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Note

Extracellular synthesis of silver nanoparticles by the fungus *Emericella nidulans* EV4 and its application

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Over the last decade, nanotechnology has potentiated remarkable growth, particularly in the field of biomedical sciences including pharmacology, precisely drug delivery and surgery. It has lead to the increased demand for synthesis of nanoparticles. Biological synthesis adopting green chemistry procedures involving microorganisms, fungi and even plants took centre stage. Here, we tried out synthesis of silver nanoparticles using the fungus *Emericella nidulans* EV4 and studied their antibacterial activity against *Pseudomonas aeruginosa* NCIM 5029. Silver nanoparticles were synthesised when the cell free filtrate of the fungus *E. nidulans* EV4 was treated with 1.0 mM silver nitrate solution. The UV-visible spectrum of the silver nanoparticles showed a Surface Plasmon Resonance (SPR) peak at 420 nm. High resolution transmission electron microscopy analysis indicated that the nanoparticles were spherical in shape with a size range of 10-20 nm. X-ray diffraction analysis revealed the formation of face-centred cubic structure of the silver with average crystallite size of ~3.5 nm. The synthesized silver nanoparticles in solution were found to be stable for a period of 12 months without any stabilizing agents. The silver nanoparticles, synthesized as detailed above, demonstrated control over the growth of *Pseudomonas aeruginosa* NCIM 5029.

Keywords: Antibacterial activity, Electron microscopy, Fungi, *Pseudomonas aeruginosa*, X-ray techniques

Nanotechnology is a rapidly advancing field in Science and Technology, particularly in pharmacology and medicine, precisely drug development and surgical applications. Synthesis and assembly of nanoparticles would benefit from the development of clean, nontoxic and environmentally acceptable ‘‘green chemistry’’ procedures, probably involving organisms ranging from bacteria to fungi and even plants[1,2]. Fungi have been known to secrete much high amount of bioactive substances than bacteria, which make fungi more suitable for large-scale production of nanoparticles[3]. Recently, Ballabh and Nara[4] have reported an ecofriendly and clean synthesis of gold nanotubes using *Escherichia coli*, with potential biomedical applications such as drug delivery, diagnostics and therapy. Silver has been known to possess strong biocidal properties both in its metallic and nanoparticle forms; hence, it has found a variety of application in different fields[5]. The extracellular synthesis of silver nanoparticles using the fungi *Aspergillus* sp. EV-4[6], *Aspergillus fumigatus*[7] and *Aspergillus flavus*[8] have already been reported. In the present study, we explored the prospects of synthesizing of silver nanoparticles using the fungus *Emericella nidulans* EV4 and check their antibacterial activity against *Pseudomonas aeruginosa* NCIM 5029.

Methodology

The fungus *Emericella nidulans* EV-4 was isolated from soil samples collected near metal cottage industry, Madurai. The isolated fungus was grown in Potato Dextrose Broth (PDB) and incubated on orbital shaker at 120 rpm and 45°C for 3 days. The biomass was harvested after 72 h by sieving through sterile sieves (~100 micron size). About 5 g of wet biomass was transferred to sterile double distilled water after repeated washing. The flask was agitated under same condition as described earlier. After the incubation period, the cell free filtrate was obtained by passing it through Whatman filter paper no.1 and further used for nanoparticle synthesis. For the extracellular synthesis of silver nanoparticles, 1 mM silver nitrate was mixed with fungal cell free filtrate (1:1 v/v as final concentration) in 250 mL conical flask and agitated at 45°C (120 rpm) in dark condition. Control (without silver nitrate) was also run along with the experimental flasks.

Preliminary characterization of silver nanoparticles was carried out using Systronics Double beam UV-Visible spectrophotometer. Further, the synthesized silver nanoparticles were analysed by EDX and the particle size distribution by Dynamic Light Scattering (DLS) measurement, FTIR spectroscopy and

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Transmission Electron Microscopy. The antibacterial activity of synthesized silver nanoparticles was assessed against *Pseudomonas aeruginosa* NCIM 5029. Approximately, 1×10^8 CFU/mL cells were swabbed uniformly in Muller Hinton Agar (MHA) plates.

Data of antibacterial activity was analysed statistically by analysis of variance (ANOVA) and Tukey's post hoc test at a significance level of 5% using the software IBM SPSS Statistics 20.

