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Biosynthesis and Characterization of Silver Nanoparticles

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A B S T R A C T

The development of reliable, eco-friendly process for the synthesis of nanoparticles is an important aspect of nanotechnology. In the present study, the extracellular synthesis of Silver nanoparticles was done by reducing aqueous Ag+ with the culture supernatant of four different bacterial strains viz. *Bacillus flexus*, *Bacillus pseudomycoides*, *Cronobacte runiversalis*, and *Kocuria rosea*. The formation of silver nanoparticles was confirmed by the change in colour from colorless to brown. The synthesized nanoparticles were characterized adopting suggested instrumentations. In case of UV-Visible spectroscopy, four strong peaks were observed at 430, 410, 420 and 420 nm respectively which confirmed the synthesis of AgNPs. FTIR analysis confirms the presence of elemental silver and reveals the dual function of biological molecule responsible for the reduction and stabilization of AgNPs in the aqueous medium. The XRD showed that silver nanoparticles produced are crystalline in nature with size ranges from 30 to 70 nm. The SEM, shows that produced silver nanoparticles are spherical, Pseudo spherical in shape. However, it also showed an indeterminate morphology. The silver nanoparticle also showed traces of agglomeration. Silver nanoparticles synthesized through this biosynthesis method have been known to posses' great interest in recent times due to their reported advantageous properties as well as application in a diversity of fields.

Introduction

Nanotechnology is the creation of functional materials, devices and system through control of matter on the nanometer length scale (1-100nm) and exploitation of novel phenomenon and properties at that length scale"(Nasa's definition). Nanotechnology, on the other hand, simply denotes the man-made use of these nano-sized particles, for industrial and medical purposes. Now-a-days we are using nanoproducts in various fields. Different types of nanomaterials like copper, zinc, titanium (Retchkiman-Schabes *et al*., 2006), magnesium, gold (Gu *et al*., 2003), alginate (Ahmad *et al*., 2005) and silver have come up but silver nanoparticles have proved to be most effective as it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic micro-organisms (Gong *et al*., 2007). Zhao and Stevens in 1998 worked on the antimicrobial activity of silver nanoparticles (Ag-NPs), which appears significantly high and also reported that Silver is more toxic element to microorganisms than many other metals. The synthesis of nanoparticles extensively studied by using chemical and physical methods, but the development of reliable technology to produce nanoparticles is an important aspect

of nanotechnology. Many studies have reported successful synthesis of silver nanoparticle using microorganisms and biological systems (Korbekandi *et al*., 2009; Sastry *et al*., 2003; Iravani, 2011).

It was reported that highly stable silver nanoparticles (40 nm) could be synthesized by bioreduction of aqueous silver ions with a culture supernatant of some non pathogenic and pathogenic Bacteria viz. *Bacillus licheniformis* (Kalishwaralal *et al*., 2008b), *B. subtilis* (Saifuddin *et al*., 2009), *Pseudomonas stutzeri* AG259 (Klaus *et al*., 1999), *Klebsilla pneumoniae* (Mokhtari *et al*., 2009), *E. coli*, *Enterobacter cloacae* (Shahverdi *et al*., 2007) and *Lactobacillus* (Nair and Pradeep, 2002).

Many studies have enlightened the biological synthesis of Silver nanoparticles from bacteria; however, biological synthesis of silver nanoparticles by using Bacterial isolates was scantily studied. Hence, the present study was carried out on synthesis of Silver nanoparticles by bacteria. The generated nanoparticles were characterized by an instrumental analysis *viz.*, UV- Visible spectroscopy, Fourier transform infrared spectroscopy (FT-IR), Scanning electron microscopy (SEM) and X-ray diffraction (XRD).

Materials and Methods

Biosynthesis and characterization of silver nanoparticles

The silver nanoparticles were synthesized from four different silver resistant bacterial isolates viz. *Bacillus flexus*, *Bacillus pseudomycoides*, *Cronobacter universalis*, and *Kocuria rosea.*, adopting the method suggested by Das *et al*., (2013) with slight modifications.

Preparation of bacterial cell free extract

For the biosynthesis of silver nanoparticle, selected bacterial isolates were separately inoculated in to 250 ml conical flasks containing 100 ml LB broth and maintained in incubatory shaker (Remi make) at 220 rpm. The growth parameters were adjusted at pH 6 and Temperature 40^0C. Followed by incubation the enriched cultures were subjected to ultra centrifugation at 20,000 rpm for 10minutes. The supernatant material was separated out and use as crude source of reductase enzyme for the extracellular synthesis of nanoparticles.

