Production of silver nanoparticles (AgNPs) by certain bacterial strains and their characterization

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Abstract

Development of biologically inspired experimental processes for the biosynthesis of nanoparticles (NPs) is an evolving important branch of nanotechnology. In the present work, we studied the potential of four bacterial species for extracellular production of nanosilver (AgNPs) from 3 mM concentration of silver nitrate ($AgNO_3$) after incubation for 4h at 85°C. Biosynthesized AgNPs were characterized by using different methods such as; UV/vis spectroscopy, Transmission electron microscope (TEM), X-Ray Diffraction (XRD) and Fourier Transform Infra-red (FTIR) spectroscopy. Results of UV–vis spectroscopy showed maximum absorption at 401-432 nm, which represents the characteristic surface plasmon resonance of AgNPs. TEM demonstrated that the size range of these NPs ranged approximately from 7.8- 13.4 nm. Representing the XRD pattern obtained for the AgNPs, a number of Fcc structures of silver Bragg reflections corresponding to (100), (110), (111), (200) and (220) planes were observed. FTIR results recorded a downward shift of absorption bands between 400–4000 cm^{-1} indicating the formation of AgNPs. Finally we concluded that the extracellular biosynthesis of AgNPs by the four bacterial species; *Ochrobactrum* sp. (MAM-C9), *Achromobacter xylosoxidans* (MAM-29), *Pseusomonas aeruginosa* (MAM-42) and *Bacillus cereus* (MAM-I.11) were confirmed. This study recorded that bacterial biosynthesis of AgNPs is useful to avoid adverse effects of chemical and physical methods that are non-suitable for medical applications.

Keywords: Nanosilver, Green biosynthesis, Bacterial isolates, UV/vis spectroscopy, TEM, XRD, FTIR.

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1. Introduction

For NPs production, there is a need to develop ecofriendly procedures to avoid the toxic chemicals used in the old synthesis protocols, hence avoid their adverse effects in case of medical applications of these NPs. Recently, the researchers were inspired to produce benign NPs using microorganisms and plant extracts as biological systems that were termed as the ''green chemistry approach'' (Sinha *et al*., 2009; Salar *et al*., 2015; Sarsar *et al*., 2015).

Several physical and chemical methods are widely used for the synthesis of NPs. However, although these methods offer higher production rate and better size control of the synthesized NPs, they are unsuitable due to their high capital cost, energy requirements, the use of toxic chemicals and the formation of non-ecofriendly wastes (Fariq *et al*., 2017). This led to a growing awareness for the need of developing clean, nontoxic and ecofriendly procedures. High demands for NPs led to their production on large scales. According to Thakkar *et al*., (2010), only one gram of AgNPs is known to impart antibacterial properties to hundreds of square meters of substrate materials.

Bio-applications of NPs have been extensively increased during last decades. Nanoparticles of noble metals such as gold, platinum and especially silver are widely applied in medical and pharmaceutical applications. Although varieties of physical and chemical methods have been developed for production of these metal NPs, however because of their destructive effects on environment, several biosynthetic routes have been suggested as novel alternatives.

Mohanpuria *et al*., (2008) reported that NPs are being viewed as fundamental building blocks of nanotechnology. The most important and distinct property of these NPs is their large surface area to volume ratio, thus increasing their antimicrobial potential as they would interact better with the microbial cell surface at low concentrations (Narayanan and Sakthivel, 2010). Specifically in the case of AgNPs, the broad spectrum antimicrobial activity encourages their use in biomedical applications, water and air purification, food production, cosmetics, clothing and numerous household products (Marambio-Jones and Hoek, 2010).

Ahmed and Ikram, (2016) reported that synthesis of NPs by biological methods affords economic, nontoxic and eco-friendly alternative to the different physical and chemical methods. Microorganisms such as bacteria, mold fungi and yeasts are mostly preferred for NPs biosynthesis due to their fast rate of growth, ease of cultivation and their ability to grow at available conditions of temperature, pH and pressure (Fariq *et al*., 2017). In an earlier study, Mohseniazar *et al*., (2011) pointed that bacterial spp. have different ranges of potentiality to uptake metal ions and produce NPs during detoxification processes. Creation of NPs with suitable size, shape and dispersity is one of the major challenges of present nanotechnology (Husseiny *et al*., 2007).

The current work aimed to produce AgNPs by an ecofriendly manner using four bacterial spp., and then characterization of these biosynthesized AgNPs using different physical methods as a primary step towards their use in medical applications.

