ISSN(Online) : 2319-8753
ISSN (Print) : 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 9, September 2016

Extracellular Synthesis of Gold Nano Particles from *Pseudomonas stutzeri* (MTCC 8362) and its Antimicrobial Activity

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ABSTRACT: In the present study, the extracellular synthesis of gold nanoparticles were made by making use of *pseudomonas stutzeri*. sodium citrate is used as a stabilizing and capping agent for the synthesis. Synthesis of gold nano particles was preliminary confirmed by the change in colour. The nanoparticles obtained were characterized by UV–VIS spectrophotometer, Scanning electron microscopy (SEM), particle size and antimicrobial activity. Synthesized nanoparticles peak obtained by spectrophotometer on 530nm ,scanning electron microscopy shows the images of nano particles. Particle size was obtained having two major peak one of the peak between 50 to 60 and another peak between 550. Antimicrobial activity against two gram positive and two gram positive bacteria. The synthesis of gold nanoparticles by microbial source is the most reliable method of production and yield**.**

KEYWORDS: Nano particles, UV VIS spectrophotometer, microorganism ,scanning electron microscopy, extracellular synthesis.

I. INTRODUCTION

Nanotechnology has experienced a rapid growth recently because nanostructures exhibit physical and chemical properties that are distinctly different from the bulk solid [1]. In particular, metallic nanostructure materials have been the subject of intense scientific research by virtue of their fundamental importance and potential applications [2]. Among noble metals, gold nanoparticles have been extensively investigated in the last 10 years because of their potential applications in optics, electronics, and catalysis [3,4]. The applications of the nanoparticles are mainly due to the quantum size effect, which is the function of the number of free electrons in the nanoparticles [5]. Few reports are also available which show that the gold nanoparticles exhibit good antimicrobial activity [6–8]. Their predominant antimicrobial activity can be attributed to the strong cytotoxicity to various bacterial cells. They can interact with the functional groups on the bacterial cell surface to inactivate bacteria and destroy them [9]. Various methods have been used to produce the nanoparticles, like chemical reduction [10], photolytic reduction [11], radiolytic reduction [12] and metal evaporation [13]. Gold and silver nanoparticles have also been used in biological optical imaging and sensing applications [14–16] due to the excellent elastic light scattering properties of metal nanoparticles [14,17,18]. nanoparticles could be characterized spectroscopically on the basis of their sizes as their concentration depends on the method of synthesis [19–22]. Various chemicals serve as reducing agents in the process of nanoparticles production, such as sodium/potassium borohydrate [23], hydrazine [24], salts of tartarate [25], sodium citrate [26], ascorbic acid [27,28] and amino acids [29–31]. When the nanoparticles are formed, they need to be stabilized for further applications. Various reagents have also been reported to act as stabilizing agent such as polyethylene glycol [32,33], polyvinyl alcohol [34], polyvinyl pyrollidine [35,36], chitosane [37] and various surfactants viz, sodium dodecyl sulphate [38–40], Triton-X [41] etc. Numerous processes have been reported in literature for the synthesis of gold nanoparticles, for example, citrate was used as a reducing agent as well as a stabilizer for the synthesis of gold nanoparticles [42,43]. Reverse microemulsion method [44,45], polymeric matrix [46] and polyelectrolyte systems [47] . The development of reliable, ecofreindly processes for the synthesis of nanoscale material is an important aspect of nanotechnology. Nanoparticles attract greater attention due to their various applications in different fields. In recent years, the synthesis of gold nanoparticles has been the focus of interest because of their emerging application in a number of areas such as bio imaging, bio labels, biosensors, biomedicine and so forth[48]. Gold nanoparticles have gained increasing interest

