of cell free supernatant in clean, sterile test tube. Three replicates were used for each strain. The cell free supernatant without addition of $AgNo_3$ used as control.

The bio-reduction of silver ions was monitored by visual colour change and spectrophotometrically by UV/vis spectrometer for reaction mixture. Based on the rapid reduction of AgNo3 into AgNPs, the most efficient bacterial strain was selected to be identified by 16S-rRNA and characterization of AgNPs

2.4 Identification of the most efficient bacterial strain by 16S-rRNA

2.4.1 DNA extraction

The most efficient bacterial isolated strain MAM-42 DNA extraction have been done according to protocol of Gene Jet genomic DNA purification kit (thermo ko721). The microcentrifuge containing maximum hot start master mix (2x) was vortexed and following components for each 50ul reaction at room temperature was added as indicated in table (1).

The thermal cycling conditions was performed as indicated in table (2)

F-AGA GTT TGA TCC TGG CTC AG

R-GGT TAG CTT GTT ACG ACT T

2.4.3 DNA sequencing

The purified PCR product was extracted from gel and sequenced by GATC Company using ABI Prism Big Dye™ terminator cycle sequencing 3730/3730 XL DNA

sequencer (AME, Bioscience, USA).

Table 1: PCR reaction mixture

Maxima® Hot Start PCR Master Mix (2X)	25µl
16S-rRNA Forward primer	1ul (20uM)
16S-rRNA Reverse primer(of each 8 primer)	1ul(20uM)
Template DNA	5ul
Water, nuclease-free	18µl
Total volume	50µl

Table 2: Cycling conditions

Step	Temperature, C	Time	Number of cycles
Initial denaturation / enzyme activation	95	10 min	1
Denaturation	95	30 s	35
Annealing	65	1min	
Extension	72	1 min 30s	
Final Extension	72	10 min	1

2.4.4 Phylogenetic analysis

The 16S- rRNA DNA sequence was submitted to the National Center for Biotechnology Information (NCBI) data based and the sequence was compared to other available 16S- rRNA sequence using an automatic alignment tool (Blastn). The construction of the Phylogenetic tree was generated by Phy ML and the visualization of the tree by Tree Dyn using the online program www.Phylogeny.fr. The MEGA program was used for drawing the tree.

2.5 Characterization of AgNPs

Extracellular synthesized silver nanoparticles produced by cell free cells supernatant of bacterial isolated strains were characterized by UV/VIS scaning spectroscopy in range of 200-700 nm. Also, a topographic structure of AgNPs was characterized by Atomic Forced Microscopy (AFM) of model Agilent 5500 AFM Scanning Probe Microscopy (USA), the clear microscopic images were observed and documented in different ranges of magnifications with silicon cantilever with force constant 42 N/M .

2.6 Factors affecting AgNPs production

Microbial synthesis of AgNPs by the most efficient bacterial isolated strains MAM-24 have been investigated under different conditions. Bioreduction of silver ions by the cell free supernatants at pH range (1.5-11.0) using 0.2M NaoH and 0.1 M HCl. The incubation periods from zero time to 48h. to determine the time needed for maximum AgNPs accumulation at 37C° and pH 7.0 have been determined. Incubation temperature (20 C° -97 C°) for 48h. and pH 7.0, and different concentrations of silver nitrate (0.5-9.0 mM $AgNO_3$) were used to determine the optimum conditions for maximum accumulation of AgNPs.

3. Results and Discussion

3.1 Microbial production of AgNPs

The four bacterial isolated strains were screened for production of AgNPs using their extracellular (cell free supernatant) of bacterial cultures.

Table 3: Scaning for the maximum absorbance of brown colour formed by extracellular supernatants of different bacterial strains.

Strain code	wavelength (nm)
MAM-24	393
MAM-29	412
MAM-42	430
MAM-68	356

Table 4: The absorbance of colour formed by different bacterial strains at 420 nm.

Strain code	absorbance at 420 nm
MAM-24	0.326
MAM-29	0.900
MAM-42	0.990
MAM-68	0.361

The primary sight for AgNPs formation was change of colour of reaction mixture from (pale yellow to dark brown). This change in colour could be noticed by nicked eye. As the colour intensity increased, the accumulation of AgNPs increased (deep brown colour). The four extracellular culture supernatants and 1.0 mM $AgNO_3$ mixture (formed colour) were scanned by spectrophotometer (200-700nm) by measuring the maximum absorbances,as indicated in tables (3,4) . The most efficient bacterial isolate MAM-42 scane was shown in figure (1).

