Nawfal Hussein Aldujaili et al /J. Pharm. Sci. & Res. Vol. 9(7), 2017, 1220-1228

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Antibiofilm Antibacterial and Antioxidant Activity of Biosynthesized Silver Nanoparticles using *Pantoea agglomerans*

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Abstract

The biological methods for nanoparticles synthesis is vital area due to their benefits over chemical and physical methods of synthesis. The aim of the present study is the biosynthesis of silver nanoparticles using *Pantoea agglomerans* and evaluating their biomedical activity. AgNPs were biosynthesized by adding $AgNO_3$ to cell free supernatant of *P.agglomerans* at concentration (10 mM). The PCR was accomplished to molecular identification of *P. agglomerans* using specific primers for 16S rRNA gene, foreword primer (8fpl) and reverse primer (1492rpl)

Biosynthesis of AgNPs was firstly indicated by the colour alteration of reaction mixture from yellow to reddish brown. The characterization completed by XRD, SEM, EDS, and AFM. The XRD presented the size of AgNPs was 16nm.The SEM was offered the shape was spherical and homogenous and the size was ranged between 64-100nm. The occurrence of elemental silver was analysed by EDS. The AFM displayed three dimensional structures of silver nanoparticles and the average diameter was 98.78 nm. Biosynthesized AgNPs showed antibacterial activity against multidrug resistant bacteria (MDR) of both gram positive and gram negative bacteria (*S.aureus, S.pyogenes, E.coli, K.pneumoniae, E.aerogens ,S.typhi, A.baumannii, P.auroginosa and P.mirabilis*) .

All tested bacterial isolates revealed their ability to form biofilm in the form of biofilm using tube and Congo red method without treated by the nanoparticles except *P.mirabils*, *A.boumanii* and *E.aerogenes* ,but when treated with AgNPs this ability was prevented and removed in *K.pneumoniae, Ps.auroginosa* and *C.neoformans*

The AgNPs at two concentration 1mg/ml and 2mg/ml bared their antioxidant capacity *in vitro* by scavenging DPPH free radicles, the largest inhibition titer found in the mixture of DPPH with biogenic AgNPs at concentration 2mg/ml (87.97).

Keywords. Biosynthesis AgNPs, *P. agglomerans* , antibiofilm, Antimicrobial, antioxidant Activity,

INTRODUCTION

Nanobiotechnology deals with creation and use of nanoparticles having a size of 1-100nm.Nanoparticles have been planned because of their unique physicochemical features including antimicrobial, catalytic, optical, electronic, and magnetic properties(Bharathi *et al*.,2015; Murugan *et al*.,2014)

Silver nanoparticles have expected substantial helpfulness because of their antimicrobial action and inhibition the biofilm creation, as well as their unique physicochemical, biological properties, and their applications in medicine, electronics, and optics (Bhimba *et al*., 2015; Kannaiyan *et al*., 2015).

There are number of physical, chemical, biological, and hybrid techniques for synthesizing types of nanoparticles. Physical and chemical methods are more costly, energy unbearable and actually toxic to the environment (Narayanan *et al*., 2011). Growth of dependable, nontoxic, and green techniques for synthesis of nanoparticles is the most essential to multiply their biomedical uses. One of the options to achieve this objective is to use microorganisms to synthesize nanoparticles (Jha *et al*., 2010; Thampi *et al*., 2015). The extracellular synthesis of silver nanoparticles using bacterial species acts to be acceptable to many applications (Mohanpuria *et al*., 2008; Chaudhari *et al*., 2012). and to improve new effective antimicrobial agents that overcome the MDR microorganisms (Karthick *et al*., 2016) . Therefore the present study has been designed to biosynthesis of silver nanoparticles using *P.agglomerans* species and studies their antimicrobial activity .

EXPERIMENTAL

Preparation of cell free supernatant of *P.agglomerans*

The *P.agglomerans* was selected from screening of many different bacterial species from different sources according to their resistance to commercial AgNP and their ability to extracellular production (supernatant) to AgNP (data not shown) .Brain Heart Infusion broth (BHI) was inoculated with *P.agglomerans* and incubated under aerobic condition at 37°C for 24 hrs. Colonies were picked up and confirmed as *P.agglomerans* depending on morphological, biochemical tests and PCR (Holt *et al*., 1994).