Biosynthesis of silver nanoparticles

In a typical biosynthesis production scheme of silver nanoparticles, 2 ml of supernatant from each bacterial culture was mixed separately with 100ml of 1mM aqueous solutions of filtered sterilized $AgNO_3$, in 250ml conical flasks. Further the reaction mixture is placed at 150 rpm in incubatory shaker (Remi make) shaker at 37^oC up to 72 hours and allowed for reduction. The set without $AgNO_3$ was maintained as Control and use to compare with the test samples.

Extraction and Purification of Silver Nanoparticles

The silver nanoparticles were purified by three successive ultra Centrifugations at 20,000 rpm for 15 minutes at 4^0 C, the supernatant were separated out. The supernatant clear suspension was redispersed in sterile deionised water to remove the residual biological molecules. The process of centrifugation and redispersion in sterile deionised water was repeated thrice for complete removal of the residual entities from the silver nanoparticles. The purified solution was then dried using hot air oven at 60^0C for overnight (Nagarajan and Kuppusamy, 2013).

The dried powder of Silver nanoparticles was then mixed with 10 ml of deionized water and kept on a sonicator to prevent aggregation of molecules and further used for Characterization.

Characterization of silver nanoparticles

UV- Visible spectroscopic analysis

During the assay, approximately 1 ml of sample (crude extract) was withdrawn in standard quartz cuvette (1cm). The absorbance spectra of the AgNPs were analyzed by determining the maximum absorbance of the samples in the range of 350 to 450 nm, with UV–Vis spectrometer Shimadzu-UV 1800 using double deionized distilled water as a reference.

FT-IR analysis

The characterization of functional groups on the surface of AgNPs was investigated by FTIR analysis (Nicklet 380). The samples were prepared by dispersing the synthesized AgNPs uniformly in a matrix of dry KBr (in the ratio of 1:100), compressed to form an almost transparent disc FTIR spectrum of all samples was recorded in Nicolet Impact 400FT-IR spectrophotometer instrument with a diffuse reflectance mode (DRS8000) attachment. The spectra were scanned in the range of 4000–400 $cm^{-1}$with the resolution of 4 cm^{-1}.The spectra thus obtained were used to determined the associated compounds with nanoparticles (Aguilar-Mendez *et al*., 2011)

XRD analysis

XRD measurements of the reduced AgNPs perform were recorded by using D8 advanced model X-ray diffractometer instrument made in Bruker. In XRD Diffractometer, all the synthesized samples were operating at a voltage of 40 kV and current of 30 mA with Cu K (α) radiation to determine the crystalline phase and material identification.

The samples were scanned in the 2θranges of 10-80 degree in continuous scan mode. The Scan rate was adjusted at 0.20/ second. The crystalline size of the synthesized silver nanoparticles was determined from X-ray line broadening using the Scherrer's equation formula reported by Cullity and Rita John (2009),

$$D = 0.9 \lambda / \beta \cos\theta \quad \ldots\ldots(1)$$

Where 'λ is wave length of X-Ray (0.1541 nm), β is FWHM (full width at half maximum), θ is the diffraction angle and 'D' is particle diameter size.

SEM analysis

The Morphological characterization of the samples was done using JEOL 5400. Silver nanoparticles in its powder form were separately sonicated with distilled water further the small amount of sample was spread on glass slide and allow drying and a thin film of gold was coated to make the sample conductive. The machine was operated at a vacuum and accelerated voltage of the microscope was kept in the range 30 kv. The images thus obtained were used for determination of particle shape (Pavani *et al*., 2013).

Results and Discussion

Biosynthesis of silver nanoparticles

In all the test samples AK1, AK2, AK3 and AK4 the Silver nanoparticles were produced showing the positive reduction of Ag+ ions. This has been confirmed by visualize change in color from colourless to dark brown. Further the Silver nanoparticles were separated out by ultracentrifugation process. The purified solutions were then dried and all four AgNPs powder samples (viz. AK1, AK2, AK3 and AK4) were subjected for characterization.