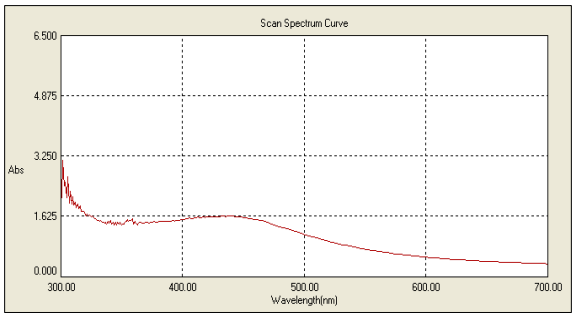

Figure 1: Scan for the most efficient bacterial isolate MAM-24.

The the results revealed that isolated bacterial strain MAM-42 was the most efficient bacterial strain in production of AgNPs (0.990) followed by isolated bacterial strain MAM-

29(0.900).

The primary conformation of synthesis of nanoparticles in the medium was characterized by the changes in color from yellowish white to brown , the knowledge about the reduction of silver ions and formation of silver nanoparticles were still not clear, but believe that protein molecules and enzyme, includes nitrate reductase enzyme act as good regulating agent in silver nanoparticles synthesis [12]. As cleared by [4] biological method of synthesis of silver nanoparticles exhibit strong absorption of electromagnetic waves in the visible range due to their optical resonant property, called Surface Plasmon Resonance (SPR).

The SPR is highly influenced by shape and size of the nanoparticles. Likewise, the microorganisms has the metal-microbe interaction to produce inorganic metal ions, and have several applications in biotechnological fields, includes bioremediation, biomineralization, bioleaching and microbial corrosion [26].

The IR spectrum results showed the amide linkage of the protein has the stronger ability to bind silver so that the protein could most possibly to form a coat covering around AgNPs and it stabilize the aqueous medium. This evidence suggests that the biomolecules present in the cell free supernatant of B. flexus could possibly perform the function for the formation of stable AgNPs [30].

It was well known that the protein can bind to AgNPs through free amide groups. High negative f-potential of AgNPs due to capping with negatively charged proteins may be the reason for stabilization of AgNPs [35]. In a study of silver nanoparticle synthesis, it was observed that most of particles clearly attached on the surface of the cytoplasmic membrane [16].

3.2 Identification of the most efficient bacterial isolate MAM-42.

The bacterial strains used in the present work were isolated from Egyptian soil samples contaminated with petroleum crude oil. Two of these isolates MAM-24 and MAM-29 were previously identified by 16S-rRNA. The most efficient isolated strain was MaM-42 which was isolated from soil contaminated with crude petroleum oil in Suze Canal, Suze Governorate Egypt. This most efficient strain MAM-42 was Gram negative, short rods and identified also by 16S-rRNA. The result of DNA electrophoresed by agarose gel as indicated in Fig (2) revealed that DNA was 1500 bp. The results of forward and reverse sequencing of DNA of strain MAM-42 were indicated in Fig (3, 4).

The phylogenetic tree of strain MAM-42 with other revealed strain according to the similarity with their sequences have been shown in Fig. (5) .

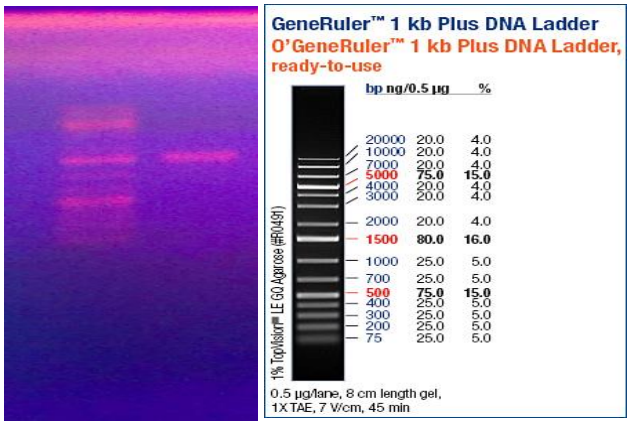

Figure 2: Showed the gel electrophoresis for strain 42 given at 1500 bp.

