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Sporotrichum thermophile culture extract-mediated greener synthesis of silver nanoparticles: Eco-friendly functional group transformation and anti-bacterial study

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ABSTRACT

Thermophilic mould *Sporotrichum thermophile* BJTLRMDU7 played the role in greener synthesis of the silver nanoparticles (AgNPs) extracellularly using silver nitrate. The change in colour from transparent to dark brown primarily indicated the formation of AgNPs due to reduction of Ag (I) ions to Ag by the fungal culture extract. Nanoparticles synthetic process was optimized using "one variable at a time" approach. Sucrose containing medium having pH 8.0 supported the synthesis of AgNPs by the mould at 45 °C. Furthermore, presence of light significantly accelerated the formation of silver nanoparticles. AgNPs were characterized by various techniques like UV–vis & FT-IR spectroscopy, dynamic light scattering (DLS) and X-ray diffraction (XRD). Appearance of a band at 426 nm in UV–vis spectrum revealed the reduction of Ag(I) ions to Ag (0) by mould's culture filtrate. DLS data showed that AgNPs bear an average size of 70 nm, while XRD data revealed particle size of 40 nm. The application of AgNPs with their functional group transformation has successfully reduced *p*-nitrophenol into *p*-aminophenol in an eco-friendly manner in the presence of light and $NaBH_4$. Further, the synthesized AgNPs showed anti-bacterial potential against Gram positive and Gram negative bacteria.

1. Introduction

Nanotechnology is the field of science which deals a variety of nanostructures useful in the health industry, electronics, manufacturing, environment, agriculture and different biomedical industries. Nanoparticles (NPs) are nano scale particles ranging between 1 and 100 nm in size and or smaller in dimension. Metal nanoparticles like silver, gold, iron etc. are very fine and strong particles having potential in various fields like the medical sector, drug delivery, biolabeling, nanocomposites, antimicrobial agents, intercalating materials for electrical items in addition to act as catalysts in chemical reactions. Globally, biological synthesis is preferred over chemical and physical methods, which are capital intensive and toxic. Biological methods are easy, non-hazardous, cost-effective and biocompatible and thus encouraged the society to explore the use of non-pathogenic biological sources for green nano-biotechnological applications [1]. This green synthesis of $AgNO_3$ methodology depends of biological as well as physical parameter such as solvent, medium, temperature, light, pressure and pH condition [2].

It is reported in literature that microorganisms including bacteria [3], fungi [4,6], actinomycetes [4], and even higher plants leaves [5] have been used for the synthesis of nanoparticles. Among microorganisms, filamentous fungi has been considered as most suitable for the synthesis of NPs as it secretes higher amount of enzymes, which are more easier to handle and grow on simple or complex media. Several reports are available on the biosynthesis of silver NPs (AgNPs) using filamentous fungi like *Phanerochaete chrysosporium* [6], *Aspergillus* sp. [7], *Aspergillus flavus* [8], *Fusarium oxysporum* [9], *Fusarium graminearum* [10], *Penicillium polonicum* [11], *Penicillium aculeatum* [4] and other filamentous

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Table 1
Compositions of different media used for the cultivation of thermophilic mould.

Medium	Component	Composition (g/L)
Yeast extract potato soluble starch (YpSs)	Starch	15
	Yeast extract	4.0
	K_2HPO_4	1.0
	$MgSO_4.7H_2O$	0.5
Potato dextrose broth (PDB)	Potato	200
	Dextrose	20
Glucose medium	Glucose	15
	Ammonium sulphate	4.0
	K_2HPO_4	1.0
	$MgSO_4.7H_2O$	0.5
Sucrose medium	Sucrose	15
	Yeast extract	4.0
	K_2HPO_4	1.0
	$MgSO_4.7H_2O$	0.5