The culture was centrifuged at 6000 rpm for 25 min, 4°C to prepare the cell free supernatant from *P.agglomerans* . Cell free supernatants were collected for using in the biosynthesis of silver nanoparticles (Chaudhari *et al*., 2012).

Molecular Identification of *P.agglomerans*

Extraction of DNA from *P. agglomerans* was accomplished according to the kit of extraction FavorPrep Genomic DNA Extraction Mini Kit Favorgen / Korea. The concentration of DNA was determined

spectrophotometrically by measuring its OD at 260 nm (Extintion coefficient of dsDNA is 50 µg/ml at 260 nm. The purity is indicated by OD260/OD280 which is in the range of 1.8± 0.2 for pure DNA (Stephenson,2003) .

The PCR was done to identify *P. agglomerans* using specific primers for 16S rRNA gene, foreword primer (8fpl) 5`AGAGTTTGATCCTGGCTCAG 3` and reverse primer (1492rpl) 5`GGTTACCTTGTTACGACTT 3`.

A PCR mix was 2.5 ul (10µM) of forward and reverse primers, with tube of Accupower ®PCR-Pre Mix-Kit ((1unit of Top DNA polymerase, 250 mM Each: dNTP (dATP, dCTP, dGTP, dTTP), 10 mM Tris-HCl (pH 9.0), 30 mM KCl, 1.5 mM $MgCl_2$, Stabilizer and tracking dye)), and 8µL (50ng) of DNA template, volume was adjusted to 20ul of DW.

The reaction was run at 94 °C for 5 min followed by 35 cycles of 1min at 94 °C , 1min at 56 °C , and 2min at 72 °C and 7min at 72°C in a thermocycler. PCR Products (1500 bp) were electrophoresed on a 1.5% agarose gel stained ethidium bromide and photographed under Ultraviolet transilluminator.

Biosynthesis of silver nanoparticles using cell free supernatant

$AgNO_3$ was as precursor for biosynthesis of silver nanoparticles by *P.agglomerans.* $AgNO_3$ was added with concentration (1, 3, 5and 10 mM) to cell free supernatant which mixed. This step was prepared in dark condition to avoid oxidation of $AgNO_3$.The pH of the reaction mixture was adjusted to 8. The resultant solutions were incubated in shaking incubator 150 rpm at 37° C for 24 hrs.

After incubation the colour alteration was detected and the reaction mix was centrifuged at 6000 rpm for 25 min,4°C ,the supernatant was discarded and replaced with deionized distil water and re centrifuged three times at the same conditions to remove remained supernatant ,the pellet represent collection of nanoparticles was dried in oven at 40 °C for 18-24 hours.The dried powder was collected carefully and stored for further analysis (Chaudhari *et al* .,2012; Sarvamangala *et al* ., 2013).

Characterization of silver nanoparticles

XRD analysis

The X-ray diffraction was used for characterization of silver nanoparticle in department of Geology, Faculty of Science/ Baghdad University

SEM analysis

SEM (Inspect S50. FEI) was used for characterization the morphology of nanoparticles in electron microscope unit, Faculty of Science/ Kufa University. The microscope operated at an accelerated voltage at 15 KV and different magnification, low vacuum, a spot size 4 and working distances5-10mm (Natarajan *et al* .,2014) .

EDS analysis

Elemental analysis of single particle was carried out using Bruker EDS attached with SEM in electron microscope unit, Faculty of Science/ Kufa University. EDS performed for point analysis with accelerating voltage 10 KV,spote size 5, working distances 10mm, this analysis was used to detect presence of elements nanoparticles (Sarvamangala *et al* ., 2013) .

AFM analysis

Atomic force microscope (AFM) was used for characterization the silver nanoparticle in department of chemistry Faculty of Science /Baghdad University.

Antibacterial activity of nanoparticles

Antibacterial activity of biogenic AgNPs was carried by agar well diffusion method against different kinds of pathogenic multidrug resistant bacteria of both gram positive and gram negative(table 1).Standardized suspension of each tested bacteria ($1.5x10^8$cfu/ml) by McFarland standard (0.5N) then swabbed separately onto sterile Muller-Hinton Agar (MHA) plates using sterile cotton swabs.