Results and Discussion

Due to unique properties, nanoparticles were significantly used in many fields such as biomedical, agriculture, energy, etc. Molecular identification of test fungus EV-4 has been identified as *Emericella nidulans* EV4 (GenBank Accession No. KC684926) and it was used for extracellular synthesis of silver nanoparticles. The cell free filtrate of *E. nidulans* EV-4 when incubated with silver nitrate in dark condition, the colourless aqueous solution gradually changed into dark brown indicating the synthesis of silver nanoparticles in contrast to the control (cell free filtrate alone), which showed no colour change. The change of colour is primarily due to the excitation of surface plasmon vibrations in the silver nanoparticles, a characteristic property of the nanoparticles[9].

The UV-Visible spectra recorded at different time intervals showed increased absorbance with increasing incubation time (Fig. 1). A strong surface plasmon resonance (SPR) peak centered at 420 nm corresponding to the SPR of silver nanoparticles and a peak at 220 nm was also observed. The silver nanoparticles synthesized by *Emericella nidulans* EV4 in solution were stable for more than 12 months with no change in peak position and aggregation. The peak at 220 nm could be attributed to amide bond that might have stabilized the nanoparticles. The secretion of proteomic components by the fungal biomass into the medium play an important role in the reduction of the metal ions in the form of nanoparticles and may also play a major role in the stability. To the best of our knowledge, there are no prior reports on the stability of nanoparticles that extends up to 12 months. In Bhimba *et al*.[10], the silver nanoparticles synthesized using *Gracilaria corticata* extract showed an absorption peak at 424 nm and also it reported a peak at 220 nm which was due to presence of amide bond. The UV spectral peak obtained in this study corroborates with the result obtained with the extract of *Tridax procumbens* with the absorption spectra range at 410-430 nm[11]. Energy Dispersive X-ray (EDX) spectrum exhibited a peak at ~3 keV indicating the presence of silver in the sample.

The X-ray diffraction pattern (Fig. 2) confirmed the presence of pure silver metal with face-centred cubic (fcc) symmetry. X-ray diffraction method is used for determining the mean size of nano crystallites inside the particles[12]. The FTIR spectrum (Fig. 3) showed

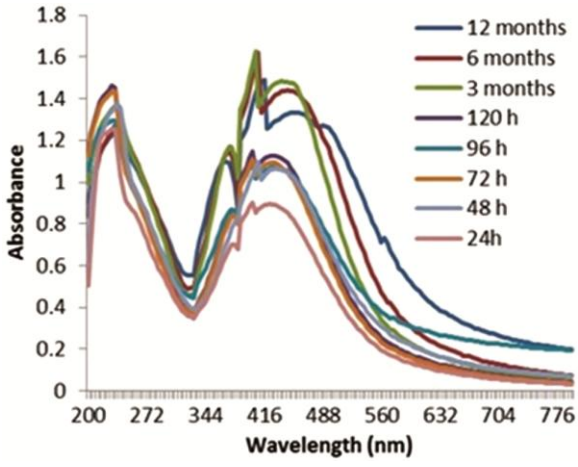

Fig. 1—Time dependent UV-Visible spectra of silver nanoparticles

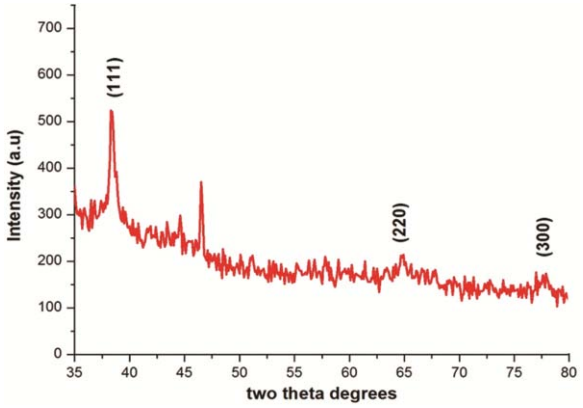

Fig. 2—XRD pattern of silver nanoparticles.

