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ORIGINAL ARTICLE

Preparation and Characterization of Silver Nanoparticles Biosynthesis by *Pseudomonas stutzeri* Environmental Bacteria Isolated from Oil Fields and Their Antimicrobial Activity

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ABSTRACT

Objectives: This study investigate The bacteria of *Pseudomonas stutzerii* was used for biosynthesized of AgNPs role of as antibacterial against burns and wounds infection pathogens.

Methods: the characters of AgNPs were tested by visible ultraviolet (UV) spectroscopy, Fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM) analyzes.
the antibacterial potential was achieved using agar well diffusion method against 90 samples were collected from burns and wounds patients the pathogenic isolates were diagnosed using a VITEK. The biological activity of AgNPs was tested against pathogenic bacteria by two methods including agar well diffusion method at concentrations (1000, 500, 250, 100 µg / ml) and dilution method at concentrations (1000, 750, 500, 250, 100 µg / ml).

Results: The results of The results of genetic identification showed that the bacteria of *Pseudomonas stutzerii* was under the strain *Pseudomonas stutzerii* 0106 ,The fabricated AgNPs were found that SPR peak for AgNPs was at a wavelength of (417)nm and the FTIR many biomolecules that are responsible for the conversion of silver ion into AgNPs the shape of AgNPs were spherical with a size in the range of (22-47) nm. Identification of pathogenic bacteria revealed isolate of 15 species include *Pseudomonas aeruginosa* 6(12%) ,followed by *Proteus mirabilis* and *E.coli* 5(10%),*Enterobacter cloacae*, *Burkholderia cepacia* and *Staphylococcus areus* 4(8%), *Staphylococcus Xylosus*, *Pseudomonas fluerescents* and *Pseudomonas luteola* 3(6%), *Staphylococcus lugdnnensis*, *Enterococcus columnpae*, *Acinetobkter buamannii* and 2(4%) lastly *Granulicatella elegans* and *Morganella morganii* 1(2%).

Conclusion: Silver nanoparticles Ag-NPs generated by *Pseudomonas stutzreii* extract were monodispersed and spherical in morphology with size a range from 15 to 30 nm. The biosynthesizedi Ag-NPs were not aggregated, indicating that the Ag-NPs have been stabilized by a cappingi agent from the bacterial extract . Ag-NPs displayed potent bactericidal activities against both Gram-positive and negative bacteria with effect on Cram-negative bacteria more than Cram-positive bacteria.

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INTRODUCTION

Nanotechnology involvinga the synthesisa and applicationa ofa nanoscalea materialsa isa an emerginga fielda of nanosciencea witha majora applicationsa in biologya, medicinea anda electronicsa due to theira unusual particlea sizea and shapea-dependenta physicala, chemicala and biologicala propertiesa [1] among the metal nanoparticles application in various fields, such as antimicrobial activity [2] therapeutics Bio-molecular detection [3], silver nano coated medical devices [4] optical receptors [5] and water treatment [6]. Its assumed that its will be the ultimate solution for many of most serious diseases in the future, such as cancer, liver disease and kidney failure and solve problems caused by antibacterial antibiotic resistance [7].

The synthesisa of nanoparticles involves physicala and chemical methods including the usea of biologically and environmentallya hazardous chemicals, so many studies have produceda nanoparticles using green synthesisa methods (plant) or the use ofa biological methods (microorganisms) sucha as bacteria, fungi, yeastsa and viruses [8]. Bacteria area onee off the mosta importanta biologicala sourcess for nanoparticlesa becausea of their aadvantages, sucha as the secretiona of extracellulara enzymess that work to reducea metala ions [9] rapida growth, easy cultivation anda in vitro preservation [10]. In the casea of burn and wounda infections, surgical treatmenta usually is carried out witha the use of antibioticsa and antiseptics asa accompaniment therapies. Nevertheless, long-terma use of these agents can be rendereda ineffective by resistancea developing in the target organisms.

MATERIALS AND METHODS

Bacterial isolate

The environmental bacteria *Pseudomonas stutzrii* were used as a source for the biosynthesis of silver nanoparticles were obtained from Applied Microbiology Laboratory -College of Science -University of Basrah [11].

Genetic diagnosis of *Pseudomonas stutzeri*

A. DNA Extraction from bacteria: Bacterial DNA was extracted from bacterial isolate according to (Presto™ Mini gDNA Bacteria Kit, Geneaid -Taiwan), then visualized using 0.8 % agarose gel electrophoresis technique.

B. Polymerase Chain Reaction (PCR): Identification of *Pseudomonas stutzeri* isolate were confirmed by using universal primers on 16SrDNA sequence alignment [12].