Agar was punched with sterilized cork borer 6 mm and 100µl (150µg/ml) from commercial SNPs and biogenic AgNPs was added into each well, incubated for 24 hrs at 37°C, after incubation the inhibition zones were measured (Rajeshkumar and Malarkodi .,2014).number of bacteria used as indicator strains, *Acinetobacter baumannii, Enterobacter aerogens ,Escherichia coli ,Klebseilla pneumonia ,Proteus mirabilis Pseudomonas aeruginosa ,Salmonella typhi ,Staphylcoccus aureus ,Steptococcus pyogenes* .

Antibiofilm activity of silver nanoparticles

Tube and Congo red agar methods were used for qualitative assessment of biofilm formation and antibiofilm activity by nanoparticles as described by (Kumar *et al*., 2012; Mathur *et al*., 2006: freeman *et al*., 1989).

Antioxidant activity of biogeneic silver nanoparticles in vitro

DPPH is a free radical scavenging assay was used to evaluate the ability of the commercial AgNPs and biogenic AgNPs annihilate the DPPH free radical. The method described by Harbone and Baxter, (1995) with some modification was used.

The biogenic AgNPs were added to DPPH (0.1mM) at concentration 1 and 2mg/ml , 0.5ml of both supernatant and microorganism growth of *S.boulardii* and *P. agglomerans* were added to 2ml of metabolic solution of DPPH (0.1mM).

The reaction mixture was incubated for 30 min in dark room at 37°C and the absorbance (A) was read at 517 nm in spectrophotometer. The experiment was repeated for three times .DPPH solution was used as a control (without sample) and ethanol 99.8% as blank .The inhibition of the DPPH radical by biogenic AgNPs was calculated according to the following formula:

% of Inhibition = ((Abs of control – Abs of test))/(Abs of control) X100

RESULT

Identification by PCR

Result revealed the single band with 1500bp (fig1)

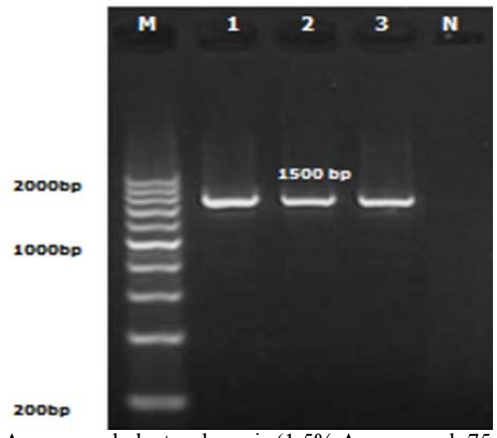


Fig. 1: Agarose gel electrophoresis (1.5% Agarose gel, 75 volts to 1 hours) for PCR product (1500bp) of 16S rRNA gene of *P.agglomerans* Lane (M) :size marker (200 bp DNA Even Ladder); Lane 1-3 PCR product.

Biosynthesis of Silver nanoparticles

P.agglomerans exhibited their ability in the extracellular biosynthesis of AgNPs using cell free supernatant and $AgNO_3$ (10Mm) as a precursor. After shaking incubation for 24 hrs at 37°C at 150rpm, *P.agglomerans* have the ability in changing the colour (figure 2) of reaction mixture from yellow to reddish brown which denotes as indicator for biosynthesis the AgNPs.

XRD analysis of nanoparticles

XRD showed that, *P.agglomerans* produced silver nanoparticles with average size 16nm (fig.2).

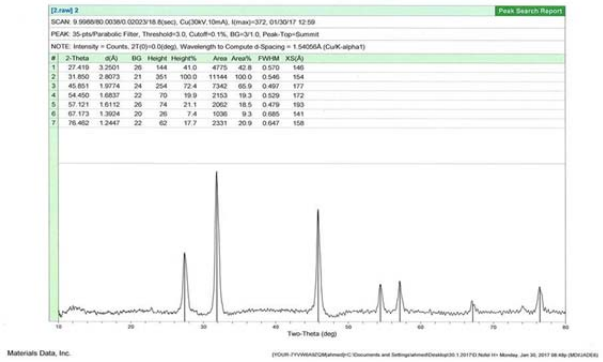


Fig.2 : XRD analysis of Biosynthesized nanoparticles using *P.agglomerans* (average diameter 16.73nm)

SEM analysis of nanoparticles

SEM results showed well-dispersed nanoparticles and homogenous with diameter of 60-100nm for AgNPs, with variable shapes most of them present in spherical form (fig 3).