2. Materials and Methods

2.1. Chemicals: Silver nitrate ($AgNO_3$) was purchased from Sigma Aldrich (St. Louis, USA), and LB broth culture medium from Oxoid (UK).

2.2. Bacterial isolates

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The four bacterial isolates used in the present work were kindly provided by Dr. Abo-State (EAEA, Nasr City, Cairo, Egypt). MAM-9 and MAM-29 strains were isolated from soil and water samples of Cairo Oil Refining Company, Mostorod, Al-Qalyubia, Egypt, while MAM-42 was isolated from soil sample contaminated with crude petroleum oil from Suez Canal, Egypt. MAM-I.11 was isolated from textile waste water I, El-Mahala El- Khobra textile industry Company, Egypt. These isolates were identified by 16-S-rRNA molecular technique in previous studies of Abo-State *et al*., (2013); Abo-State and Partila, (2015).

2.3. Extracellular biosynthesis of AgNPs by different bacterial spp.

For biosynthesis of AgNPs, a loopful of each bacterial sp. was inoculated into 100 ml Erlenmeyer flask containing 40.0 ml LB broth medium (Martin *et al*., 1981). The inoculated flasks were incubated on an orbital shaker at 37°C and agitated at 150 rpm for 48h. The grown cultures were centrifuged at 8000 rpm for 10 min. The supernatants (cell free extracts) were incubated for 4h at 85°C with 3mM AgNo$_3$ in darkness (Abo-State and Partila, 2015).

2.4. Characterization of AgNPs

2.4.1. UV/vis spectrophotometer

The formation of AgNPs (bio-reduction of silver ions) was monitored by scanning UV/vis spectrophotometer (LW-V-RS UV/VIS, Germany) for optical absorption spectra of AgNPs. Scanning ran between 300 and 700 nm for each AgNPs produced by the 4 bacterial isolates separately (Abo-State and Partila, 2015).

2.4.2. Transmission Electron Microscope (TEM)

In reference to Priyadarshinia *et al*., (2013), samples of AgNPs solutions were dropped onto carbon-coated cupper TEM grid. The films on the TEM grids was allowed to dry prior to the determination of shape, size and morphologies of bio-produced AgNPs using TEM (JEOL, JEM 100 CX, Japan).

2.4.3. X-Ray Diffraction (XRD)

XRD for dried powder of AgNPs was determined by Shimadzu – XRD-6000 diffractometer (Cu.Ka, λ = 1.54056 Å). Scans were run from 2 theta 4º - 90º. The hydrodynamic diameter of AgNPs was measured by scattered dynamic light (Ciftci *et al*., 2013) using Scherrer's equation:

$$D = 0.89 \, \lambda / \beta \cos \Theta$$

Where D is the average of particle size;

β is the full width at half maximum of x-ray reflection in terms of 2Θ in radians, and 2Θ is the position of the different peaks in the diffractograms (Galande *et al*., 2011).

2.4.4. Fourier Transform Infra-red (FTIR) spectroscopy

FTIR spectroscopy measurements were carried out to identify the biomolecules of the produced AgNPs. These AgNPs were centrifuged at 10,000 rpm for 20 min., and then the pellets were re-suspended in sterile double distilled water. The suspension was re-centrifuged at 10,000 rpm for 20 min., and then the pellets were dried. The dried samples were grounded with Potassium bromide (KBr) pellets and compacted. The compacted samples were analyzed by an ATI Mattson (Genesis series, Unicam, England) FTIR spectroscopy. Scan and measure the absorption spectra of range (400-4000 cm^{-1}) at room temperature at a resolution of 4 cm^{-1} according to Priyadarshinia *et al*., (2013).

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3. Results

3.1. Identification of bacterial isolates

Isolates MAM-9, MAM-29, MAM-42 and MAM-I-11 were identified as; *Ochrobactrum* sp., *Achromobacter xylosoxidans* with accession No. JN038055, *Pseudomonas aeruginosa* with accession No. 3NP0614 and *Bacillus cereus*, respectively. The green synthesis of AgNPs by the four bacterial isolates was indicated by the

formation of dark brown color from colorless $AgNO_3$ solution.