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due to their specific features[49] such as unusual optical and electronic properties, noncytotoxicity[50] high stability, biological compatibility, controllable morphology, size dispersion and easy surface functionalization. Gold nanoparticles scatter light with an intensely which is much brighter than that of chemical flourophores. The brilliant colour exhibited by gold nanoparticles in the visible and near infra red spectral region is due to their surface Plasmon resonance (SPR) properties [49]. Recent studies have shown that gold nanoparticles have immense potential for cancer diagnosis and therapy on account of their SPR enhanced light scattering and absorption [51]. Plant-mediated synthesis of silver and gold nanoparticles was also well-known method [52-55]. Biosynthesis of Au, Ag and Au-Ag nanoparticles using edible mushroom extract was also in follow [56]. The use of microorganisms in the synthesis of gold nanoparticles emerges as an ecofreindly and exciting approach. Biosynthesis of metal nanoparticles using fungi and actinomycete has also got importance [57,58]. The main interest is production of nanoparticles using a biological method from a cheap resource, uniform production of gold nanoparticles. Utilizing a biological source gives an easy approach, easy multiplication, and easy increase of biomass and size uniformity. Though numerous chemical methods prevailed for nanoparticles production, numerous problems are often experienced with stability of product, control of the crystal growth and aggregation of particles on long term exposure. In the present study *pseudomonas stutzeri* a gram negative bacteria(MTCC 8362)is used for the extra cellular synthesis of gold nano particle The extra cellular synthesis of gold nanoparticles[59] of about 8 nm diameter has also been reported by using the alkalothermophilic actinomycete Thermomonospora sp[54]. In earlier reports, synthesis of gold nanoparticles have been shown by reduction of aqueous chloroaurate ions using extracts from Embilica officinalis[54], and Terminata catappa[52]. Intracellular recovery of gold by microbial reduction of AuCl4- ions using the anaerobic bacterium, shewanella algae has been investigated[60]. Synthesis of au nanoparticles by H2O2 reduction of HauCl4 was studied[61]. Investigation was done on loading of gold nanoparticles inside the DPPC bilayers of liposome and their effects on membrane Fluidities, Colloids and Surface[62].

II. RELATED WORK

Materials and methods :-

P. stutzeri. Cultures were grown up in a conical flask containing 100 mL of nutrient broth in a shaker incubator at 37 °C. After 24-48 h of incubation, biomass developed on the medium. Nutrient broth was prepared by mixing the following contents; 2.5 g of peptone, 1.5 g of yeast extract 2.5 g of NaCl in 500 mL of deionised water. pH was adjusted to 5.5-6.5. 50 mL of 10-3 M aqueous Auric Chloride (HAuCl4) was added into the culture m edium. The broth was inoculated with sodium citrate as a capping and stabilization agent Then the reaction mixture was left for a further 24-48 h in a shaker incubator at 37 °C. Biotransformation took place after incubation i.e., chloroaurate ions were reduced to gold nanoparticles. The accumulation and reduction of gold were examined by visual observation of the medium. The medium turned pale yellow to purple which was a clear indication of the formation of gold nanoparticles. The synthesized gold nanoparticles were characterized by UV-Visible Spectroscopy, Scanning Electron Microscopy (SEM), particle size and antimicrobial activity was performed against two gram positive and two gram negative bacterial culture.

III. RESULTS

In this investigation, the gram negative l bacterium *P. Stutzeri* was screened and found successful in producing gold nanoparticles and quit stable in the solution. The bacterium, P.stutzeri incubated with broth containing auric chloride solution at 37 °C for 48 h. The pH value was ranging from 6-7. The auric chloride ions were reduced during the exposure to bacterial biomass and as a result biotransformation took place. The colour of the reaction solution turned from pale yellow to deep red indicating the formation of gold nanoparticles (Figure 1). The result demonstrated that gold nanoparticles are showing two peaks in particle size and even less . There is no change in control, indicating that the production of gold nanoparticles was obtained by the reduction of microorganisms indeed. The reaction was completed after 48hrs of incubation indicating it as a slow reaction. The colour of the solution remained deep pink without any changes so long.