fungi [12]. It has been reported that the presence of biomolecules like enzymes/proteins, polysaccharides, vitamins, and amino acids in the culture filtrate of mould is majorly responsible for the reduction of Ag^+ to Ag^0 [13–15]. Among filamentous fungi, there are more reports on mesophilic fungi but use of theromphilic moulds is less explored as compared to their mesophilic counterparts. Researchers reported, first time, the biosynthesis of AgNPs by the thermophilic mould *Humicola* sp. [16]. They also reported the thermophilic *Humicola* sp. which synthesized the extracellular gadolinium oxide nanoparticles which were bioconjugated with taxol for the treatment of cancer [17]. Nowadays AgNPs are used to enhance the catalytic efficiency in oxidation and reduction process and the conversion of nitroaromatics has great delight because amino group of compound used for the synthesis of dyes, herbicide, pharmaceuticals and other useful compounds. Singh and co-worker used the nitrophenol which act as water pollutant with high toxicity and it AgNPs of tulsi extract were convert the 4-NP to 4-AP [18]. This is the first report on the extracellular biosynthesis of silver nanoparticles using a thermophilic mould *Sporotrichum thermophile*. The present investigation first time reports the biosynthesis of silver nanoparticles by using a thermophilic mould *S. thermophile* and their characterization. Further, antibacterial and catalytic potential of nanoparticles has also been studied.

2. Materials and methods

2.1. Thermophilic mould and culture conditions

Sporotrichum thermophile BJTLRMDU7 was inoculated on yeast extract potato soluble starch (YpSs) agar medium at 45 °C in a BOD incubator and stored on the petriplate at 4 °C and also at −20 °C in glycerol. Conidial suspension was prepared by growing on YpSs agar at 45 °C for 4 days. Normal saline containing 0.1% Tween 80 was used for collecting spores and counted using a haemocytometer. YpSs broth was used for the growth after inoculating with 1 mL spore suspension having 2.4×10^7 CFU/mL.

2.2. Production medium and silver nanoparticle synthesis

The mould was grown in Erlenmeyer flasks containing 50 ml YpSs broth (pH 5.5) and kept in BOD incubator under shaking condition at 45 °C for 3 days. Fungal biomass was filtered by Whatman No.1 filter paper discs and the obtained cell-free culture filtrate was further clarified using centrifugation at 10,000 rpm and 4 °C for 10 min. Then, culture filtrate was used to synthesize AgNPs by mixing 0.5 ml culture filtrate with 20 ml of 1 mM silver nitrate solution followed by incubation at room temperature (30 °C) for 4 h in the presence of white light. The resultant brown colour sample was scanned in the range of 300–600 nm by a UV–vis spectrophotometer.

2.3. Optimization of synthetic process for silver nanoparticles

Different physicochemical conditions were optimized by 'one variable at a time' approach for the synthesis of nanoparticles. Fungal culture was grown in the four different media to select a suitable medium (Table 1). Effects of different pH (5.0–8.0) and temperature (35–50 °C) were studied on the growth of the fungal culture used for synthesis of AgNPs. Furthermore, effects of light and dark conditions, different reaction times (2–8 h), different amounts of culture filtrates (25–200 µl) and different concentrations of silver nitrate (0.3–2 mM) have also been studied. A control condition containing culture filtrate and $AgNO_3$ solution was also monitored simultaneously with each set of the experiments.

2.4. Characterization of silver nanoparticles

Characterization of AgNPs was carried out on the basis of FT-IR, UV–vis spectroscopic and X-ray diffraction techniques. The synthesized AgNPs were checked with the help of double beam UV–vis spectrophotometer (Shimadzu-UV 1800) by recording the absorbance spectrum in the wavelength range of 300–600 nm. Control samples of 1 mM $AgNO_3$ solution and culture filtrate were also scanned in the same range.

Further, thoroughly washed silver NPs were scanned in the range of 375–4000 cm^{-1} on FT-IR spectrophotometer (Bruker, Germany). A high beam of X-ray diffractometer (XRD, Rigaku MiniFlex 600) was also used to confirm the crystalline nature of AgNPs. The size distribution analysis of biosynthesized silver nanoparticles was carried out using DLS (Spectroscatter RiNA, FmbH class 3B) at 25 °C for 10 cycles. The dynamic size measurement was completed in a disposable sizing cuvette and the mean of three sizes was taken.