EDS analysis of nanoparticles

The presence of elemental silver designated the reduction of silver ions in mix by *P.agglomerans* supernatant. The EDS spectrum was recorded in the point and map mode, strong signals from the Ag atoms were observed while medium signals from oxygen and weaker signals from other atoms. The weight percentage of elemental constituents for AgNPs that was96.62% silver (fig.4).

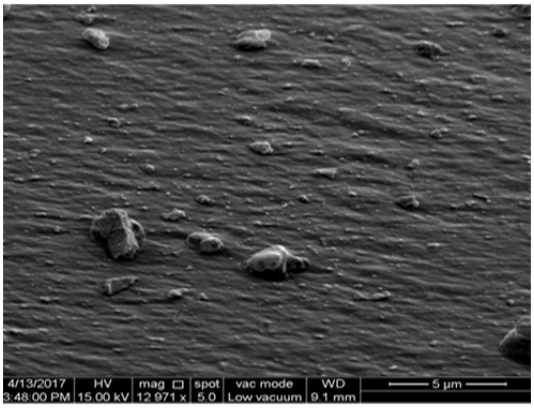
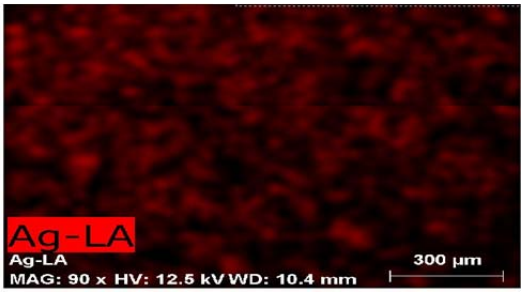
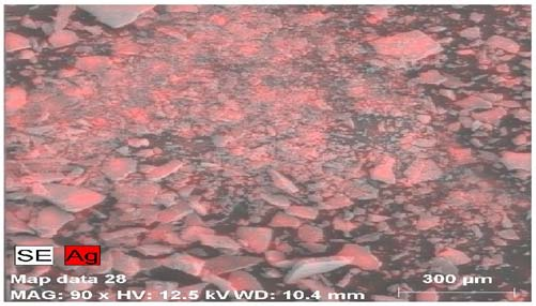
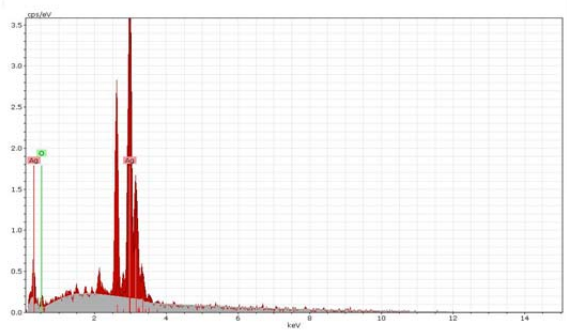


Fig. 3: SEM micrograph of biogenic silver nanoparticles. The shape of AgNPs was spherical and homogenous, size between (64-100nm).







Spectrum: Acquisition

Element	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]
Silver	L-series	63.26	96.62	80.93
Oxygen	K-series	2.21	3.38	19.07
	Total:	65.47	100.00	100.00

Fig.4. EDS analysis (point and mapping) of biogenic silver nanoparticles: Illustrated strong signals from the Ag, medium signal from O2, the optical absorption peak of Ag was observed at 3Kev, the weight percentage of silver (96.62%)