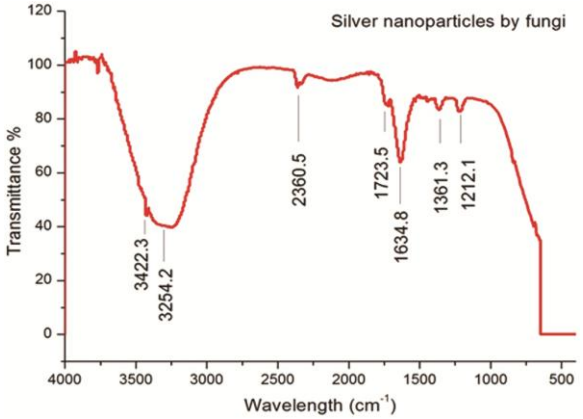

Fig. 3—FTIR spectrum of Silver nanoparticles

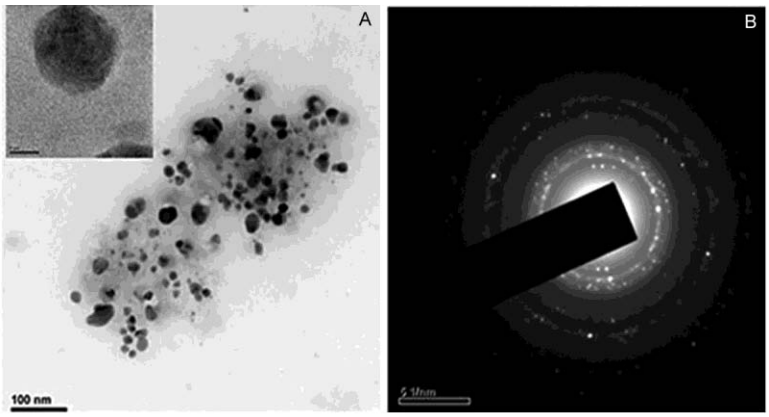

Fig. 4 (A)—HRTEM image; and (B) SAED pattern of silver nanoparticles.

Table 1—Antibacterial effect of silver nanoparticles as tested on *Pseudomonas aeruginosa* NCIM 5029

Nanoparticles/ antibiotic	Zone of Inhibition (mm) at different concentrations			
	25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL
Silver nitrate[a]	7.2±0.58	8.0±2.08	9.0±1.0	10.0±0.26
Silver nanoparticles[b]	19.0±2.21	20.0±1.0	21.0±1.15	23.6±1.05
Amikacin[b]	15.3±0.26	18.1±0.15	20.2±0.34	23.0±0.11

Values are the Mean ± Standard deviation of two measurements. In column with the same letter are not significantly different (ANOVA, $P \leq 0.05$)

prominent peaks corresponded to amide I and amide II regions that are characteristic of proteins and enzymes that are responsible for the reduction of metal ions for the synthesis of metal nanoparticles[13].

Zeta potential measures the potential stability of the particles in the colloidal suspension. The value of zeta potential of the silver nanoparticle is −28.3 mV, signified the presence of electrostatic repulsion among the synthesized nanoparticles, resulting in stability. Our findings justified the earlier results obtained using leaf extract of *Abelmoschus moschatus*[14]. The High Resolution Transmission Electron Microscopy (HRTEM) image (Fig. 4A) and the insert picture showed that majority of nanoparticles were spherical in shape with dispersed silver nanoparticles in the size of 10–20 nm. The Selected Area Electron Diffraction (SAED) pattern (Fig. 4B) confirms the 'fcc' crystalline structure of metallic silver.

The antibacterial activity of silver nanoparticles and silver nitrate was analysed against *Pseudomonas aeruginosa* NCIM 5029 and compared with most potent commercial antibiotic amikacin (Table 1). Silver nanoparticles at 25 µg/mL exhibited higher inhibition zone of 19 mm when compared to $AgNO_3$ (7.2 mm) and amikacin (15.3 mm). The antibacterial activity of silver nitrate was significantly different when compared to silver nanoparticles and amikacin ($P \leq 0.05$). The bacterial cell in contact with silver nanoparticles takes in silver ions, which inhibit respiratory enzymes, facilitating the generation of reactive oxygen species and consequently damaging the cell[15].

Conclusion

The biological synthesis of metal nanoparticles may replace some of the physical and chemical methods in use for nanoparticle production. An extracellular biosynthesis process using *E. nidulans* EV-4 resulted in spherical silver nanoparticles with the average size of 15 nm that are quite stable for more than 6 months, without the addition of any capping or stabilizing agents. The synthesised silver nanoparticles were found to be inhibitory against *P. aeruginosa* NCIM 5029 compared to amikacin and silver nitrate.

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