3.2. Scanning spectrophotometry (UV/vis)

Scanning by UV/vis spectrophotometer of the AgNPs produced by MAM-9, MAM-29, MAM-42 and MAM-I.11 isolates showed a characteristic surface plasmon absorption bands at 412, 401, 409, 432 and 423 nm, respectively, as indicated in Fig.'s 1(a), (b), (c) and (d).
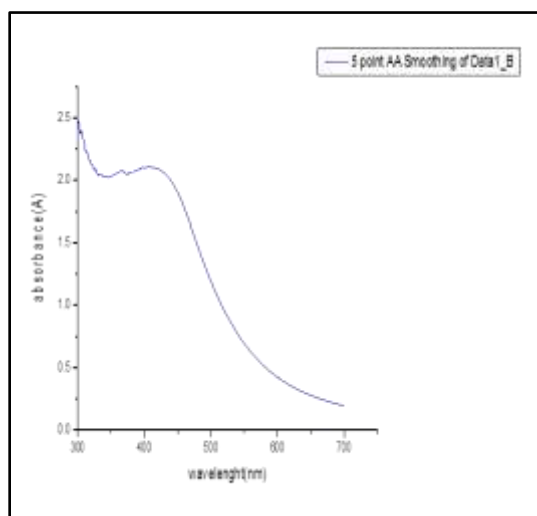

Fig. 1(a): Scanning UV-vis spectrum of AgNPs synthesized by *Ochrobactrum* sp. (MAM-9) extract
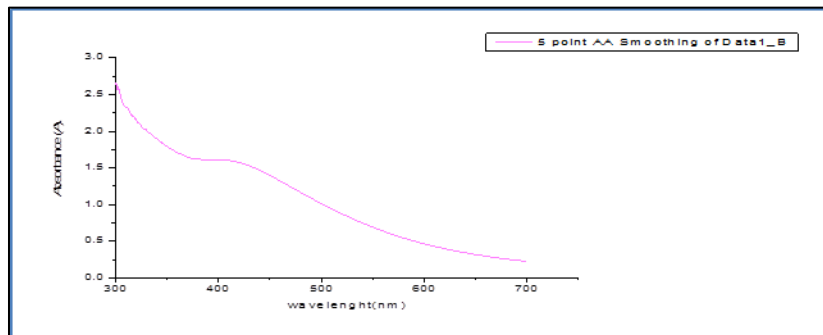

Fig. 1(b): Scanning UV-vis spectrum of AgNPs synthesized by *Achromobacter xylosoxidans* (MAM-29) extract
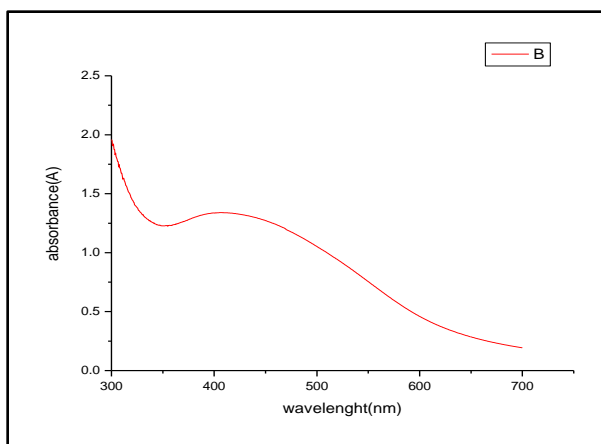
Fig. 1(c): Scanning UV-vis spectrum of AgNPs synthesized by *Pseudomonas aeruginosa* (MAM-42) extract
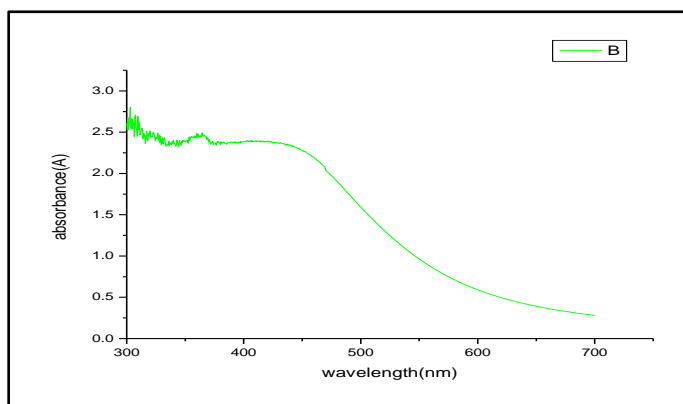

Fig. 1(d): Scanning UV-vis spectrum of AgNPs synthesized by *Bacillus cereus* (MAM-I-11) extract

However, results of intensity of absorbance of AgNPs measured at 420 nm revealed that the concentrations of AgNPs synthesized by the bacterial isolates were in the order of MAM-9> MAM-I.11>MAM-42>MAM-29, corresponding to 1.303, 1.132, 1.101, 1.056 and 0.667 nm, respectively. This means that the concentration of AgNPs formed was strain dependent.