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International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

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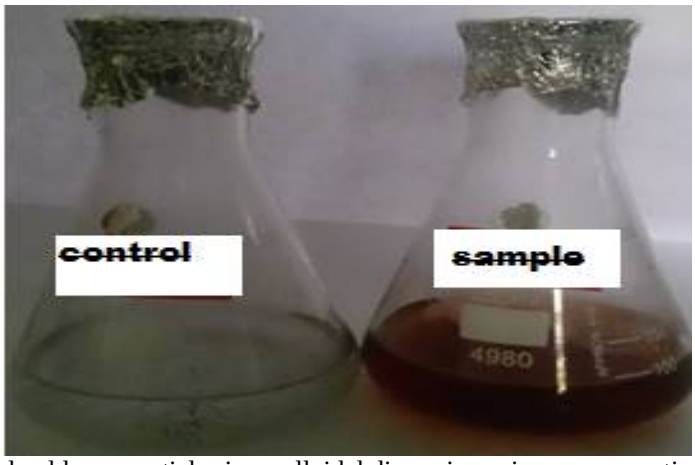

Figure 1. Biosynthesised gold nanoparticles in a colloidal dispersion using gram negative bacterium *P .stutzeri*.

UV-Vis spectroscopy studies:-

The formation of gold nanoparticles was monitored by UV-Visible spectroscopy. As the size of the gold nanoparticles increases, the colour of the solution varied from deep red to purple. The different colours of gold nanoparticles solution are due to their Surface Plasmon Resonance properties. Nanoparticles can experience SPR in the visible portion of the electromagnetic spectrum. This means that a certain portion of a visible wavelength will be absorbed and while another portion will reflect. The portion reflected will lend the material a certain colour. After 48 h of incubation, the spectroscopic studies revealed the absorption maxima of 530 nm.

Table showing UV-VIS visible spectrophotometer.

WL	ABS	WL	ABS	WL	ABS	WL	ABS
300	0.9172	400	0.6047	500	0.7417	600	0.2951
305	0.8844	405	0.5989	505	0.7789	605	0.2774
310	0.8549	410	0.5964	510	0.8135	610	0.2615
315	0.8332	415	0.5913	515	0.8444	615	0.2473
320	0.8129	420	0.5853	520	0.8641	620	0.2363
325	0.7938	425	0.583	525	0.8677	625	0.2269
330	0.7768	430	0.5822	530	0.8575	630	0.218
335	0.7622	435	0.5782	535	0.8311	635	0.2104
340	0.7488	440	0.5767	540	0.7893	640	0.2016
345	0.7353	445	0.5787	545	0.7386	650	0.191
350	0.7252	450	0.5788	550	0.6851	655	0.1851
355	0.7151	455	0.5812	555	0.6271	660	0.1813
360	0.7059	460	0.5873	560	0.5689	665	0.178
365	0.6935	465	0.5923	565	0.5175	670	0.1736
370	0.6837	470	0.6005	570	0.4718	675	0.1693
375	0.6791	475	0.6133	575	0.429	680	0.1651
380	0.6139	480	0.6298	580	0.3926	685	0.1623
385	0.6172	485	0.6494	585	0.3616	690	0.1603
390	0.6158	490	0.6743	590	0.3357	695	0.1582
395	0.6072	495	0.7053	595	0.3132	700	0.1555