Bio-Synthesis of Silver Nanoparticles by *Pseudomonas stutzeri*

Biomass Production: The biosynthesis of silver nanoparticles from isolate of *Pseudomonas stutzrii* was performed by inoculum of freshly colony bacteria in 250 ml Erlenmeyer flasks containing nutrient broth and was incubated at 37℃ with shacking incubator at 150 rpma for 24h.

Purificationo of Silver Nanoparticles: After oincubation, the cell filtrateo was obtained by centrifugationo at 6,000 rpmo for 10 min. The finalo concentration of 1 mM $AgNO_3$ waso added into 100 ml of supernatanto in a 250 ml flask. Theno the flask was incubatedo in a darka rooma conditiono up toa 72 h. The controlo was a maintained withouto the additionn of $AgNO_3$ witho the experimentall flasko containinga the onlyo supernatant. After incubationo the color was changedo and turbidity was ooccurred, that indicated the presenceo of silver nanoparticles ino the culture. The formedo of silver nanoparticleso solution was centrifugedo at 6000 rpmo for 25 min., then the supernatanto was discarded and replacedo with deionized distilled watero to be washed three timeso. while the pellet that foundo in the bottom of theo tube was dried at 40°Co and collected driedo powder gently ando stored for othero tests [13].

Characterization of Biosynthesized Ag NPs: Several techniques were used for characterizing of silver nanoparticles synthesized by *Pseudomonas stutzrii*, which are absorption spectrophotometer (UV-VIS), scanning electron microscope (SEM) and fourier transformso infrared spectroscopyo (FT-IR).

UVa-Vis Spectroscopy Analysiss: The optical characteristics of biosynthesized silverr nanoparticless wass determined of Ag^{+2} reduction in sample which monitored by UV-Vis spectral analysis from (200- 700) nm using UV-Vis spectrophotometer, control sample was usedo as blank reagent [14]. This was processed at the Departmentt of Chemistry /Collegee of Sciencee/Universityy of Basrah.

Fourierr Transformm Infraredd Spectroscopyy (FTIR): The FTIRd spectrophotometerr wass usedd dto conductt characterizationn dof the interactionn betweenn dAg NPs andd biomoleculess [15]. This was processed at the Departmentt oof Chemistry /Collegee of Sciencee/Universityy of Basrah.

Scanning a Electron a Microscope (SEM): Scanning electrons microscope (SEM) was used in thes electron microscope unit, Iran's / University of Tehran to characterizes the morphologicals and size of silver nanoparticles [16].

Sample Collection of pathogenic bacteria.

Sample Collection: The samples were collected from the differentt clinicall specimenss takenn from aAL-Gemhhorey hospitall andd thee AL- Fyehaa hospital from wound and burns patients, in Al-Basrah. A total of 90 samples were collected from patients. samples were collectedi by using sterile cotton swabs whichi are moistened with sterilei saline to prevent dryingi. For each specimen, onei swabs were used. The swabsi were brought to the laboratoryi in a sterile container withini one hour after the collectioni and processed immediatelyi.

Culturing of Samples: The swabs were collected inoculated on Mac-Conkeyi agar and blood agari plates for isolating the pathogens. The inoculated platesi were incubated at 37 ºC for 24 hri. After incubation, the plates werei observed for growth and thei isolated colonies were examined by morphological characteristics and checkedd for purityy by Grama stainінga undera phasea contrasta microscopya, after that colonies were kept on nutrient agar slants.

Diagnosed the Pathogenic Bacteria: The pathogenic bacterial was diagnosed by the vitek in al-Money a hospital.

Biomedicali Applicationi of Biosynthesizedi Silver Nanoparticles

The Antimicrobiall Activityi of AgNPs .

Agar well diffusion method: Agarr welll diffusionn methodd wass followedd forr thee determinationi of Antimicrobiall activityy. The isolatess weree grownn in Nutrientt i agarr mediumm at 37 ºC forr 24 hrs. Bacteriall suspensionm (inoculumm) wass i dilutedd withh a sterilee physiologicall solutionn to 10^8 cfu mL^{-1} withh referencee to the Mc farland i turbidometry. The bacteriall i suspensionn wass addedd to eachi platee containingg Mullerr Hintonn Agarr (MHA) by i sterilee cottonn swabb andd allowedd to remainn in contact for 1 minn. the AgNPs was dissolved by using Dimethyl sulfoxide DMSO at concentration i of (1000, 500, 250,100), wellss weree madee on the platess usingg i a coree borerr withh a diameterr of 8 mm ; these wellss filledd by additionn 100 µl of eachh AgNPs concentrationn withh the use of DMSO aas a controll .the platess i were incubatedd at 37 ºC for 24 hrs i. the inhibitionn zonee aroundd i each well wass measuredd i by mm [17].