3.3. Transmission Electron Microscope (TEM)

The morphology and size of the benign AgNPs synthesized by the different bacterial isolates were observed by TEM. The sizes of biosynthesized AgNPs were found between 7.8 to 13.4 nm and seemed to be spherical in shape.

3.4. X-Ray Diffraction (XRD)

The diffraction patterns was recorded by Cu-Kα1 radiation with ⅄ of 1.54056 A° in the region of 2 Ө from 4° to 90°. The crystalline nature of AgNPs synthesized by isolate MAM-9 was indicated in Fig. 2(a). Results revealed that a number of strong Bragg s diffracted peaks at 2 Ө of 31.64 A° , 32.08 A° and 66.19 A° corresponding to (110), (200) and (220) of the

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face centered cubic lattice of silver (FCC) were observed. The sizes of AgNPs were 11.1, 4.7 and 10.1 nm, respectively, with mean size of 8.6 nm.

On the other hand, XRD of AgNPs biosynthesized by isolate MAM-29 revealed that the strongest peaks at 2 Ө were 31.63 A° , 32.11 A° and 31.96 A° corresponding to (110), (200) and (200) of FCC lattice of silver and is shown in Fig. 2(b). The sizes of AgNPs were 9.2, 7.0 and 6.8 nm with mean size of 7.8 nm. Fig. 2(c) showed that the strongest peaks of AgNPs synthesized by isolate MAM-42 at 2Ө were 31.64 A°, 32.14 A° and 25.41 A° corresponding to (110), (200) and (100) of FCC lattice of silver. The sizes of AgNPs were 10.6, 9.6 and 20.0 nm, respectively. The mean size was 13.4 nm.

In case of XRD pattern of AgNPs biosynthesized by isolate MAM-I.11, Fig 2(d) revealed that the strongest Bragg`s diffracted peaks at 2Ө were 28.15 A°, 31.66 A° and 66.23 A° which correspond to (111), (110) and (220) of FCC lattice of silver. The calculated sizes of AgNPs were 9.01 nm, 10.9 nm and 11.7 nm with mean size of 10.5 nm. The slight shift in the peaks positions indicated the presence of AgNPs in the crystalline structure, and also due to their biosynthesis using different bacterial spp. In this study the sizes of AgNPs were ranging from 7.8 nm to 13.4 nm. Results of sizes of AgNPs determined by XRD measurements and calculations were in agreement with those determined by TEM.
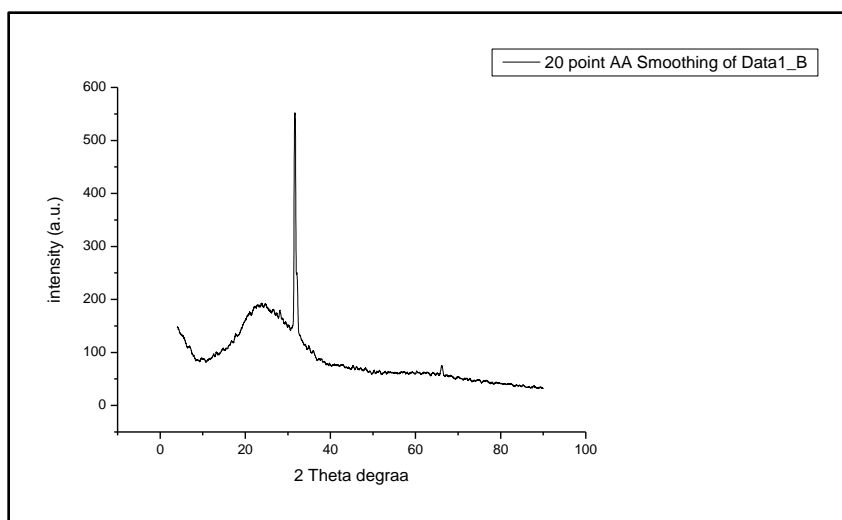