>AM_384998-45_Ab42-group6-_FP-group6-_A06.ab
TNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNANCCNGGCGCANNAGGNNNANC
TAGGTATCCGTAGAGTAATGCCNANNCAATCTGCCTGGTAGTGGGGGATAACGTCCGGAA
ACGGGCGCTAATACCGCATACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGC
TATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACG
ATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGA|ACTGAGACACGGTCCAGACTC
CTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGC
CGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTA
AGTTAATACCTTGCTGTTTTGACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAG
CAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCG
TAGGTGGTTCAGCAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAA
ACTACTGAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGT
AGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAG
GTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGAT
GTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACC
GCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAG
CGGTGGAGCATGTGGNTTTAATTCGAAGCAACGCGAAGAACNTTACCTGGNNTTGACNTG
CTGAGAACTTTCCAGANATGGATTGGTGCCTTNGGGAACTCNGACACAGNTGCTGCATGG
NTGNCGTCAGCTCGTGTCGTGAGATGTTNGGNNNAGTCCNNTAACGAGCNCNNNNTNNNN
NNNNTTNCNGCNCNTNNNNNGCNCTCTANGNANACTGNNNNNNANNACNNNNNNNNGGNN
NNNNNNNAGTCATCNTNNNNNNGNCNNNNANNNNCTANANNNNNGNNNNAAGNNNNNN
NNNNNNNNNNGNNNN

Figure 3: The forward sequence for strain 42.

>AM_384998-46_Ab42-group6-_RP-group6-_B06.ab1
NNNNNNNNNNNNNNNNNNNNNNNNGNANNNNGGANNNNTNGNNNGACTTANGGAGCNN
GGANNAAACTCTACGTGNTAGGCGTTGTGTACAAGGCCCGGGAACGTATTCACCGTGACA
TTCTGATTCACGATTACTAGCGATTCCGACTTCACGCAGTCGAGTTGCAGACTGCGATCCG
GACTACGATCGGTTTTATGGGATTAGCTCCACCTCGCGGCTTGGCAACCCTTTGTACCGAC
CATTGTAGCACGTGTGTAGCCCTGGCCGTAAGGGCCATGATGACTTGACGTCATCCCCACC
TTCCTCCGGTTTGTCACCGGCAGTCTCCTTAGAGTGCCCACCCGAGGTGCTGGTAACTAAG
GACAAGGGTTGCGCTCGTTACGGGACTTAACCCAACATCTCACGACACGAGCTGACGACA
GCCATGCAGCACCTGTGTCTGAGTTCCCGAAGGCACCAATCCATCTCTGGAAAGTTCTCAG
CATGTCAAGGCCAGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCT
TGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTC
GACTTATCGCGTTAGCTGCGCCACTAAGATCTCAAGGATCCCAACGGCTAGTCGACATCGT
TTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGT
GTCAGTATCAGTCCAGGTGGTCGCCTTCGCCACTGGTGTTCCTTCCTATATCTACGCATTTC
ACCGCTACACAGGAAATTCCACCACCCTCTACCGTACTCTAGCTCAGTAGTTTTGGATGCA
GTTCCCAGGTTGAGCCCGGGGANTTCACATCCAACTTGCTGAACCACCTACGCGCGCTTTA
CGCCCAGTAATTCCNATTAACGCTTGNACCCTTCGTATTACCGCGGCTGCTGGCACNAAGT
TAGCCNGGTGCTTATTNNNNNGNAACGTCNAAANAGCAAGGNNTNACTTACTGNCCNTCC
TCCCANCTNNNNNGNTNNNNANCCGANNANCTNNNTNNNNNNNNNNNNNATNNNGNNCN
NNNTNNCCCANTNNCNNNNNTCCCNNNNNCTGCNTCCNNNANNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNGNANNNNCCNNNNNNNNNNNNGNNNNNNNCGCNNNTNNNNN
NN

Figure 4: The reversed sequence for strain 42.

