Summary and Conclusion
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In the present study, fungi from soils and the ones associated with plants, especially the plants used in ethnomedicinal practices in NE region of India, were explored for their potential to synthesise silver nanoparticles. Literature suggests that soil fungi have been reported to be known to reduce silver ion into nanoparticles. However, information on fungi endophytic to ethno-medicinal plants is scarce as far as their biosynthesis of the silver nanoparticles is concerned. Thus, the present study was attempted keeping in mind the soil fungi as well as the endophytic fungi isolated from the medicinal plants, Potentilla fulgens L. and Gloriosa superba L. for their biological ability for extracellular reduction of silver ions to form nanoparticles. A total of eighty morphologically distinct fungal isolates were isolated from the soil samples collected from various microhabitats of the NE region of India. Of these, sixty fungal isolates were from soil and twenty isolates were isolated from the plant parts (root, stem and leaves) of Potentilla fulgens L. and Gloriosa superba L. All eighty fungal isolates were screened by treating the mycelium-free filtrate of the fungal isolates with the silver nitrate solution. Bioreduction of the silver ion to silver nanoparticles were indicated by the visible colour change of the silver nitrate solution. The six soil fungal isolates namely RS1, MP5, SP5, SF1, NH6 and KL1 showed potential for mycosynthesis of silver nanoparticles. Among the endophytic fungal isolates, six isolates namely, PFR6, PFR8, PS4, PFL2, GS1 and GS2 were found to be potential for mycosynthesis of silver nanoparticles.

The promising fungal isolates for the biosynthesis of silver nanoparticles were tentatively identified based on microscopic and macroscopic characteristics. The six soil
fungal isolates namely RS1, MP5, SP5, SF1, NH6 and KL1 showing potential for mycosynthesis of silver nanoparticles were identified as *Cladosporium* sp, *Fusarium* sp, *Aspergillus fumigatus*, *Paecilomyces* sp, *Aspergillus niger*, *Arthrinium* sp respectively. The other six endophytic fungal isolates namely, PFL2, PS4, PFR6, PFR8, GS1 and GS2 were identified as *Aspergillus* sp, *Cryptosporiopsis* sp, *Aspergillus niger*, *Penicillium* sp, *Alternaria* sp and *Penicillium* sp respectively. Fungal isolates were further characterized upto species level as *Cladosporium cladosporioides* RS1, *Fusarium oxysporum* MP5, *Aspergillus fumigatus* SP5, *Paecilomyces lilacinus* SF1, *Aspergillus niger* NH6, *Arthrinium* sp KL1, *Aspergillus tamarii* PFL2, *Cryptosporiopsis ericae* PS4, *Aspergillus niger* PFR6, *Penicillium ochrochloron* PFR8, *Alternaria solani* GS1 and *Penicillium funiculosum* GS2 based on the molecular approaches by using the sequence analysis of the ITS (ITS1, 5.8S and ITS2) gene.

The silver nanoparticles in fungal filtrate were characterized using UV–Vis spectrophotometer based on its characteristic surface plasmon resonance. The UV–Vis spectrophotometer analysis of the filtrates (MP5, NH6, SF1, KL1, SP5, RS1 PFL2, PFR6, PFR8, PS4, GS1 and GS2) treated with AgNO₃ showed a characteristic surface plasmon absorption band at 421, 426, 419, 409, 412, 413, 419, 430, 430, 440, 415, and 403 nm respectively. Apart from this, the absorption peaks at around 208, 207 and 203 nm was assigned to the strong absorption of peptide bonds in filtrate indicating the presence of aromatic acid such as tryptophan and tyrosine residues in the protein. This observation indicated the release of proteins into the filtrate which suggested possible mechanism for the reduction of silver ions present in the solution. The biosynthesized
nanoparticles were found to be extremely stable at room temperature, with no evidence of flocculation of particles as determined by UV-Vis spectroscopy measurements.