2.5. Anti-bacterial activity of silver nanoparticles

The myco-synthesized AgNPs using *S. thermophile* BJTLRMDU7 culture extract were screened for their anti-bacterial potential against Gram-positive (*Bacillus subtilis*, *Micrococcus luteus*) and Gram-negative (*Escherichia coli* DH5α) bacterial strains. Anti-bacterial activity study was done by the agar well diffusion assay method. The bacterial cultures were mixed in 0.8% molten nutrient agar and poured on simple nutrient agar petriplates. After solidification, agar wells of 8 mm were made with a cork borer and different concentrations of (25–200 µl) of silver NPs were added in the wells. The zone of inhibition was recorded after incubation at 30 °C for 24 h.

2.6. Catalytic activity of AgNPs

The catalytic potential of the myco-synthesized AgNPs was assessed for the reduction of *p*-nitrophenol to *p*-aminophenol. Briefly, 150 µL of *p*-nitrophenol (2 mM) was mixed with 1 mL freshly prepared $NaBH_4$ solution (30 mM). To reach the final reaction volume, 50 µL mycosynthesized silver NPs were added to a 3 mL reaction mixture to initiate catalytic reduction process at room temperature. The catalytic reduction of *p*-nitrophenol was monitored by UV–vis spectrophotometer (Shimadzu-UV 1800) and the decrease in an absorption peak at 405 nm and increase in peak at 301 nm were monitored at different time intervals (0–30 min). Control reaction was performed under the identical conditions without AgNPs.

3. Results and discussion

3.1. Mycosynthesis of silver nanoparticles

Sporotrichum thermophile BJTLRMDU7 has been reported for the synthesis of various hydrolases and biomolecules of industrial importance [17,18]. This mould has not been explored for the synthesis of silver nanoparticles. Therefore, we have reported first time the ability of