The results of phylogenetic tree revealed that the isolated

strain MAM-42 was *Pseudomonas aeruginosa* accession No. 3NP0614 with similarity 98 % similarity with P. *aeruginosa accession* No. 3NP0614.

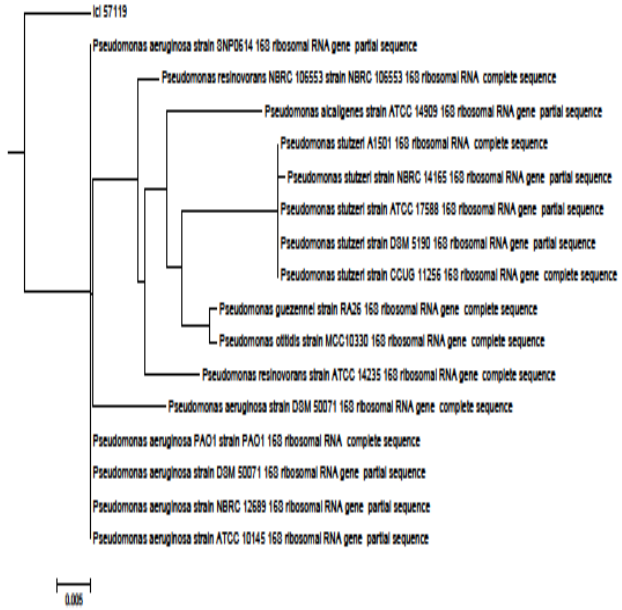

Figure 5: Phylogenetic tree for strain 42 indicate high similarity to *Pseudomonas aeruginosa*.

3.3 Characterization of AgNPs produced by the most efficient bacterial strain

The most efficient bacterial isolated strains (MAM-42 and MAM-29) were characterized by AFM. Fig (6) indicated that AgNPs formed by MAM-42 was in a nano size ranged from 15-35 nm and was spherical in shape. However, figure (7) showed triangular AgNPs in a range from 55 to 120nm produced by MAM-29 cell free extract. [29] Demonstrated that AgNPs exhibit different shapes and the truncated triangular silver nanoplates with a lattice plane as the basal plane displayed the strongest bio-cidal action, compared with spherical and rod-shaped nanoparticles.

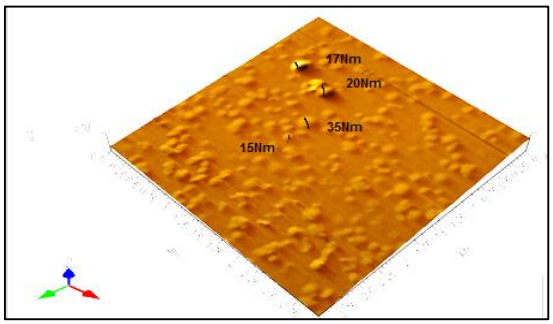

Figure 6: AFM images of AgNPs synthesized using cell free extract of *Pseudomonas aeroginosa* (MAM-42) showed the spherical shaped particle size from 15 nm and 35 nm.

This result parallel with the result by [10] by TEM micrograph of silver nanoparticles obtained after 24 h of incubation showed nanoparticles with variable shape,the size of the particle ranged from 5 to 25 nm. And with [30] who proved The synthesized AgNPs using cell free supernatant that found to be predominantly spherical and triangular shaped AgNPs ranges from 12 and 61 nm. [8] Was referred, the fabricated silver nanoparticles were imaged by AFM to understand the exact configuration of the fabricated silver nanoparticles and also used to verify that the silver nanoparticles were more or less homogenous in size.

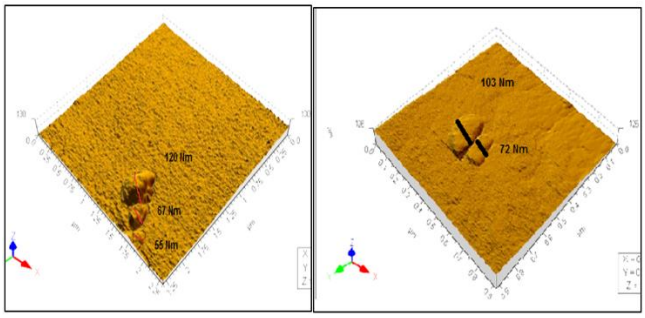

Figure 7: AFM images of AgNPs synthesized using cell free extract of *Achromobacter xylosoxidance* MAM-29 showed the spherical shaped particle size from 55 nm to 120 nm