Dilution Method: Briefly, AgNPs was diluted into various concentrations, (1000, 750, 500, 250 and 100) µg/ml, in sterile nutrient broth in test tubes. 100µl of each activated pathogen isolate compared with McFarland solution (0.5 McFarland standard) was inoculated into test tubes containing 1 ml of the various concentrations of AgNPs in nutrient broth. Nutrient broth and bacterial isolate without AgNPs was used as control. The tubes were incubated in shaker at 37°C for 24 hr, The tubes are \/*incubated and examined for turbidity which determined by optical density using spectrophotometer at wave length of 600 nm absorbance [18].

RESULTS

Genetic identification of *pseudomonas stutzeri*:

Genomicc DNAa extractionn: The techniquee of electrophoresiss for DNA extractionn showedd clearr isolatedd DNA of *pseudomonas stutzeri*, in Figure 1.

PCR amplification of 16srDNA gene: The results exhibit obtaining of the expected bands of 16srDNA gene for isolate with electrophoresed ladder in region of 1500 pb (Figure 2).

Sequencing of the 16SrDNA gene: The approximately 1500 pb 16srDNA of *P. stutzeri* isolate was purified and sequencing at Macrogen company/Korea the sequencing was thenn alignedd withh known 16sr DNA sequencess Genn bankk using the BLAST at NCBI for comparision with reference species of Bacteria found in genomic database. The results showed that the isolate was under the species of *P. stutzeri* 0106 .

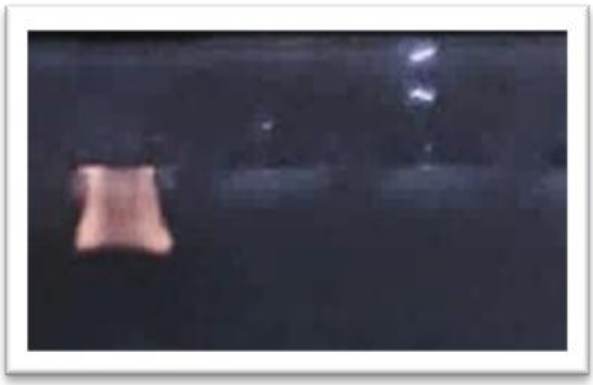

Figure 1. **Electrophoresis of genomic DNA**.

Figure 2. **The electrophoresis of PCR.**

Biosynthesis of Silver Nanoparticles AgNPs: The visual observation of extracellular biosynthesis of AgNPs by *p. stutzeri* 0106 isolate showed that culture supernatant color was change in mixturee of Bacteriaa supernatantt with 1Mm $AgNO_3$ afterr 72 hours of incubationn in the dark state compared with control. The color at first varies from lightt yellowa too dark brown Figure 3 then the color goes up. Figure 4 showed AgNPs harvest obtained from isolate.

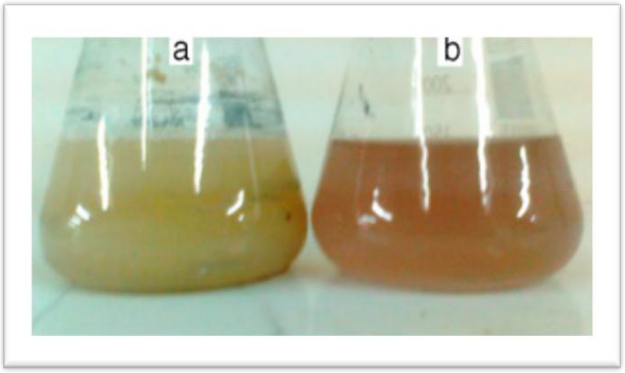

Figure 3**. Formation of AgNPs after 72 h.**
a- without 1Mm $AgNO_3$ b- with 1mm $AgNO_3$

Figure 4. **Nanoparticles synthesized by *p. stutzeri*.**

Characterization of Biosynthesis Silver Nanoparticles

UV-Visible spectrophotometry analysis: Reduction of silver ions as nitrate salt into silver nanoparticles by bacterial culture supernatant was observed as a result of the color change. With light wave 417 nm.

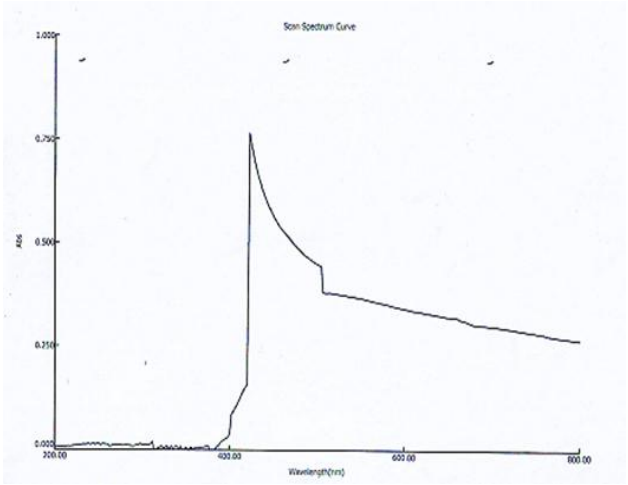

Figure 5. **UV-Vis spectrophotometeri analysis of biosynthesizedi silver nanoparticles.**

Characterization of biosynthesized AgNPs by Scanningg Electron Microscopyy (SEM).