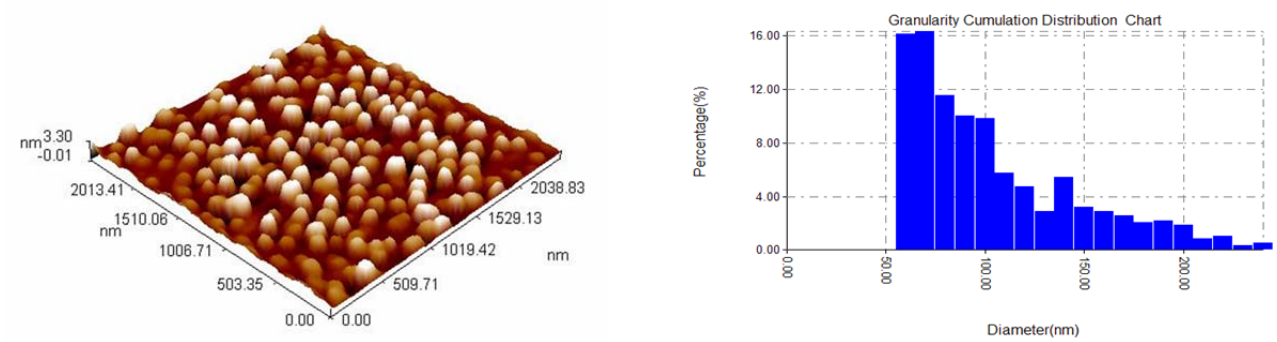


Fig.5. Atomic Force Microscopic analysis of biosynthesized silver nanoparticle from *P.agglomerans* (Average Diameter: 98.78 nm)
A-three dimension of nanoparticles
B-Granularity cumulation distribution chart of nanoparticles

Table (1): Inhibition Zones of pathogenic bacteria in mm by AgNPs at concentration 150ug/ml

Tested microorganisms	**Commercial AgNPs**	**AgNPs *P.agglomerans***
A.baumannii	0	12
A.fumigatus	0	0
A.niger	0	0
C.neoformans	0	0
E.aerogenes	11	14
E.coli	14	20
K.pneumoniae	0	19
p. aeruginosa	15	23
P. agglomerans	0	16
P.chrysogenium	0	0
P.mirabilis	0	15
S.aureus	0	15
S.pyogen	16	23
S.typhi	18	30

Table (2) Biofilm activity using congo red method for different microorgansims

Microorganism	**Without AgNPs**	**Commercial AgNPs**	**Biogenic AgNPs from *P.agglomerans***
A.boumanii	Weak	Weak	None
C.neoformans	Moderate	Moderate	Weak
E.aerogenes	None	None	None
E.coli	Moderate	Strong	None
K.pneumonia	Strong	Modetera	Moderate
P.agglomerans	Strong	moderate	Weak
P.mirabils	None	None	None
Ps.auroginosa	Moderate	Weak	Weak
S.aureas	Weak	Weak	Weak
S.boulardii	Strong	Moderate	Weak
S.pyogen	Weak	Moderate	Weak
S.typhi	Weak	Moderate	Weak

AFM analysis of nanoparticles

(AFM) analysis seemed, *P.agglomerans* produced AgNPs with average diameter 98.78% (fig.5).

Antibacterial activity

Results showed that AgNPs has the ability to inhibit the bacterial growth gram positive and gram negative bacteria. The inhibition zone was greater in gram negative than in gram positive bacteria (table 2). The largest inhibition zone of commercial AgNPs in Gram negative bacteria was 15mm in *Ps. auroginosa* with concentration 150µg/ml**,** while the largest inhibition zone in Gram positive bacteria was 16mm in *Streptococcus* with same concentration, while the SNPs synthesized from *P. agglomerans* showed large inhibition zone in Gram negative bacteria which was 30mmin *S.typhi* ;and large inhibition zone in Gram positive bacteria was 23mm in *S.pyogen* at the same concentration 150ug/ml in addition the antibacterial activity in other bacteria were also different in their sensitivity to AgNPs when exposed to the same concentration. (table 1).

Antibiofilm activity of silver nanoparticles

Congo red method

The experiment was tested 12 isolates two of them was not producing biofilm (*E.aerogenes and P.mirablis*) , the biogenic AgNPs from *P.agglomerans* was prevented the formation of biofilm in many isolates such as *A.boumanii and E.coli* while some isolates was inhibited but not preventing the formation of biofilm such as *C.neoformans* and *K.pneumonia* also but the biogenic AgNPs not have effect on the formation of biofilm in *S.aureas* (table 2)

Tube method

Twelve isolates was used, two of them was not producing biofilm (*E.aerogenes and P.mirablis*) , the biogenic AgNPs from *P.agglomerans* was prevented the formation of biofilm in many isolates such as *C.neoformans* and *K.pneumonia*while some isolates is inhibited but not preventing the formation of biofilm such as *S.aureas* and *S.typhi* (table 3).

Antioxidant activity of biogenic AgNPs nanoparticles

After adding the nanoparticles and *S. boulardii* to DPPH solution (0.1M).The results revealed ability of nanoparticles and *S. boulardii* to scavenging DPPH free radicles that indicated by observing the colour change from the original colour of DPPH purple into yellow colour.