Electron microscopy techniques were used to determine the morphology of the synthesized nanoparticles. Based on the scanning electron micrograph, the morphology of all the biosynthesized silver nanoparticles were approximately spherical. Transmission electron micrographs provided further insight into the morphology and particle size distribution profile of the mycosynthesized silver nanoparticles. TEM analysis confirmed the biosynthesis of spherical silver nanoparticles. The nanoparticles synthesized using *Fusarium oxysporum* MP5, *Aspergillus niger* NH6, *Paecilomyces lilacinus* SF1, *Arthrinium* sp KL1, *Aspergillus fumigatus* SP5, *Cladosporium cladosporioides* RS1, *Aspergillus tamarii* PFL2, *Aspergillus niger* PFR6, *Penicillium ochrochloron* PFR8, *Cryptosporiopsis ericae* PS4, *Alternaria solani* GS1 and *Penicillium funiculosum* GS2 were found to be of different size ranges (3.5 ± 3nm to 16.3 ± 6.4 nm). The selected area diffraction pattern (SAED) patterns recorded from single particle in the aggregates of all the nanoparticles samples revealed the crystalline nature of the biosynthesized silver nanoparticles. Energy Dispersive X-ray spectroscopy (EDS) analysis confirmed the presence of elemental silver in the samples. The EDS profiles of the sample solutions showed the presence of characteristic silver signal at approximately 3 keV, which is typical for the absorption of silver nanoparticles due to surface plasmon resonance confirming successful biosynthesis of silver nanoparticles.

To evaluate the antimicrobial efficacy of silver nanoparticles obtained using soil and endophytic fungi namely, *Fusarium oxysporum* MP5, *Aspergillus niger* NH6, *Paecilomyces lilacinus* SF1, *Arthrinium* sp KL1, *Aspergillus fumigatus* SP5,
*Cladosporium cladosporioides* RS1, *Aspergillus tamarii* PFL2, *Aspergillus niger* PFR6, *Penicillium ochrochloron* PFR8, *Cryptosporiopsis ericae* PS4, *Alternaria solani* GS1 and *Penicillium funiculosum* GS2, *in vitro* antimicrobial activity assay was carried out against Gram positive and Gram negative bacteria namely, *Staphylococcus aureus* MTCC96, *Enterococcus faecalis* MTCC2729, *Salmonella enterica* MTCC735 and *Escherichia coli* MTCC730 as well as a pathogenic fungus *Candida albicans* MTCC183. The mycosynthesized silver nanoparticles showed potent antimicrobial activity against both Gram positive and Gram negative pathogenic strains and also against the pathogenic fungal strain. However, the biosynthesized nanoparticles were found to have maximum antimicrobial activity against the fungal strain *Candida albicans* MTCC183 followed by Gram negative bacterial strains *Escherichia coli* MTCC730 and *Salmonella enterica* MTCC735. Silver nanoparticles had a minimal microbiocidal activity on Gram positive bacteria. Among the twelve fungal isolates, the silver nanoparticles synthesized using *Cryptosporiopsis ericae* PS4 with the average particle size 5.5 ± 3.1nm gave the maximum antimicrobial activity against all the tested pathogenic strains viz., *Staphylococcus aureus* MTCC96, *Enterococcus faecalis* MTCC2729, *Salmonella enterica* MTCC735, *Escherichia coli* MTCC730 and *Candida albicans* MTCC183 with inhibition zone diameters of 14mm, 14mm, 15mm, 17mm and 19mm respectively.