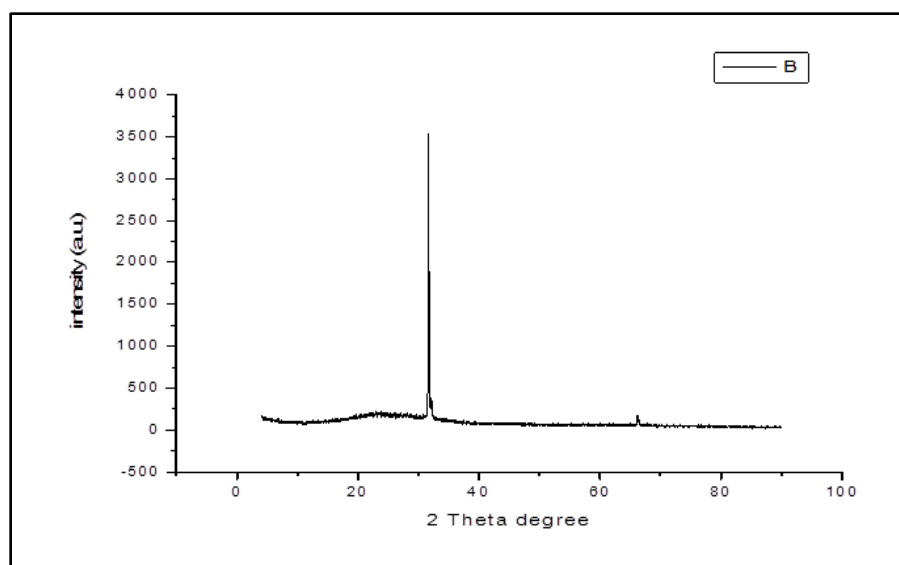**Fig. 2(a):** XRD pattern of AgNPs synthesized by *Ochrobactrum* sp. (MAM-9) extract

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Fig. 2(b): XRD pattern of AgNPs synthesized by *Achromobacter xylosoxidans* (MAM-29) extract
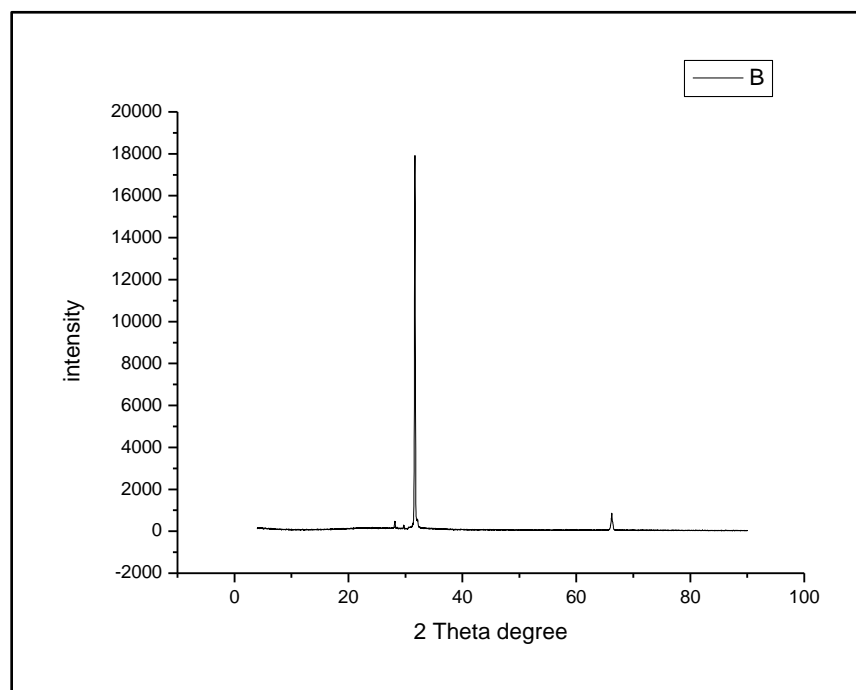

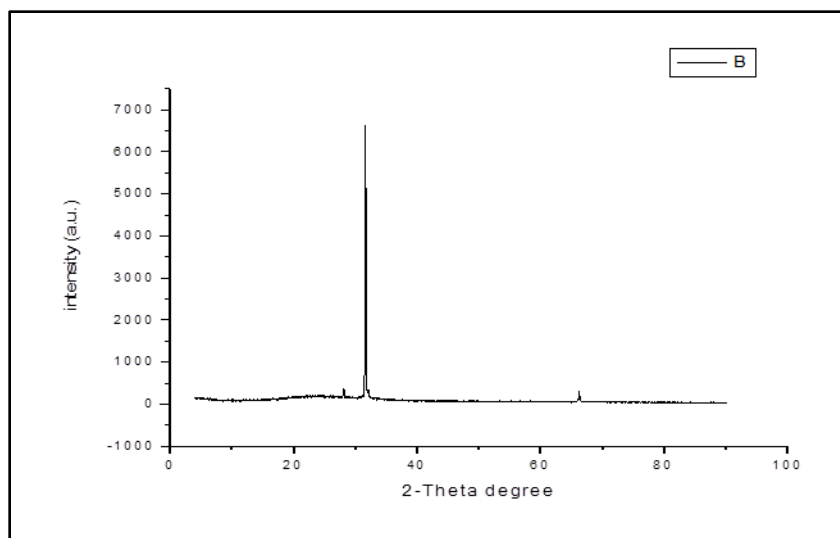**Fig. 2(c):** XRD pattern of AgNPs synthesized by *Pseudomonas aeruginosa* (MAM-42) extract