These results demonstrated the antioxidant activity of biosynthesized AgNPs and *S. boulardii in vitro* and led to evaluation the competency of nanoparticles and *S. boulardii* for antioxidant activity *in vitro*. The largest inhibition titer found in the mixture of DPPH with Silver biosynthesized from *P.agglomerans* at concentration 2mg/ml (87.97);

DISCUSSION

The microorganisms which have the "Silver resistance machinery" can make AgNPs. Extracts from microorganisms may act both as reducing and capping means in AgNPs synthesis. The reduction of silver ions by mixtures of biomolecules such as enzymes/proteins, amino acids, polysaccharides and vitamins is environmentally benign and chemically complex (Thakkar *et al*., 2010).

The appearance of a brown color in solution is a clear indication of the formation of AgNPs in the mixture due to reduction of Ag+ ions to Ag metal by the reducing agents such as enzymes, proteins, amino acid, polysaccharides etc (Natarajan *et al* .,2014; Sreedevi *et al* .,2015). The color exhibited by metallic nanoparticles is a result of the coherent excitation of entire free electrons within the conduction band, leading to surface plasmon resonance (SPR) (Gurunathan *et al*., 2009).

The environment of the culture supernatant can be simply improved and conserved than the cell, where the constituents in the cytoplasm would try to retain constant environment such as heat shock proteins and necessitate more purification Therefore, supernatant can be used for the synthesis of silver nanoparticles rather than cells itself (Kalimuthu *et al* .,2008).

A number of studies directed that NADH and NADH-dependent enzymes are factors in the biosynthesis of metal nanoparticles. The reduction looks to be initiated by electron transfer from the NADH by NADH-dependent reductase as electron carrier (Ranganath *et al* .,2012; Joerger *et al* .,2000).

The morphology are dependent on several chemical and physical parameters, e.g., incubation time, pH, composition of the culture medium, and growth in the light or dark (Durán *et al*., 2011). Shape and size controlled nanoparticles could be synthesized by modulating the pH or the temperature of the reaction mixture (Gericke and Pinches 2006). at at 65Cº, fewer amounts of nanoparticles were synthesized, whereas at 35Cº more amount of nanoparticles were synthesized. At acidic pH, the AgNPs synthesis decreased due to the alkaline ion (-OH) is very much required for the reduction of metal ions there will be less nucleation for silver crystal formation on which new incoming silver atoms deposit to form larger sized particles .While as the pH increase towards alkaline region, the dynamics of the ions and synthesis enhances and reaches the maximum at pH 10 and more nucleation regions are formed due to the availability of –OH ions. The conversion of Ag+ to Ag0 increases followed by increase in the kinetics of the deposition of the silver atoms (Sanghi and Verma ,2009).

Table (3) Biofilm activity using tube method for different microorganisms

Tested microorganism	Without AgNPs	Commercial AgNPs	Biogenic AgNPs from *P.agglomerans*
A.boumanii	moderate	Weak	None
C.neoformans	Strong	Strong	None
E.aerogenes	none	None	None
E.coli	Weak	Strong	Weak
K.pneumonia	moderate	Strong	None
P.agglomerans	moderate	Weak	None
P.mirabils	None	None	None
Ps.auroginosa	Strong	Strong	None
S.aureas	Strong	Moderate	Weak
S.boulardii	Strong	Moderate	None
S.pyogen	Strong	Moderate	Weak
S.typhi	Weak	Strong	Weak

XRD analysis detected the average size of silver nanoparticle; the AgNPs biosynthesized from *P.agglomerans* was 16.73 nm and this size is acceptable in comparison with other studies (Abdulhassan, 2016).

SEM used to determine the shape and size of biogenic nanoparticles, experimental results displayed well-dispersed nanoparticles with diameter of 64-100nm for silver nanoparticles biosynthesized using *P.agglomerans* with variable shapes most of them present in spherical form the previous study on the biosynthesis of silver nanoparticle from *Lactobacillus sp.* produced nanoparticles with size (30-100 nm) related with (Aldujaili et al.,2015; Abdulhassan ,2016).

EDS analysis detected the presence of elemental silver which indicated the reduction of silver ions to silver metals in the reaction mixture, the weight percentage of silver was 96.62% for AgNPs biosynthesized from *P.agglomerans* .The optical absorption peak was observed at 3keV which is a typical absorption of metallic AgNPs (Caroling et al.,2013; Bhakya et al.,2015).