Characterization of synthesized Silver Nanoparticles

UV- Visible Spectrophotometric analysis

The reduction of Silver salt to their respective silver ions was monitored by UV-Visible spectrum, presented in Table (1) and graphically represented in figure 1. Four strong peaks were observed at 430nm, 410nm, 420nm and 420nm confirming the synthesis of AgNPs using four different bacterial strains (*viz. Bacillus flexus*, *Bacillus pseudomycoides*, *Cronobacter universalis* and *Kocuriarosea*), the results on the obtained UV-Visible spectrum are in accordance with the revived reports of Henglein (1993), who reported that a SPR peak located between 410 and 440nm has been observed for AgNPs and is well documented for Ag (metal) nanoparticles with sizes from 2 to 100nm. Our findings also correlated with the reports of He *et al*., (2007) and Portner (2007).

FT-IR spectroscopy analysis

The results of FTIR for all four AgNPs samples (*viz.* AK1, AK2, AK3 and AK4) were represented in figure (2.1 to 2.4). In case of Sample AK1 and AK3, the band seen at 3398.02 and 3398.29 were assigned to NH2 stretching of primary amide, band at 1530.38 and 1530.55 corresponds to N-H bending and C-N stretching of secondary amide, where as the band at 1143.48 and 1143.22 corresponds to C-N stretching of aliphatic amine respectively. In sample AK 1 alone the band at 773.77 is characteristic of NH2 wagging and twisting. Where as In case of Sample AK2 and AK4, the band at 3231.19 and 3285.24 were assigned to N-H stretching of secondary amide respectively, in sample AK4 the band at 1078.85, 1018.21 correspond to C-N stretching of aromatic amines. The bands obtained were compared with band range given by Stuart (2004). It has also been reported earlier by Gole *et al*., (2001), that protein can bind to silver nanoparticle either through their free amine groups or cysteine residues. Therefore stabilization of silver nanoparticles by proteins is a clear possibility. Hence the FTIR analysis confirms the presence of elemental silver, in biosynthesized samples of AgNPs as it is generated from inorganic silver salt and reveals the dual function of biological molecule responsible for the reduction and stabilization of AgNps in the aqueous medium. Our results correlate with Senapati *et al.*, (2005), Jeeven *et al.*, (2012) and Vijayaraj *et al.,* (2012) they also reported the presence of silver nanoparticles by determining the interaction between silver salt and protein molecules.

XRD analysis

The XRD pattern obtained for all four AgNPS samples (viz. AK1, AK2, AK3 and AK4) were represented in figure (3.1 to 3.4) Comparisons of XRD spectrum with the standard powder diffraction card of Joint Committee on Powder Diffraction Standards (JCPDS), silver file No. 04–0 783, confirms that the silver nanoparticles found in the present study were in the form of nano-crystals as evident from the peak at 2θ values of all the samples, these all values are correspond to (111), (200), (220), (311) respectively for silver represented in table 2. Pande *et al*., (2012) reported that these typical XRD peak occurs due to the presence of Face centered cubic (fcc) of the crystalline silver nanoparticles.

Table.1 UV-Visible absorbance spectra of synthesized silver nanoparticles

Optical (nm)density at	Mean optical density of synthesized silver nanoparticles			
	SampleAK1	**SampleAK2**	**SampleAK3**	**SampleAK4**
350	0.11	0.10	0.10	0.12
360	0.2	0.22	0.18	0.20
370	0.35	0.31	0.29	0.29
380	0.41	0.39	0.37	0.34
390	0.47	0.44	0.43	0.40
400	0.53	0.52	0.52	0.48
410	0.59	**0.65**	0.61	0.56
420	0.65	0.60	**0.69**	**0.66**
430	**0.71**	0.51	0.60	0.58
440	0.64	0.46	0.52	0.51
450	0.52	0.39	0.46	0.45
λ max	**430**	**410**	**420**	**420**