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Fig. 2(d): XRD pattern of AgNPs synthesized by *Bacillus cereus* (MAM-I-11) extract

3.4. Fourier Transform Infra-red (FTIR) spectroscopy

FTIR measurement of the AgNPs synthesized by isolate MAM-9 (biomolecule) showed reduction and stabilization of metal nanoparticles and a band at 1060-1062 cm^{-1} corresponding to C-O stretching alcohol, the peak at 1380-1403 cm^{-1} indicated bending C-H, whereas peak at 1633-1641 cm^{-1} showed C=C which indicated the formation of alkene and aromatic ring. The band at 2915 – 2919 cm^{-1} corresponded to the formation of the multi bended strong Sp^3 carbon for C-H, while the peak at 3399 – 3401 cm^{-1} revealed the formation of amine amino group (N-H) by the bacterial extract as clear in Fig. 3(a). AgNPs biomolecules synthesized by isolate MAM-29 showed a peak at 1051-1085 cm^{-1} corresponding to C-O stretching alcohol, the peak at 1402 cm^{-1} indicated C-H, whereas the peak at 1635-1658 cm^{-1} indicated C=C group for alkene and aromatic ring. However the band at 2848 cm^{-1} corresponded to C-H Sp^3 (carbon multi bended),

the peak at 3399- 3400 cm^{-1} was N-H group (amine amino group) or O-H (alcohol group) as indicated in Fig. 3(b). In case of AgNPs biomolecules synthesized by isolate MAM-42, they have a peak at 1078 cm^{-1} corresponding to C-O stretching group of alcohol, while peak at 1383 cm^{-1} indicated bending C-H. However peaks at 1635-1641 cm^{-1} and 1658 cm^{-1} corresponded to C=C for alkene and aromatic ring. The peak at 2850 indicated C-H Sp^3 (carbon strong broad multi bended) or broad OH (carboxylic acid), while broad peak at 3372 – 3700 cm^{-1} states of N-H (amine amino group) or O-H (alcohol strong) as observed in Fig. 3(c). Finally, AgNPs synthesized by isolate MAM-I-11 as observed in Fig. 3(d) revealed that peaks at 1105-1116 cm^{-1} corresponding to C-O, peak at 1384 cm^{-1} for C-H, peaks at 1637-1639 and 1656 cm^{-1} corresponded to C=C for alkene and aromatic ring, respectively. The peak at 2848-2850 cm^{-1} corresponding to C-H Sp^3, and peak at 3342 – 3345 cm^{-1} were for N-H or O-H groups.
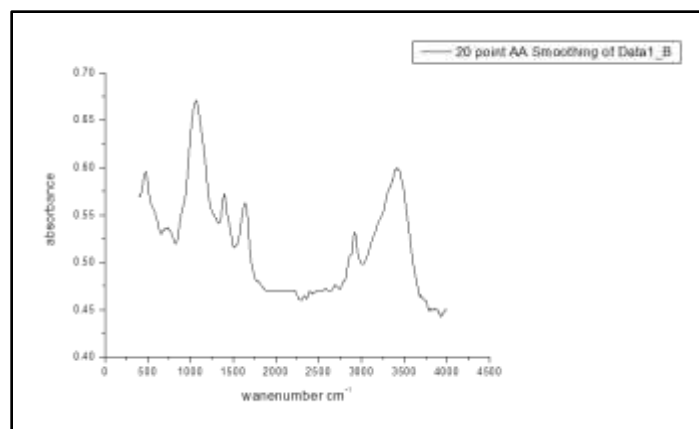
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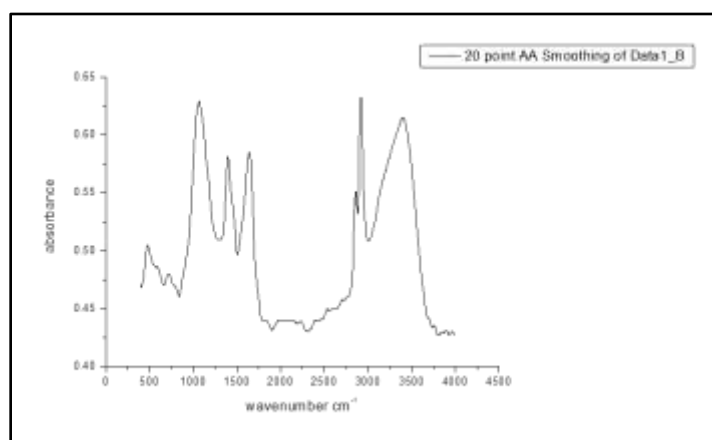**Fig. 3(a):** FTIR spectra of AgNPs synthesized by *Ochrobactrum* sp. (MAM-9) extract