The SEM micrographs (Figure 5) demonstrated the morphology and size of the biosynthesis of AgNPs which appear spherical in shape with varied size ranging from (22–46)nm. Furthermore, the biosynthesized nanoparticles were well dispersed without accumulation or morphological variations (Figure 6).

FT-IRa analysisa of thea biosynthesizeda AgNPs: FT-IRa measurementsa werea showeda to reveall the possiblee potentiall biomoleculess that contributed in the bio reduction of silverr ions and stabilizationn of AgNPs. The FTIR spectrum analysis Figure 6 shows that the supernatant of *P. stutzeri* 0106 contains biomolecules that are responsible for the conversion of silver ions into AgNPs and revealed the appearance of 10 different stretches bands which are : 3443.41, 2980.81, 2927.46, 1636.65, 1523.55, 1458.42, 1386.99, 1230.60, 1067.01, 848.61 (cm-1) (Figure 7).

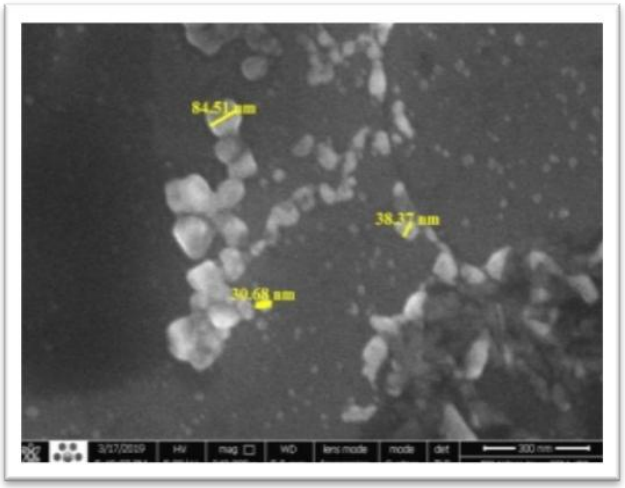

Figure 6. **Imagee of electronn microscopya of the AgNPs particleas produceda froma the *P. stutezri* 0106.**

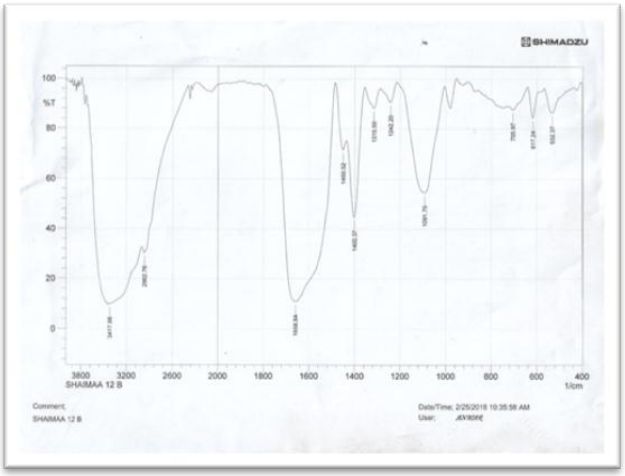

Figure 7. **FTIR spectrumm of AgNPs synthesizedd by *P. stutzeri* 0106 withh distinctt peakss.**

Isolation and Identification of pathogenic bacteria from wounds and burns infection.

A total of 90 swabs were collected from wounds and burns patients, cultured and isolated bacteria diagnosed by Vitek .the results showed different types ofi bacteria as shown in Table 1. The bacterial isolates werei found in 50(55.5%) wounds and burns Swabs whereas 40(44.4%) showed no bacterial growth**.**

The results of identification showed many species of bacteria about 15 and this denote the highh contaminationn of burnn woundss in our hospitalss this agreed with [19].

Also the results revealed a high incidence of Gram negative bacteria (75.5%) 36 in compared with gram positive bacteria 12(24.4%)7 (Figure 7). This was compatible with study carried out by [20] also a previous reported by [21] showed the same results.