Table.2 Peak indexing from d – spacing and grain size of Silver nanopowder

2θ	**D**	**$1000/d^2$**	**$(1000/d^2)$ /60.62**	**Hkl**	**FWHM (β)**	**β cos θ**	**Size of particle (D) nm**
Sample AK1							
37.91	2.371	177.904	2.934	111	0.0041	0.00407	34
43.98	2.056	236.574	3.902	200	0.0048	0.00479	29
64.21	1.449	476.417	7.859	220	0.0038	0.00292	47
77.20	1.234	657.030	10.838	311	0.0034	0.00210	66
Sample AK2							
38	2.366	178.667	2.947	111	0.0041	0.00405	34
44.15	2.049	238.208	3.929	200	0.0048	0.00478	29
64.36	1.446	478.468	7.892	220	0.0038	0.00274	50
77.28	1.233	657.894	10.852	311	0.0034	0.00200	69
Sample AK3							
37.92	2.370	178.062	2.937	111	0.0041	0.00407	34
44.09	2.052	237.529	3.918	200	0.0048	0.00479	29
64.28	1.447	477.783	7.881	220	0.0038	0.00284	49
77.17	1.235	655.737	10.817	311	0.0034	0.00214	65
Sample AK4							
37.91	2.371	177.904	2.934	111	0.0041	0.00407	34
43.98	2.056	236.574	3.902	200	0.0048	0.00479	29
64.21	1.449	476.417	7.859	220	0.0038	0.00292	47
77.20	1.234	657.030	10.838	311	0.0034	0.00210	66

Fig.1

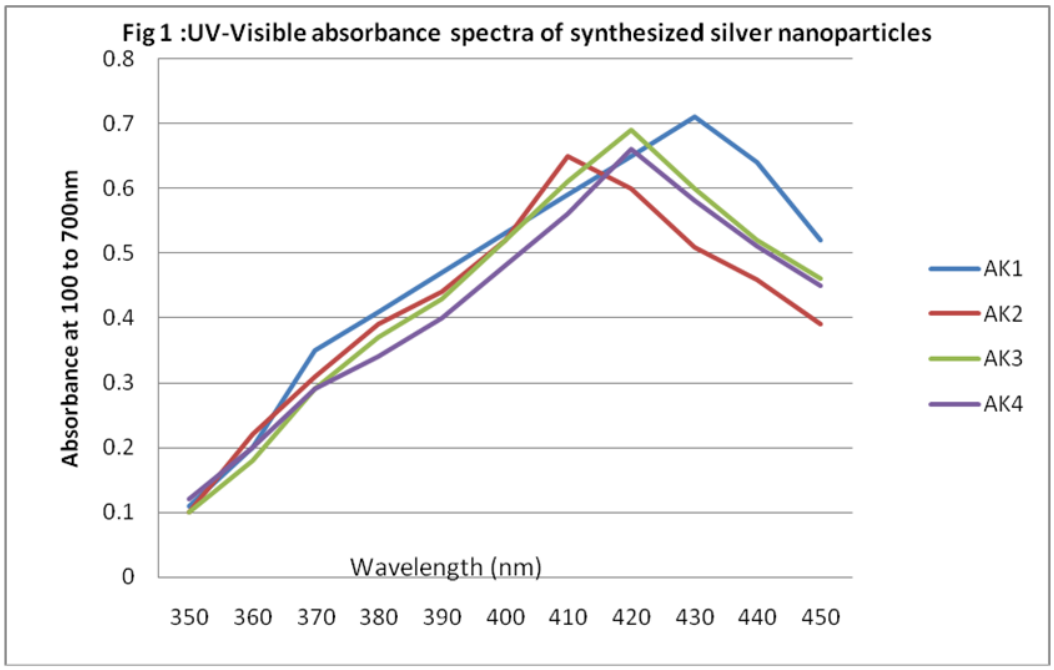


Fig.2.1-2.4 FTIR Spectrum of Silver nanoparticles synthesized from bacteria *Bacillus flexus*, *Bacillus pseudomycoides*, *Cronobacter universalis* and *Kocuria rosea* respectively