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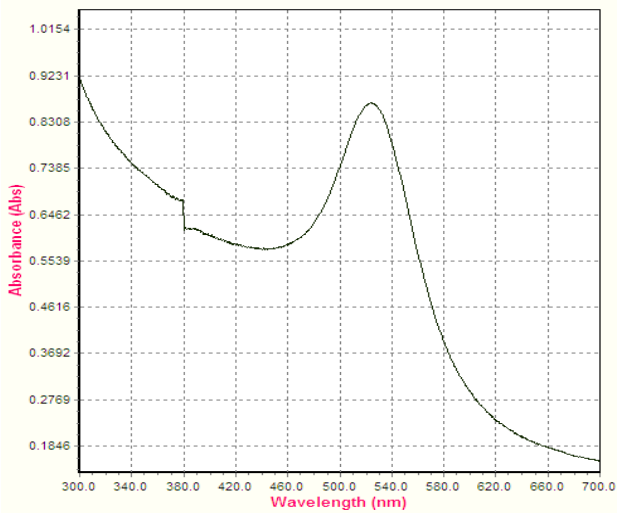
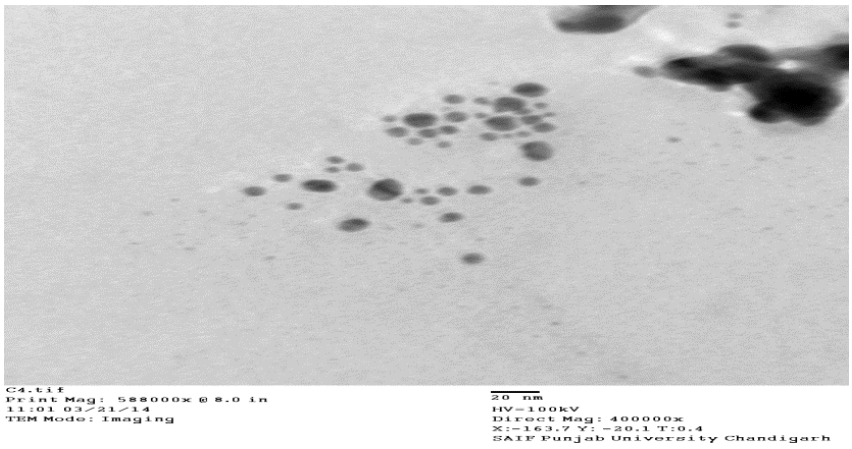
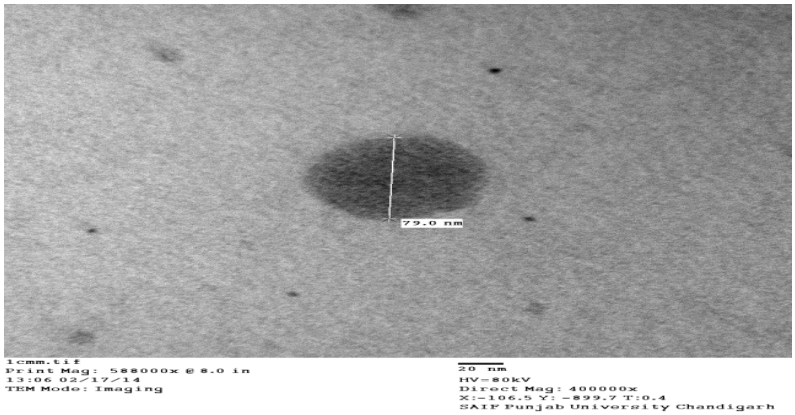


Scanning electron microscopy(SEM) :-

SEM analysis clearly shows the presence of gold nano particles of varying size. it was clearly observed that smaller size nano particles are spherical in size and some Nanoparticles in aggregates





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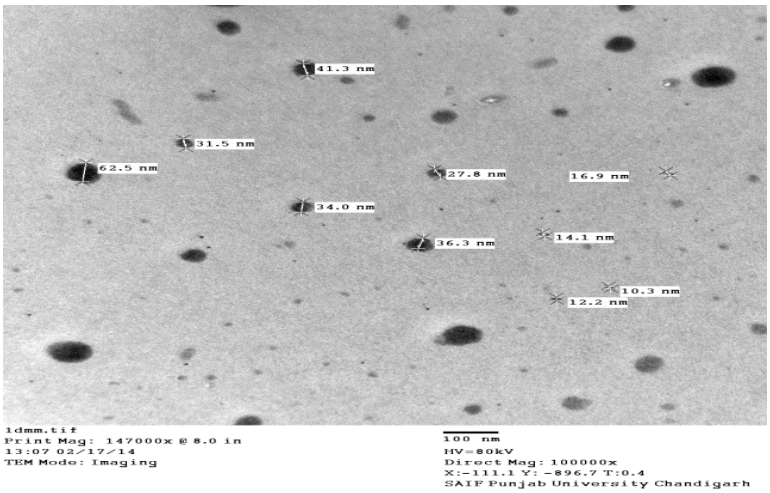