The most common bacteria isolated from patients were *Pseudomonas aeruginosa* 6(12%), followed by *Proteus mirabilis* and *E.coli* 5(10%),*Enterobacter cloacae*, *Burkholderia cepacia* and *Staphylococcus aureus*

4(8%), *Staphylococcus Xylosus*, *Pseudomonas fluerescents* and *Pseudomonas luteola* 3(6%), *Staphylococcus lugdnnensis*, *Enterococcus columnpae*, *Acinetobkter buamannii and* 2(4%) lastly *Granulicatella elegans* and *Morganella morganii* 1(2%).

Table 1. **Isolation and identification of pathogenic bacteria from wounds and burns infection**

No.	Name of Bacteria species	Repetition	Percentages %
1	*Pseudomonas aeruginosa*	6	(12%)
2	*Proteus mirabilis*	5	(10%)
3	*Escherichia coli*	5	(10%)
4	*Enterobacter cloacae*	4	(8%)
5	*Burkholderia cepacia*	4	(8%)
6	*Staphylococcus aureus*	4	(8%)
7	*Staphylococcus Xylosus*	3	(6%)
8	*Pseudomonas fluerescents*	3	(6%)
9	*Pseudomonas luteola*	3	(6%)
10	*Staphylococcus lugdnnensis*	2	(4%)
12	*Enterococcus columnpae*	2	(4%)
13	*Acinetobkter buamannii*	2	(4%)
14	*Granulicatella elegans*	1	(2%)
15	*Morganella morganii*	1	(2%)

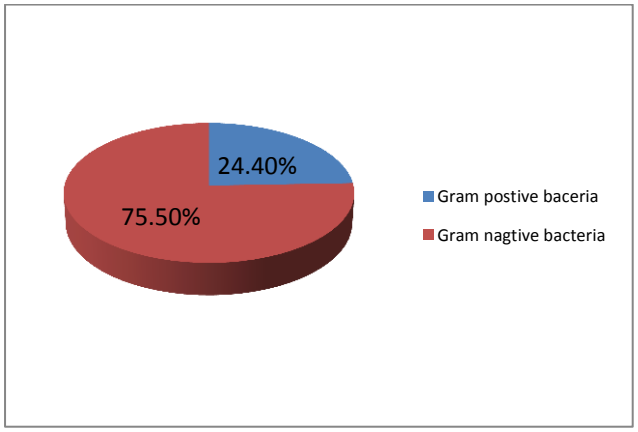

Figure 8. **Percentage % of Gram negative bacteria compared to Gram positive bacteria.**

Antibacterial Activity of AgNPs on Pathogenic Bacteria .

The Antibacterial activity of AgNPs was performed by agar well diffusion and dilution method against 15 pathogenic isolates. The results showed that antibacterial efficacy increase with increase of AgNPs concentration Table 2 and Figure 8.This was agreed with [22].

On agar plate method as shown in Table 2 and Figure 9 the highest inhibition zone (22)mm against *Enterococcus columbae*, andd the lowestt of (12) mm was producedd againstt *Staphylococcus aureus*. this results similar to [23] who showed antibacterial activity of AgNPs against both Gram negative and Gram positive bacteria . *Enterobacter columbae* was the most effected bacteria at all concentration of AgNPs with inhibition zone range between (22-20) mm followed by *Enterobacter claocoess* which recorded (22-17) mm inhibition zone to the gram negative bacteria showed rather approximate inhibition results. With regard to gram positive bacteria there was less effect of AgNPs where the isolates showed no effect of AgNP at 250 and 100 µg/ml excepted of *staphyyococcus xylosus*.

Table 2. **Antibacterial Activity of AgNPs on Pathogenic Bacteria by agar well diffusion.**

Bacteria Name	AgNPs concentration µg/ml and inhibition zone in millimeters			
	1000	**500**	**250**	**100**
Pseudomonas fluorescins	17	17	16	12
Escherichia coli	19	15	13	13
Enterobacter cloacae complex	18	17	15	15
Burkholderia cepacia group	17	13	12	12
Acinetobacter baumanii	19	15	13	13
Pseudomonas luteola	18	16	14	14
Streptococcus agalactia	14	14	-	-
Staphylococcus xylosus	18	16	16	15
Enterococcus columbae	22	22	20	20
Pseudomonas aeruginosa	18	17	15	12
Staphylococcus areus	13	12	_	_
Morganella morganii	18	18	17	15
Proteus mirabilis	18	17	17	15
Enterobacter claocoess Disolvens	22	20	17	17
Granulcatela elegans	17	16	14	14
Staphylococcus lugdnnensis	14	13	-	-