These results were confirmed with [30] who found the particle size was measured using line profile determination of individual particles in the range of 12–14 nm spherical shaped and 61 nm triangular shaped particles. Also [7] noticed that TEM analysis showed circular and some triangular nanoparticles. These shapes are common in AgNPs synthesized by microbes. [23] Referred that, the size is within the size range reported earlier for AgNPs from bacteria which varied from 5 to 50 nm in *B. subtilis*. Scanning electron microscopy micrograph showed formation of silver nanoparticles in the range of 15–50 nm. [34].

3.4 Factors affecting AgNPs production by the most efficient bacterial strain MAM-42.

3.4.1 Effect of temperature

Nanoparticles synthesized microbially by isolate MAM-42 in term of colour intensity was determined spectrophotomertically at temperatures (20.0°, 25.0°, 30.0 °, 35.0°, 37.5°, 40.0°, 60.0°,65.0°, 70.0° ,80.0°, 85.0° and 99.0°) as indicated in figure (8). The results revealed that maximum AgNPs production (1.780 at 430 nm) was at 85.0° .As the temperature of reaction mixture (cell free supernatant +0.1mM $AgNO_3$) increased, the AgNPs accumulation increased till it reached the maximum production at 85.0 and then began to decrease as temperature continued to increase.

[9] Revealed that, when the cell-free culture supernatant of *P. antarctica* was used, the stability of the AgNPs was compromised irrespective of the growth temperature of the

bacterium (8 ˚C, 22 ˚C and 30 ˚C), when the culture was grown at pH 7 and the AgNPs were synthesized either at 25 ˚C or 37 ˚C. These results indicate that the factors in the cell-free culture supernatants that facilitate AgNPs synthesis and stability vary from bactetrial species to species. It was also observed that AgNPs formed using cell-free supernatants of *A. kerguelensis* and *P. antarctica* were stable up to 8 months if stored in dark but not in the light. It has been suggested that NADH-dependent nitrate reductase are involved in the synthesis of AgNPs by the bio-reduction of silver ion to metallic silver.

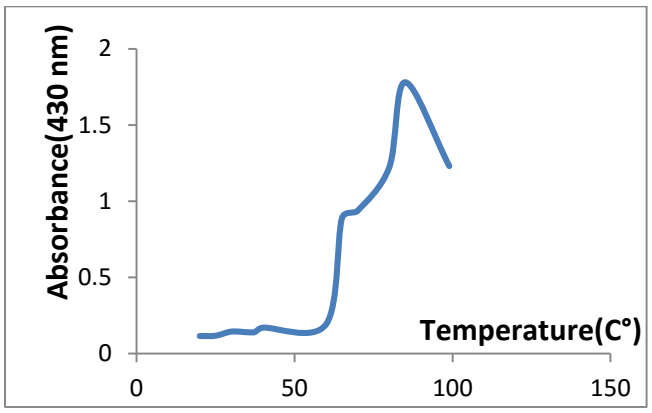

Figure 8: Effect of different temperatures on the production of AgNPs formed by cell free extract of MAM-42 as maximum absorbance of brown colour.

Thus, it is implied that the factors involved in the biosynthesis of AgNPs are present in the supernatants of bacteria used in that study.

3.4.2 Effect of different concentrations of $AgNO_3$

AgNPs produced by cell free supernatant of isolated strain MAM-42 added to different concentrations of $AgNO_3$ (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 mM) was indicated in Figure (9).

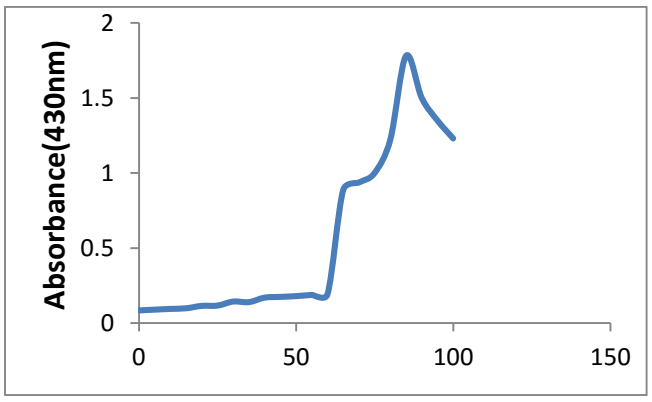

Figure 9: Effect of silver nitrate concentrations on AgNPs formation by MAM-42 cell free extract.