Particle size distribution:-

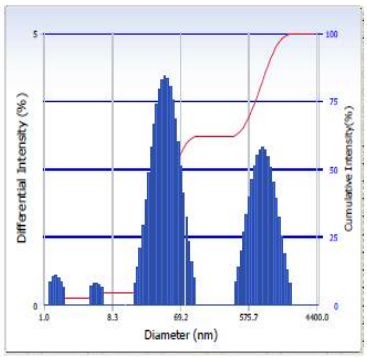
Particle size of nano particles shows two major peaks. The first peak was observed in the range of 70nm and the second peak at the range of 550 nm.

Intensity Distribution S/N : 114009

User	: Common	Group	: MZ	Repetition	: 1/1
Date	: 5/7/2016	File Name	: NANOPARTICLE_20160507_175934		
Time	: 17:59:34	Sample Information	: MZ		
SOP Name	: Sizing (general)			Security	: No Security

Version 2.21 / 2.03

Intensity Distribution



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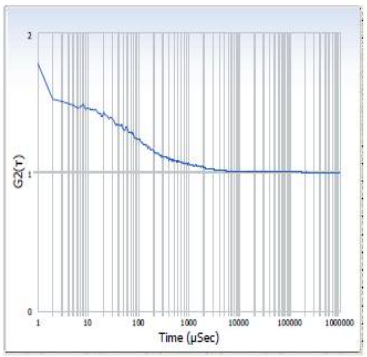

Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	1.5	0.2
2	5.0	0.6
3	45.8	19.2
4	943.9	373.2
5	0.0	0.0
Average	384.0	494.2
Residual :	2.244e-002	(N.G)

Cumulants Results

Diameter	(d)	: 301.9	(nm)
Polydispersity Index	(P.I.)	: 0.157	
Diffusion Const.	(D)	: 1.633e-008	(cm²/sec)

Measurement Condition

Temperature	: 25.1	(°C)
Diluent Name	: WATER	
Refractive Index	: 1.3328	
Viscosity	: 0.8858	(cP)
Scattering Intensity	: 8114	(cps)

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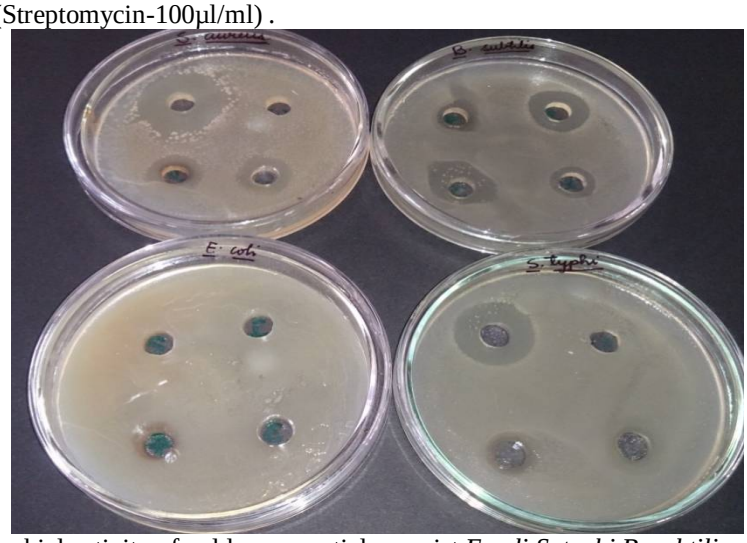
Intensity Distribution Table