AFM analysis showed the 3 dimensional shape of silver nanoparticle and the average diameter of the nanoparticle the average diameter of silver nanoparticle biosynthesized from *P.agglomerans* was 98.78 nm.

AgNPs synthesis was better in term of quality; minimum size and less polydispersity, with *P. agglomerans* ,this result may be attributed to the differences in the bio-reduction that may be return to the qualitative and quantitative of extracellular protein/enzyme and other biomolecules that presented in the culture of each microorganism ,in addition to their ability of interaction with $AgNO_3$ (Chaudhari *et al* .,2012).

Nanoparticles are a viable alternative to antibiotics and seem to have a high potential to solve this problem. AgNPs were considered particularly attractive for the production of a new class of antimicrobials (Pinto *et al* .,2009;Lara *et al*.,2011; Rai *et al* .,2012) .

The AgNPs appeared their antibacterial effects on gram positive and gram negative bacteria. The largest inhibition zone was showed in G-ve in comparison with G+ve bacteria, the maximum inhibition zone of commercial AgNPs in G-ve was (18mm) in *S.typhi* with concentration (150µg/ml) while the maximum inhibition zone in G+ve was (16mm) in *S. pyogen* with the same concentration ,while for AgNPs biosynthesized from *P.agglomerans* in G-ve was (30mm) in *S.typhi* while in G+ve was (23mm) in *S.pyogen* this difference was possibly attributed to the difference of the peptidoglycan layer of the bacterial cell between G+ve and G-ve bacteria. The Gram negative cell envelope consists of outer membrane, thin peptidoglycan layer, and cell membrane. While in the Gram positive the cell envelope consists of lipoteichoic acid containing thick peptidoglycan layer and cell membrane (Kim *et al*., 2007; Thomas *et al*., 2010; Taglietti *et al*.,2012**)** .

Also there is variances in the sensitivity of tested pathogenic bacteria to AgNPs when exposed to the same concentration(150µg/ml) of chemical AgNPs such as *E.coli*, *Ps.aeruginosa and E.aerogenes*, the inhibition zone of these bacteria was (14,15 and11 mm) respectively , while the AgNPs biosynthesized from *P.agglomerans* was (21,23,16mm) this may be return to the differences in intrinsic susceptibility of bacterial species depends on the concerted activity of several elements, what has been named as intrinsic resistome (Blake and O'Neill ,2013; Tian *et al*.,2006).

When increase the concentration of silver nanoparticles displayed increase in the antibacterial activity ,these results similar with (Shrivastava *et al* .,2007; Soo *et al* .,2011) The antimicrobial effect due to The positive charge on the silver ion as it can attract the negatively charged of microorganisms through the electrostatic interaction. This attraction probably overcomes other factors, such as size and shape that can influence the bacterial cell death(Fabrega *et al* .,2009; Badawy *et al* .,2011; Abbaszadegan *et al* ., 2015) .

pH-Dependent biosynthesized AgNPs have distinctive role in the antibacterial activity by these nanoparticles ,the smallest nanoparticles synthesized in alkaline pH displayed more antibacterial activity than the large particles which are synthesized in acidic pH (Chitra and Annadurai,2014) .

The shape of nanoparticles play very significant role in the antimicrobial activity of nanoparticles. Hexagonal AgNPs show the highest antibacterial effect when compared to other NPs shapes, this was attributed to the specific surface areas and facets reactivity (Vanaja and Annadurai ,2013; Hong *et al* .,2016) .

Silver ions bind to nucleic acid and protein negatively charge causing deformation and structural changes in the cell wall, in the membrane and in the nucleic acids of bacterial cells. Silver ion interact with a number of electron donor functional groups such as phosphates,thiols, hydroxyls, indoles and imidazoles. The AgNPs also damage membranes and induce the release of reactive oxygen species (ROS), forming free radicals with a powerful bactericidal action (Wu *et al* 2014).

The silver ions is known to mainly inhibit enzymes such as NADH dehydrogenase II in the respiratory system, which is involved as a candidate for the site of production of reactive oxygen species (Matsumura *et al*., 2003) .Small sized nanoparticles exhibited more antibacterial activity than large size particles because the small sized particles effect on a large surface area of the bacteria (Vanaja and Annadurai 2013; Chitra. and Annadurai, 2014; Wu *et al* ., 2014) .