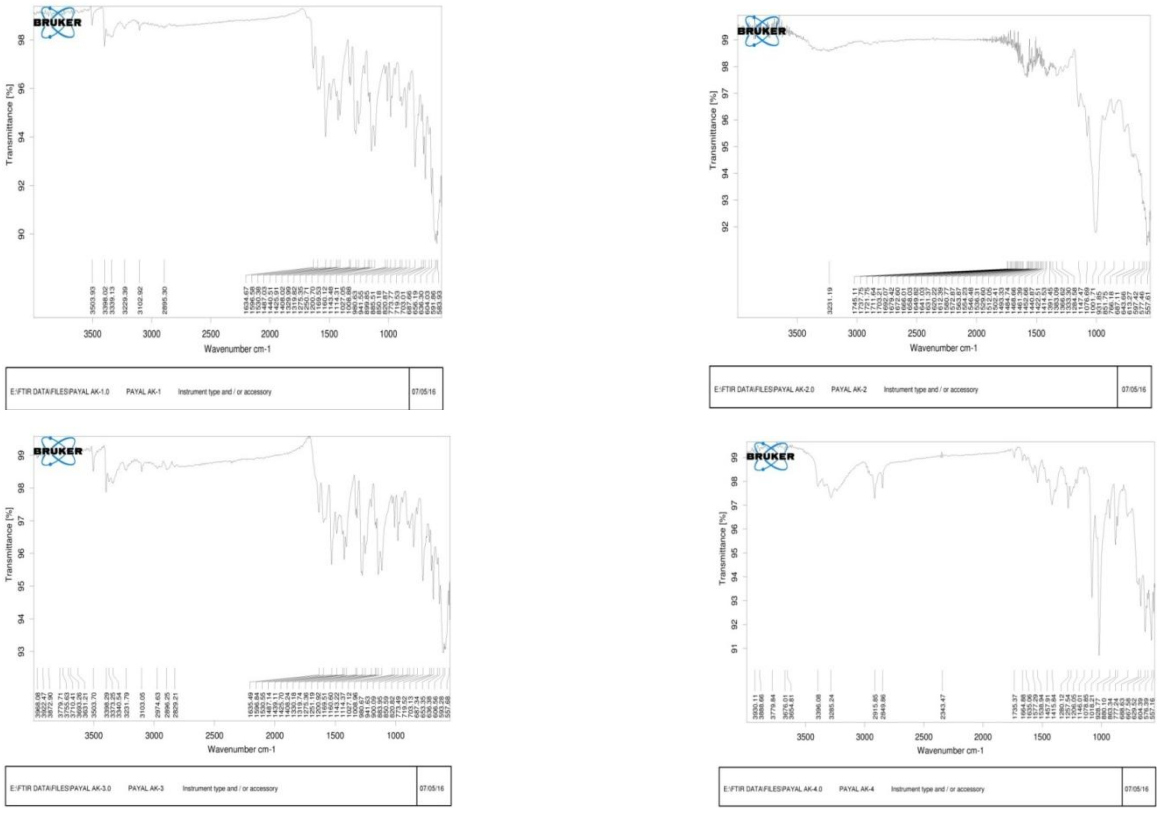
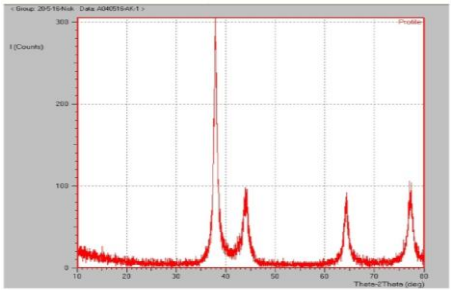
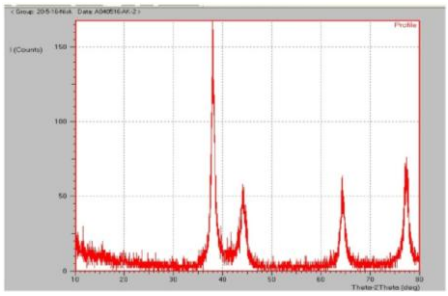


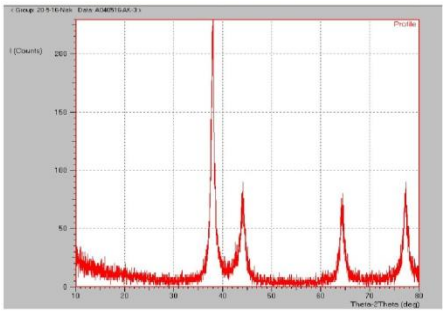
Fig.3.1-3.4 XRD Spectrum of Silver nanoparticles synthesized from bacteria *Bacillus flexus*, *Bacillus pseudomycoides*, *Cronobacter universalis* and *Kocuria rosea* respectively