d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)
1.0	0.0	0.0	8.3	0.0	4.7	69.2	2.6	56.0	575.7	2.0	69.3
1.1	0.0	0.0	9.1	0.0	4.7	75.3	2.1	58.0	626.6	2.3	71.6
1.2	0.4	0.4	9.9	0.0	4.7	82.0	1.6	59.6	682.0	2.6	74.2
1.3	0.5	1.0	10.7	0.0	4.7	89.2	1.2	60.8	742.3	2.8	76.9
1.4	0.6	1.5	11.7	0.0	4.7	97.1	0.8	61.6	808.0	2.9	79.8
1.5	0.5	2.0	12.7	0.0	4.7	105.7	0.5	62.1	879.4	2.9	82.7
1.7	0.4	2.5	13.8	0.0	4.7	115.1	0.0	62.1	957.2	2.9	85.6
1.8	0.3	2.8	15.1	0.0	4.7	125.2	0.0	62.1	1041.9	2.7	88.3
2.0	0.0	2.8	16.4	0.4	5.1	136.3	0.0	62.1	1134.0	2.5	90.9
2.1	0.0	2.8	17.8	0.7	5.7	148.4	0.0	62.1	1234.3	2.3	93.1
2.3	0.0	2.8	19.4	1.1	6.8	161.5	0.0	62.1	1343.4	2.0	95.1
2.5	0.0	2.8	21.1	1.5	8.3	175.8	0.0	62.1	1462.2	1.6	96.7
2.8	0.0	2.8	23.0	2.0	10.2	191.3	0.0	62.1	1591.6	1.3	98.0
3.0	0.0	2.8	25.0	2.4	12.7	208.2	0.0	62.1	1732.3	0.9	98.9
3.3	0.0	2.8	27.2	2.9	15.6	226.7	0.0	62.1	1885.5	0.7	99.6
3.6	0.0	2.8	29.7	3.3	18.9	246.7	0.0	62.1	2052.2	0.4	100.0
3.9	0.0	2.8	32.3	3.7	22.6	268.5	0.0	62.1	2233.7	0.0	100.0
4.2	0.4	3.1	35.1	4.0	26.6	292.3	0.0	62.1	2431.3	0.0	100.0
4.6	0.4	3.5	38.2	4.2	30.8	318.1	0.0	62.1	2646.3	0.0	100.0
5.0	0.4	3.9	41.6	4.2	35.0	346.2	0.0	62.1	2880.3	0.0	100.0

D (10%) : 22.80 (nm) D (50%) : 57.80 (nm) D (90%) : 1,101.90 (nm)

Antimicrobial activity :-

In vitro antimicrobial activity, of gold nano particles nanoparticles produced from p stutzeri was carried out against S.aureus, B.subtilis, S.typhi and E.coli by well method.. The result shows zone of inhibition differently against different microorganism i.e. S.aureus(0.1cm), B.subtilis(0.1cm), E.coli(0.1cm) and S.typhi(0.2cm) and it is compared with standard antibiotic (Streptomycin-100µl/ml) .



Antimicrobial activity of gold nano particles agaist *E.coli,S. typhi,B. subtilise,S. aureus*

Test organism	Zone of inhibition of antibiotic (in cm)	Zone of inhibition of nanoparticles. (in cm)
• S. Typhi	1.1cm	0.2 cm
• E.coli	0.9 cm	0.1 cm
• S.aureus	0.8 cm	0.1 cm
• B. subtilis	1.1 cm	0.1 cm

Table showing zone of inhibition of gold nano particles.

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International Journal of Innovative Research in Science, Engineering and Technology

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IV. CONCLUSION

It has been demonstrated that the p stutzeri. is capable of producing gold nanoparticles extracellualrly which are quite stable in solution due to capping agent sodium citrate by the proteins present in the cell filtrate. This is an efficient, eco-friendly and simple process. The gold nanoparticles showed significant antibacterial activity. Therefore, such gold nanoparticles can be used as antimicrobial agent alone after further trials on experimental animals.

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ISSN(Online) : 2319-8753
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(An ISO 3297: 2007 Certified Organization)

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