It has been suggested that AgNPs interfere with bacterial replication processes by adhering to their nucleic acids. The interaction of silver ions with sulfhydryl (–SH) groups of proteins that cause the DNA unwinding, and contact with hydrogen bonding processes are also been demonstrated lead to cell division was inhibited (Lubick 2008; Davod *et al*., 2011).

The ribosomes may be denatured by silver ions or small AgNPs as a consequence inhibition of protein synthesis as well as translation and transcription can be blocked by the binding the AgNPs with the genetic material of the bacterial cell (Morones et al .,2005; Jung *et al* 2008).

It has also been found that the nanoparticles can modulate the signal transduction in bacteria by dephosphorylate the peptide substrates on tyrosine residues,

which leads to signal transduction inhibition and thus the stoppage of growth (Shrivastava *et al*., 2007) . Nano-Ag appears to be significantly is more toxic than the Ag^+ ions towards *E. coli (*Tian *et al.,* 2006).

Biofilms are complex bacterial populations that resist the action of antibiotics and the human immune system .Due to the lack of effective antibiofilm antibiotics. Nanoparticles were used to resolve this problem, one potentially important candidate treatment uses AgNPs to show anti-biofilm activity (Markowska *et al*,2013; Vincent, *et al*.,2014).

All tested microorganisms showed their ability to form biofilm in the form of film lined the wall and bottom of tubes in the tube method without treated by the nanoparticles except *P.mirabils*, *A.boumanii* and *E.aerogenes* ,but when treated with AgNPs this ability was prevented and removed in *K.pneumoniae, Ps.auroginosa* and *C.neoformans* , AgNPs may be altered gene the expression relating to biofilm formation, as consequence they effect on microcolony formation and biofilm maturation .This lead to AgNPs could be used for prevention and treatment of biofilm-related infections (Kalishwaralal *et al*., 2010; Martinez-Gutierreza *et al*.,2013, Abdulhassan,A.J.;2016).

The antibiofilm activity of AgNPs was observed less effective against G+ve bacteria than on that of G-ve bacteria this observation may be a result of the structural differences in the composition of the cell wall in G+ve and G-ve bacteria (Fayaz *et al*., 2010), other study revealed that AgNPs have antibiofilm ability against G+ve and G-ve bacteria when catheters coated with AgNPs were tested *in vitro* observed almost complete prevention of biofilm formation by *E.coli, S.aureus* and *C. albicans* (Martines-Gutierrez *et al*., 2013).

Generated hydroxyl radicals can depolymerize polysaccharides, cause breaks in DNA, and inactivate enzymes that can compromise the EPS matrix of the biofilm architecture (Applerot *et al*.,2012;Sadekuzzaman *et al*., 2015).

The cause of remaining the biofilm in some tested bacteria such as *S.typhi and S. aureus* may be due to resistance of bacterial strain to AgNPs, some strains within a given species may be sensitive and others may be resistant or to the size of nanoparticles that may be used (Christensen *et al*.,1995;Cintas *et al*.,2001, Vanaja andAnnadurai,2013).

DPPH is a more stable and well-known free radical based on the reduction of accepting hydrogen or electron from donors. The DPPH reducing ability of the antioxidants (AgNPs and *S.boulardii*) were assessed by seeing colour change from original deep purple colour of DPPH into yellow colour after adding AgNPs in addition to the growth suspension and supernatant of *S.boulardii* to DPPH.

DPPH scavenging activity of nanoparticles increased with increasing their concentration that showed by the elevated percentage of inhibition of DPPH which increased with increase concentration of AgNPs that exhibited more inhibition (72.67% in1mg/ml) and(22.95% in 2 mg/ml) for AgNPs from *S.boulardii* respectively due to more an electron donated and accepts by DPPH (Kanipandian *et al*.,2014; Bhakya *et al*.,2015).

The inhibition proportion by bacterial suspension of *S.boulardii* was (12.56%) and cell free supernatant was 25.13% .The diverse mechanisms involved in the radical-antioxidant reactions may explain the different in scavenging potentials of the compounds . The mechanisms of antioxidants are not only by scavenging free radicals, but also by inhibiting production of free radicals (Niki,2010; Xing *et al*.,2015).

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