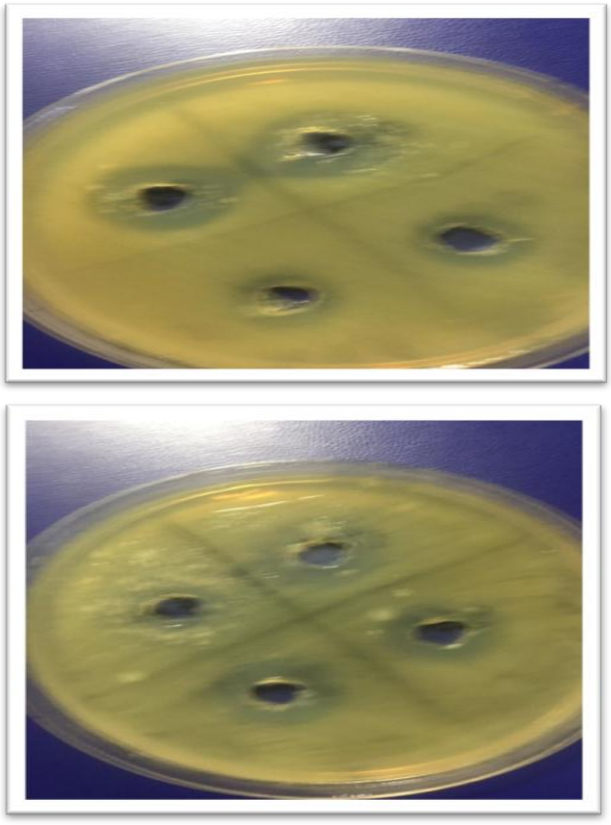

Figure 9. **The Antibacterial susceptibility of the AgNPs against to pathoginc bacteria.**

For the dilution method gave the similar results Figure 10 and Figure 11 the maximum activity against *pseudomonas luteol* at 1000 concentration of AgNPs with optical density (0.271) nm followed by *morganella morgana* with (0.381) nm at the same concentration compared with control.

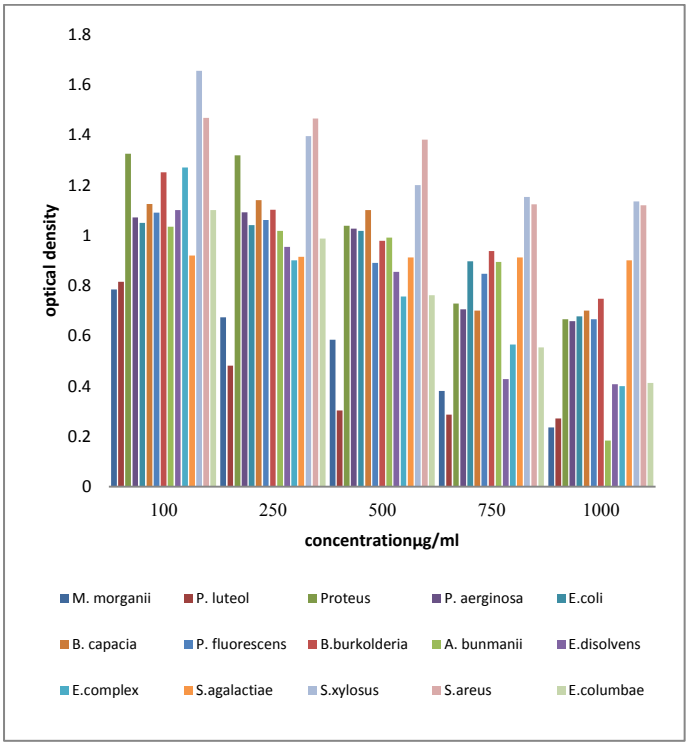

Figure 10. **A comparison of 15 isolates pathogenic bacteria and efficacy as Anti-bacterial by dilution methods.**

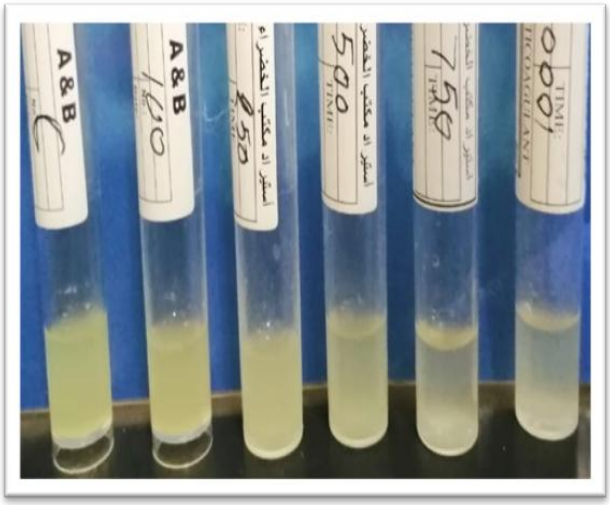

Figure 11. **Antibacterial Activity of AgNPs on pathogenic bacteria by dilution methods.**

DISCUSSION

Biosynthesis of Silver Nanoparticles AgNPs:

The change in color confirms the reduction of $AgNO_3$ to AgNPs by the culture supernatant of *P. stutzeri* 0160 , The supernatant culture has been used to synthesized of AgNPs to facilitate modification and preservation of supernatant culture factor is more than biomass [24] and to preserve cytoplasmic components proteins the Microorganismsa extracts can serve as broth reducing anda capping agent in AgNPs synthesis [25].