The results revealed that , as the concentrations of $AgNO_3$ increased , AgNPs production increased till it reached 3.0 mM $AgNO_3$ (0.389), as the concentrations of $AgNO_3$ continued to increase, AgNPs production began to decrease gradually.

3.4.3 Effect of incubation periods on AgNPs production

Production of AgNPs was monitored at different intervals (zero, 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 90.0, 120.0, 150.0, 210.0, 300.0, min, 24.0, 25.0, 27.0 and 48.0 h) by isolate MAM-42 as indicated in Figure (10). The results cleared that the most efficient bacterial isolate MAM-42 produced the higher amount of AgNPs after 30.0 min incubation period. The maximum absorbance at 430 (0.646) was recorded at 30.0 min, as the incubation period increased AgNPs production decreased gradually. In the supernatant culture color changes and synthesis of silver nanoparticles depends on incubation period, but the pellet culture takes long lag period to formation of color [6]. The color intensity increased with period of incubation due to the reduction to Ag° [12].

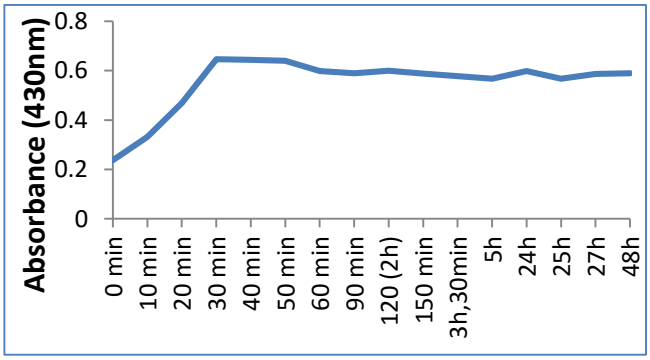

Figure 10: Effect of incubation periods on AgNPs production by cell free supernatant of isolate MAM-42.

This result near to some results of other researchers like in [10] the silver nanoparticles were synthesized within 10 min of silver ions coming in contact with the culture filtrate. And as indicated at [2] who proved that the color of the extract changed to intense brown after 24 h of incubation and there was no significant change afterwards.

In another report, Ahmad *et al.* have shown extra-cellular synthesis of silver nanoparticles within hours [1]. After 72 h of incubation, no further increase in intensity was recorded indicating complete reduction of silver ions [22].

Cell free culture supernatants of *A. kerguelensis* started synthesis of AgNPs after 2 h of incubation. This rate was faster than that reported in most of the earlier studies, where synthesis was slow as in *B. subtilis* [23]. But was comparable to the synthesis in *Morganella* sp. where it took place in 1 h [25]. The study reports rapid biosynthesis of silver nanoparticles within 20–30 min using *Cissus quadrangularis* extracts. Well dispersed and spherical shaped (50–100 nm) silver nanoparticles were determined by scanning electron microscopy (SEM) and atomic force

microscopy (AFM) [28].

The increase in colour intensity of culture filtrate was due to increasing number of nanoparticles formed as a result of reduction of silver ions present in the aqueous solution [10].

3.4.4. Effect of Different pH Values on AgNPs Production

Biosynthesis of AgNPs by the most efficient bacterial isolate MAM-42 cell free supernatant at different pH values as indicated in table (5) revealed that maximum production (0.698) was at pH 7.0. [10] Explained that pH 6.0 supported the maximum synthesis of silver nanoparticles.

Table 5: Effect of pH values on AgNO3 production by cell free supernatant of isolate MAM-42.

pH values	Absorbanse
1.5	Turbed <0.182
3.0	Turbed <0.182
4.0	Turbed <0.182
5.0	0.500
7	0.698
8	0.514
9	0.475
10	0.471
11	0.087

Acknowledgments

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