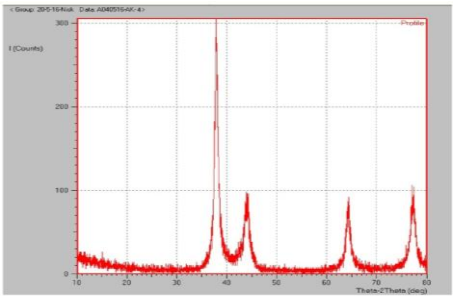
AK1



AK2

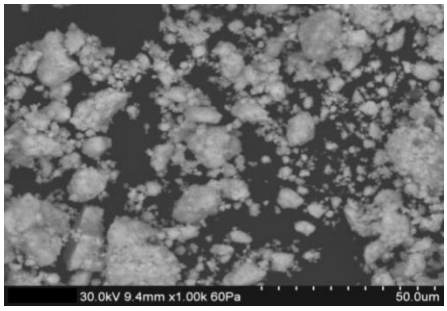


AK3

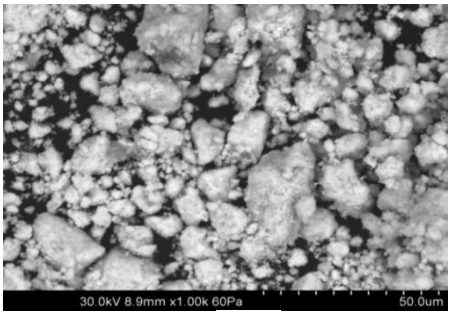


AK4

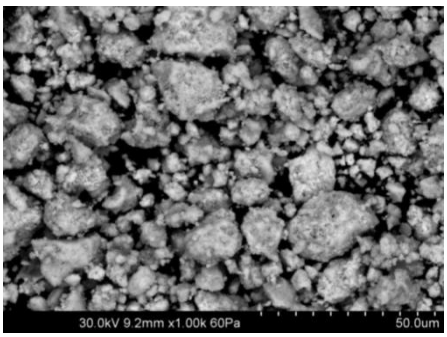
Fig.41.-4.4 SEM images of Silver Nanoparticles synthesized from bacteria bacteria *Bacillus flexus, Bacillus pseudomycoides*, *Cronobacter universalis* and *Kocuria rosea* respectively



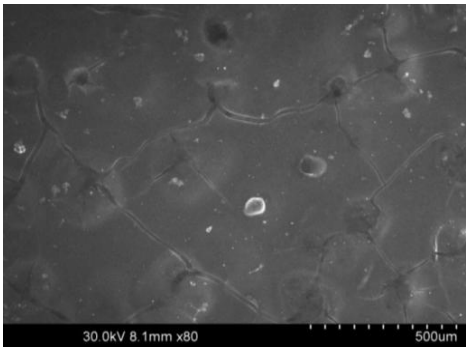
AK1



AK2



AK3



AK4

The high intense peak for FCC materials is generally (111) reflection, which is observed in all the samples. The XRD shows that silver nanoparticles formed are crystalline. The results are in correlation with the reports of, Amrut *et al.*, (2010), Prakash *et al*., (2010), Prakash *et al*., (2011), Jeevan *et al*., (2012) and Manivasagan *et al*., (2013). The calculated particle size details are displayed in Table (2), it was observed that all the samples contain four different sizes of Silver nanoparticle with size ranges from 30 to 70 nm. The overall result of XRD was correlated with the results of Theivasanthi and Alagar (2010).

SEM analysis

The image shows representative SEM images recorded at high magnifications of the biosynthesized silver nanoparticles, shown in Figure (4.1 to 4.4) in case of Sample AK1, AK2, AK3 and AK4. It was observed that the produced silver nanoparticles that present in samples were scattered as well as in aggregates of varying sizes. It was observed that produced silver nanoparticles are spherical, Pseudo spherical and of undefined morphology with traces of agglomeration. The results on the present studies on the surface morphology are in accordance with the experimental findings of Malarkodi and Annadurai (2012). They reported the spherical, Pseudo spherical and agglomeration as the surface morphological shapes. The scanning images also showed the agglomeration it may be due to the fact that silver nanoparticles have the tendency to agglomerate due to their high surface energy and high surface tension of the ultrafine nanoparticles. Larger size agglomeration was reported by Theivasanthi and Alagar (2010).

This work demonstrates the simple approach to achieve an eco-friendly way for the biosynthesis of Silver nanoparticles from bacterial isolates viz. *Bacillus flexus*, *Bacillus pseudomycoides*, *Cronobacter universalis*, and *Kocuriarosea*. Silver nanoparticles synthesized by the bacterial strains indicates the rapid synthesis of nanoparticles and hence perhaps to be used in biosynthesis process for large scale production. Several studies have reported the synthesis of silver nanoparticles using different bacterial strains. However, the reports in present study on Silver nanoparticles producing isolates could not be traced hence maybe consider as value added account.

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