There are some researchers who have used supernatant to produce nanoparticles where they used the the supernatant Which goes back to *pseudomonas aerginosa* [26] synthesised of Copper Nanoparticles using *pseudomonas fluorescens* and used *pseudomonas stutzeri* AG259 isolated from silver mines to produce AgNPs.

The color shift in supernatant of culture treated with 1mM of $AgNO_3$ was visually observed to examine the development of AgNPs Figure 3 and no further alteration in solution color occurred when the reaction ended [27]. The result Corresponding with paul and Sinha (2014) the reseans of change in color is induced by the surface plasmon ring phenomenon producedd by thee vibrationn of a groupa of electronss on the surfaces of metall NPs [28].

The amountt, structuree, and propertiesa of AgNPs are dependentt on the typee of microorganismm, culturee conditionss, and concentrationa of the reducingg agentss [29] as well as thea microbial growth phase [30].

The bacteria which could reduce metal ions showed the ability to precipitate metals at nanometer scale [31]. It iss welll establishedd that when microbess are keptt in toxicametall environmentt, theya evolvea mechanisma toa survivea in harsha conditionsa by transforminga toxica metala ionsa intoa theira correspondinga non-toxica formsa likea metala sulfide/oxidesa [32]. *P. stutezri* 0160 isolated from oil wells which is an environment rich in trace elements this isolate used in biosynthesis of AgNPs because it has the ability to resist the high concentration of heavy metals [33] used a bacterial strain isolated from industrialized area include heavy metal contaminated soil because has resistance to silver nitrate showed that In orderr to survivee in environmentss containingg highh levelss of metals, organismss havee adaptedd by evolvingg mechanismss to copee with them. These mechanismss may involves alteringa the chemical naturea of the toxic metala so that it no longer causesa toxicity, resulting in the formationa of nanoparticles of the metala concerned. Thus nanoparticle formationa is the "by-product" of a resistancea mechanism against a specifica metal, and this cana be a used as an alternativea way of producing thema.

UV-Visible spectrophotometry analysis

The color change is due to the Surface Plasmon Resonance phenomenon. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance The sharp bands of silver nanoparticles were observed around 417 nm in case of *P. stutzeri* strain 0106 Figure 4, different literatures were found that the silver nanoparticles show SPR peak at around 420 nm [34]. From our study we found the SPR peak for *P. stutzeri* 0160 was 417 nm. So we confirmed that *P. stutzeri* 0106 has more potential to reduce Ag ions into Ag nanoparticles. This characteristic

color variation is due to the excitation of the SPR in the metal nanoparticles.

Characterization of biosynthesized AgNPs by Scanning Electron Microscopy (SEM).

The biosynthesized nanoparticles were well dispersed without accumulation or morphological variations. The present data are in agreement with [35] who synthesized ecofriendly, low cost method for bio synthesis of stable silver nanoparticles from which performing botha reducing and stabilizing effect.

The present results were in compatible with [36] who used *pseudomonas putida* NCIM 2650 aqueous for productiona of AgNPs that characterizeda by smalla size and variousa shapes. whoa initiateda their discussiona on the chemicala compositiona of thea environmental bacteria whichh hada manya functional enzymes effective as reducing and stabilizinga agentsa to preventa accumulationa of nanoparticlesa.

FT-IR analysisa ofa thea biosynthesized AgNPs

P. stutzeri 0106 contains biomolecules that are responsible for the conversion of silver ions into AgNPs and revealed the appearance of 10 different stretches bands which are : 3443.41, 2980.81, 2927.46, 1636.65, 1523.55, 1458.42, 1386.99, 1230.60, 1067.01, 848.61 (cm-1).

The peak at 3443.41cm-1 is attributeda to the stretchinga vibration of OH alcohol bonda, phenols, and N-H stretch vibrationa of primary protein amidesa, the characteristica hydrogen bondeda OH group thata maya be duea to the formationa of aqueousa phasea nanoparticles.

The peak at 2980.81cm-1 could be attributed to the C-H stretch of methylene protein groups and amine salt stretching to N-H. This finding is undoubtedly associated with the modification of the methynea and methylene groups electronic environment mediated by the neighboring carbonyla and silver nanoparticles [37].