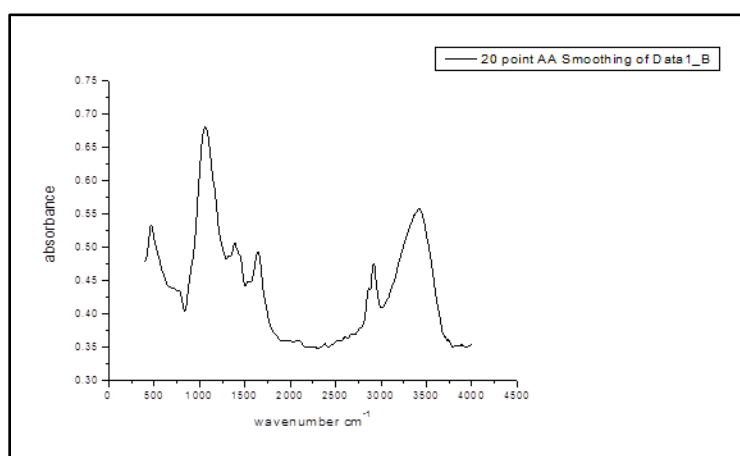**Fig. 3(b):** FTIR spectra of AgNPs synthesized by *Achromobacter xylosoxidans* (MAM-29) extract

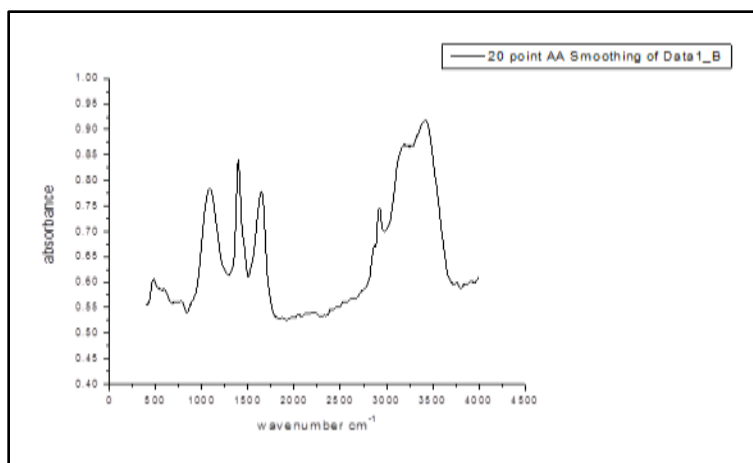**Fig. 3(c):** FTIR spectra of AgNPs synthesized by *Pseudomonas aeruginosa* (MAM-42) extract

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Fig. 3(d): FTIR spectra of AgNPs synthesized by *Bacillus cereus* (MAM-I.11) extract

4. Discussion

The observed dark brown color within 4h of incubation of AgNO$_3$ with the supernatants of the different bacterial isolates indicated the formation of AgNPs. On the contrary, no color change was observed in the control. Fariq *et al*., (2017) demonstrated that extracellular biosynthesis of NPs is mediated by different enzymes present on the microbial cell membrane or released to the growth medium. Thus the produced NPs may be adsorbed on the cell membrane or be present in the medium.

The extracellular biosynthesis of NPs in microorganisms may occur under stress free conditions as a result of the presence of metal ions in the growth medium. In addition, it provides less laborious, economic and large scale NPs synthesis by easier processing (Hosseini and Sarvi, 2015).

4.1. Scanning spectrophotometry (UV/vis)

The absorption spectra of AgNPs in the reaction media had a peak at 408 nm, broadening of this peak indicated that these NPs were polydispersed. Results of the present study

were in accordance with results of other investigators (Sastry *et al*., 1998; Awad *et al*., 2014; Abo-State and Partila, 2015). As explained by Mulvaney, (1996) the formation of reddish brown color of AgNPs in solution was due to the excitation of surface plasmon vibrations of these AgNPs. Gonzalo *et al*., (2003) added that the frequency and width of the surface plasmon absorption band depends on the size and shape of the metal NPs, as well as on the dielectric constant of the metal itself in addition to the surrounding medium.