The combined antimicrobial activity of biosynthesized silver nanoparticles with a commercially available antimicrobial agent was also studied using the indicator pathogenic bacteria and a fungus namely, *Staphylococcus aureus* MTCC96, *Enterococcus faecalis* MTCC2729, *Salmonella enterica* MTCC735, *Escherichia coli*
MTCC730 and Candida albicans MTCC183. The antibacterial activity of the broad spectrum antibiotic chloramphenicol against the tested bacterial strains increased significantly in combination with silver nanoparticles. The antifungal activity of the antifungal agent, fluconazole was also found to have increased when combined with the biosynthesized silver nanoparticles. The finding revealed that the silver nanoparticles can be considered as excellent broad-spectrum antimicrobial agent and can be potentially used alone or in combination with antibiotics. However, appropriate trials on suitable models need to be carried out to authenticate the findings. Thus, these findings suggest that the biosynthesized silver nanoparticles singly or in combination with conventional antimicrobial agents could be used as excellent broad-spectrum antimicrobial agent.

Further, antimicrobial assays were performed using the silver nanoparticles synthesized by Cryptosporiopsis ericae PS4 since this particular isolate showed maximum antimicrobial activity against the tested pathogenic strains. The MIC and MBC of the biosynthesized silver nanoparticles using Cryptosporiopsis ericae PS4 was also determined to determine its efficacy. Highest MIC and MBC value was observed in case of the Gram positive strains Staphylococcus aureus MTCC96 and Enterococcus faecalis MTCC2729 followed by Gram negative bacterial strains Escherichia coli MTCC730 and Salmonella enterica MTCC735 whereas lowest MIC and MBC value was observed in case of the fungal strain Candida albicans MTCC183.

To assess the effect of silver nanoparticles on the microbial growth, the growth of the tested strains were monitored in liquid media supplemented with different concentrations of nanoparticles. The growth curves of bacterial cells as well as the yeast
cells treated with silver nanoparticles indicated inhibition of the growth and reproduction of microbial cells by silver nanoparticles. When treated with increasing concentration of silver nanoparticles, the growth patterns of bacterial culture as well as the fungal culture showed delay in the exponential phase leading to complete arrest of growth when compared to untreated samples. The mycosynthesized silver nanoparticles were found to have lesser effect on the growth of Gram-positive bacteria as compared to Gram negative bacteria. The fungal strain *Candida abicans* MTCC183 was significantly inhibited at much lower concentration of silver nanoparticles as compared to the bacterial strains.

SEM analysis revealed the structural changes and major damages in the morphology of cells treated with silver nanoparticles. The damage of the cell membrane was much intense in case of the fungal strain *Candida albicans* MTCC183 and the Gram positive bacterial strains *Staphylococcus aureus* MTCC96 as well a *Enterococcus faecalis* MTCC2729 as compared to the Gram negative bacterial strains *Salmonella enterica* MTCC735 and *Escherichia coli* MTCC730. The morphological damage observed for all the tested microorganisms suggest that the cell damage led to cell lysis and ultimately cell death.

The findings of the present study leads to the conclusion that the soil fungi (*Cladosporium cladosporioides* RS1, *Fusarium oxysporum* MP5, *Aspergillus fumigatus* SP5, *Paecilomyces lilacinus* SF1, *Aspergillus niger* NH6 and *Arthrinium* sp KL1) as well as endophytic fungi (*Aspergillus tamarii* PFL2, *Cryptosporiopsis ericae* PS4, *Aspergillus niger* PFR6, *Penicillium ochrochloron* PFR8, *Alternaria solani* GS1 and *Penicillium funiculosum* GS2) were found to be potential candidates for biosynthesis of
silver nanoparticles. All the twelve isolates having the capacity to biosynthesize nanoparticles produced the silver nanoparticles which were found to be excellent antimicrobial agent against a broad range of microorganisms such as gram positive, gram negative bacteria as well as the fungal pathogen. The silver nanoparticles synthesized using the endophytic fungus Cryptosporiopsis ericae PS4 with the average size 5.5 ± 3.1nm exhibited best antimicrobial efficacy as compared to the rest of the biosynthesized silver nanoparticles. Thus, the silver nanoparticles synthesized using endophytic fungus Cryptosporiopsis ericae PS4 isolated from the medicinal plant Potentilla fulgens L. offers promising scope as microbial cell factories for their eco-friendly and sustainable production with potent bioprospection scope for medical and healthcare applications.