The absorption peak at 1458 cm-1 may be assigned to symmetric stretching vibrations of groups of amino acid residues with free protein carboxylate groups –COO– (carboxylate ion), 1386.99, An extreme band at 1386 cm-1 may be allocated to–C–O stretching vibrations in the carboxyl group. 1230.60 C-O groups in ester, ether+, or group of phenols, 1067 (C-O alcohol stretching oxalic acids, esters and ethers and C–N stretching of aliphatic amines). 848 cm-1 representing presence of Ag-O bond, one of the most important factors that stabilize the nanoparticles in the presence of proteins over it (the capping factors) as proved by many studies [38].

Isolation and Identification of pathogenic bacteria from wounds and burns infection.

The bacterial isolates were found in 50(55.5%) wounds and burns Swabs whereas 40(44.4%) showed no bacterial growth, this is may be due to the continuous sterilization of wounds with El-siberto and iodine for patients who are in the corridor, as well as the incidence of bacterial infections does not appear until three days after the operation. [39] showed that the a major causes of morbidity and mortality due to infectious complications of the type and amount of microorganisms on and in the injured tissues which influence wound healing. The results of identification showed many species of bacteria about 15 and this denote the high contaminationa of burn wounds in our hospitals this agreed with [40] showed that reasons for this high prevalence may be due to factors associated with the acquisition of nosocomial pathogens in patients with recurrent or long-term hospitalization, complicating illnesses, prior administration of antimicrobial agents.

The *pseudomonas aeruginosa* was the most common in burns and wounds swabs 6 (12%) this is similar to [41] who showed that most common bacteria in burn wounds was *Pseudomonas aeruginosa* also This finding in accordance with previous studies [42 , 43]. Another study reported *klebsiella sp*. to be the predominant isolated from burn wounds, whilst, many reports exhibit that *S. aureus* was the most predominate colonizing in burns and wound infection [44].

The source of infection with these bacteria may be from environment or patients gastro intestinal flora also the nosocomial infection among which contamination presence with multidrug resistance bacteria and crowding [45]. [46] reported that *pseudomonas aeruginosa* contaminated the disinfectant used in many hospitals. Further sink-traps, maps, floor, cloths are acertain subjects in hospital contaminated with this bacteria [47]. [48] showed that the differences in bacteria isolates due to variation in treatment practices of burn patients in the different geographical locations.

Antibacterial Activity of AgNPs on Pathogenic Bacteria.

Mechanicallya, the inhibition of nanoparticlesa of DNA susceptibilitya to the replicationa and gene expressiona of proteins as well as the variousa cellular proteinsa and enzymes necessary in ATP productiona leading to inhibit microbes [49].

Nanoparticlesa attacking the surfacea of the cell membranea and disrupting the permeabilitya and respiratory functions of the cell or interfering with Components of the electrona transport system for bacteria as well as lead to the creationa of gaps in the outer membranea of bacteria which effects on membranea permeability [50]. AgNPs also release ROS, which reduces the activitya of dehydrogenase (LDH), which is important in cellulara respiration [51].

As well the Gr^- bacteria clearly revealed greater antibacterial activity in comparison to Gr^+ this was similar to study of [52] Who found that Gr^- bacteria showed high antibacterial activity compared Gr^+ due to thick cell wall [53] reported that effect of AgNPs was more pronounced against to Gr^- bacteria than Gr^+ ones [54]. [55] showed the same results because of the difficulty in cell wall penetration of Gr^+ its found that 1 nm AgNPs promote thinning and permeabilisation Gram positives' active cell wall leading to bacteria cell lysis because of the destabillisation of peptidoglycan layer [56]. The negative charged of teichoic acid in Gr^+ can bind to the positively charged of AgNPs resulting weaking of cell wall [57] reported that some strain of MRSA have cell wall thicker than *S. aureas* this may explain the extended

time 5h required to achived 100% reductiona of MRSA growtha compareda to that required for *S. aureas* (1h). The mechanisma in whichi nanoparticlesa interact with bacteriala cells is that microorganismsa carry a negative chargei while nanoparticles carry a positive charge, creating an electromagnetici attraction between bacteria and the surfacea of nanoparticles, the nanoparticlesa release the ions that interact witha the total of thiol, whichi represent the proteins which transport the fooda that protrudes from the bacteriala cell membrane, leadinga to reduced permeabilitya of the membrane and thus cell death.

Gram-positive bacteriaa are characterized by the thickness of the peptidoglycan layer, whicha forms the cell wall and is made up of polysaccharide chains. This leads to a morea rigid structure and thus difficult to penetrate by silvera nanoparticles, As opposed to gram-negative bacteria, its cellulara wall is characterized by a thinner peptidoglycan layer [57].

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