4.2. Transmission Electron Microscope (TEM)

Current results were in agreement with Sastry *et al*., (1998) who found the average domain size of the AgNPs to be 12.6 nm. TEM micrograph analysis demonstrated proper dispersion of the AgNPs. This indicated that even aggregated NPs did not have direct contacts with each other. This could be due to the role of proteins present in the extract of the bacterial isolates in the biosynthesis of AgNPs (Nithya and Ragunathan, 2009).

4.3. X-Ray Diffraction (XRD)

Results of present study confirmed that the

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synthesized AgNPs were biphase in nature. Also the slight shift in the peaks position indicated the presence of AgNPs in the crystalline structure which is characteristic of nano-crystallites, as stated previously by Rajakumar and Abdul–Rahuman, (2011); Abo-State and Partila, (2015). Similar results were reported by Sarsar *et al*., (2015) using fungal strain of *Penicillium atramentosum* KM, and Salar *et al*., (2015) using aqueous leaf extract of *Ficus virens* for biosynthesis of AgNPs.

4.4. Fourier Transform Infra-red (FTIR) spectroscopy

Results of FTIR spectroscopy of the present study were in accordance with results of other investigators. As pointed by Galande *et al*., (2011) who found that during AgNPs synthesis, the relative intensity of peaks at 1092, 1164, 1240, 1412, and 2464 cm^{-1} of bacterial cell filtrate were either diminished or disappeared. In addition, Bright *et al*., (2010) previously found that the bands at 1092 and 1164 cm^{-1} were attributable to the vibration of C-O and C-OH stretch, respectively. The bands at 2464 and 1412 cm^{-1} were corresponding to the characteristic C-H and $C-H_2$ symmetric scissoring.

As mentioned earlier, the long term stability of the AgNPs in solution is believed to be an influence of proteins which are secreted from microorganisms (Mandal *et al*., 2001). Also few AgNPs were agglomerated under careful observation, because the AgNPs were surrounded by a faint thin layer of other materials.

There were reports on reductases (Kumar *et al*., 2007) and polysaccharides (Huang and Yang, 2004) as factors involved in biosynthesis and stabilization of the NPs, respectively. In agreement with Saravanana *et al*., (2018), the FTIR analysis provided evidence of protein coat

on the stabilized AgNPs. This implied that proteins of the bacterial extract have stronger affinity to bind with Ag^+ ions thus could act as capping and stabilizing agents, thereby decreasing the aggregation of NPs. Moreover, Shaligram *et al*., (2009) previously confirmed that these protein molecules may also play a significant role in the prevention of agglomeration, and in stabilization of the AgNPs in the aqueous medium by binding on to their surfaces and forming a protein coat.

In an earlier study of Bankar *et al*., (2010), after reaction of Banana peel extract (BPE) with $AgNO_3$ there was a shift in the following peaks: 3411.5 to 3420.8, 2932.6 to 2927.7, 1749 to 1742.9, 1637.6 to 1626, 1386.5 to 1383.3, 1146.5 to 1141.1, 1077 to 1076.3, 829.5 to 824.5 and 642.4 to 651.3 cm^{-1} indicating that carboxyl, hydroxyl and amide groups on the surface of this extract may be participating in the process of NPs synthesis.

Results of the present work indicated the potentials of the four bacterial isolates to synthesize AgNPs biomolecules. AgNPs were stable and produced extracellularly which eliminated the need for their harvesting from the cell enclosure. This method of AgNPs biosynthesis is simple, economic and ecofriendly. These stable and benign bio-produced AgNPs could be used in medical applications more safely than physically and chemically synthesized NPs.

5. Conclusion

Cell free extracts of four different of bacterial isolates were used in the present study to produce AgNPs extracellularly after incubation with $AgNO_3$ for 4h. Physical characterization of the bio-produced AgNPs showed that their sizes ranged from 7.8- 13.4 nm and they were crystalline with spherical shape. This is an ecofriendly method of biosynthesis,

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because the produced AgNPs were safe and benign thus could be used safely in medical applications. Therefore, we could discard using the traditional chemical and physical methods of synthesizing AgNPs as they were expensive, non-safe and non-ecofriendly.

Conflict of interests

The authors declare no conflict of interests regarding this article.

Acknowledgments

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