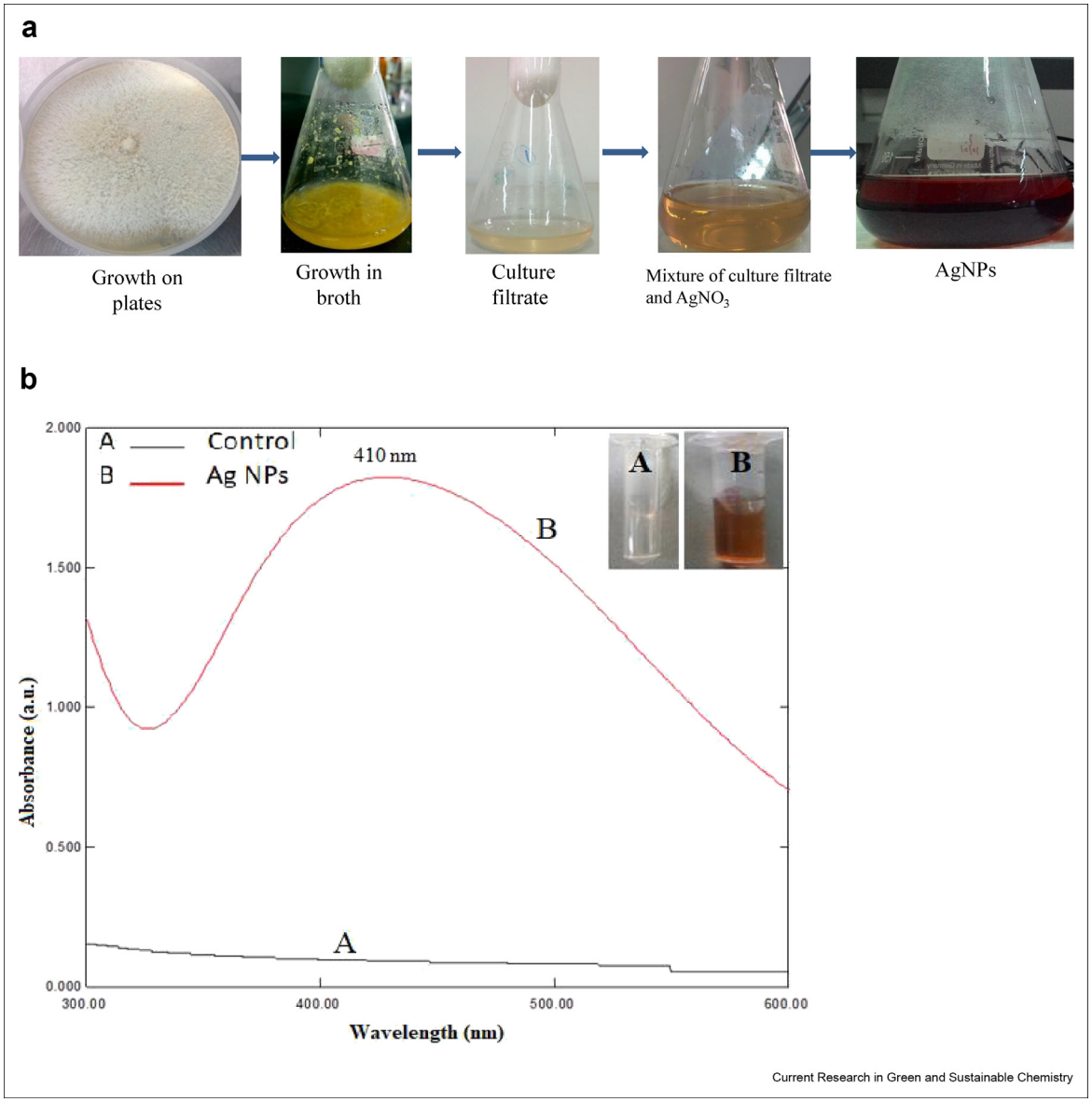

Fig. 1. (a). General steps of extracellular silver NPs synthesis by *S. thermophile* BJTLRMDU7; (b). UV–visible spectra of mycosynthesized SNPs colloid. Inset figure: colour change of filtrate from colourless (control) (A) to brown (treated) after synthesis of SNPs (B).

this mould to synthesize AgNPs extracellularly. There are various methods for the synthesis of AgNPs, but the biological methods have been preferred due to their easy accessibility, economical and eco-friendly nature [19]. Culture filtrate of the mould resulted in the reduction of silver nitrate into AgNPs confirmed due to the difference in colour from light orange to dark brown. The presence of biomolecules like enzymes/proteins, polysaccharides, vitamins, and amino acids in the culture filtrate of mould are majorly responsible for the conversion of Ag^{+} to Ag^{0} [12–14]. In order to synthesize the silver nanoparticles, an aqueous solution of silver nitrate was treated with culture filtrate of the thermophilic mould *S. thermophile* at 30 °C temperature in presence of white light. The colour change of the reaction mixture from colorless to brown was the preliminary indication of the formation of silver nanoparticles (Fig. 1 a). The colour of the reaction mixture changed to dark brown with an increase in reaction time. In UV–visible spectrum, appearance of a band at 410 nm (Fig. 1 b) was due to Surface Plasmon Resonance (SPR) of AgNPs further confirmed their formation. The SPR gives the idea about excitation of electrons in the conductive band around the silver particles [12,20,21]. The absorption band appeared at 410 nm is in close agreement to the reported value by other researchers [12–14,21]. However, UV–vis spectroscopic observation of the silver nitrate solution without cell-free extract revealed two bands at 240 and 280 nm. It has also been observed that mycosynthesis of AgNPs was extracellular only as no synthesis was observed with fungal biomass.

FT-IR spectroscopy deals the outcome about the capping and interactions of the protein with Ag^{+} ions which is majorly responsible for the stability of AgNPs. FT-IR analysis of reaction mixture was conducted to provide the information about the functional groups responsible for AgNPs formation. In the FT-IR spectrum, bands at 3293 cm^{-1}, 2118 cm^{-1}, 1900 cm^{-1}, 1636 cm^{-1}, 557 cm^{-1} and 514 cm^{-1} indicated the involvement of different functional groups of amino acids in the synthesis of AgNPs (Fig. 2 a). Appearance of a strong and narrow band at 3330-3270 cm^{-1} due to C–H stretching in the IR spectrum indicated the presence of terminal alkynes and hydrogen bonding. Further, the slightly weak band due to C–C stretching near to 2260-2100 cm^{-1} gives an idea about alkynes. Bands of AgNPs found around 1636 cm^{-1}appeared due to

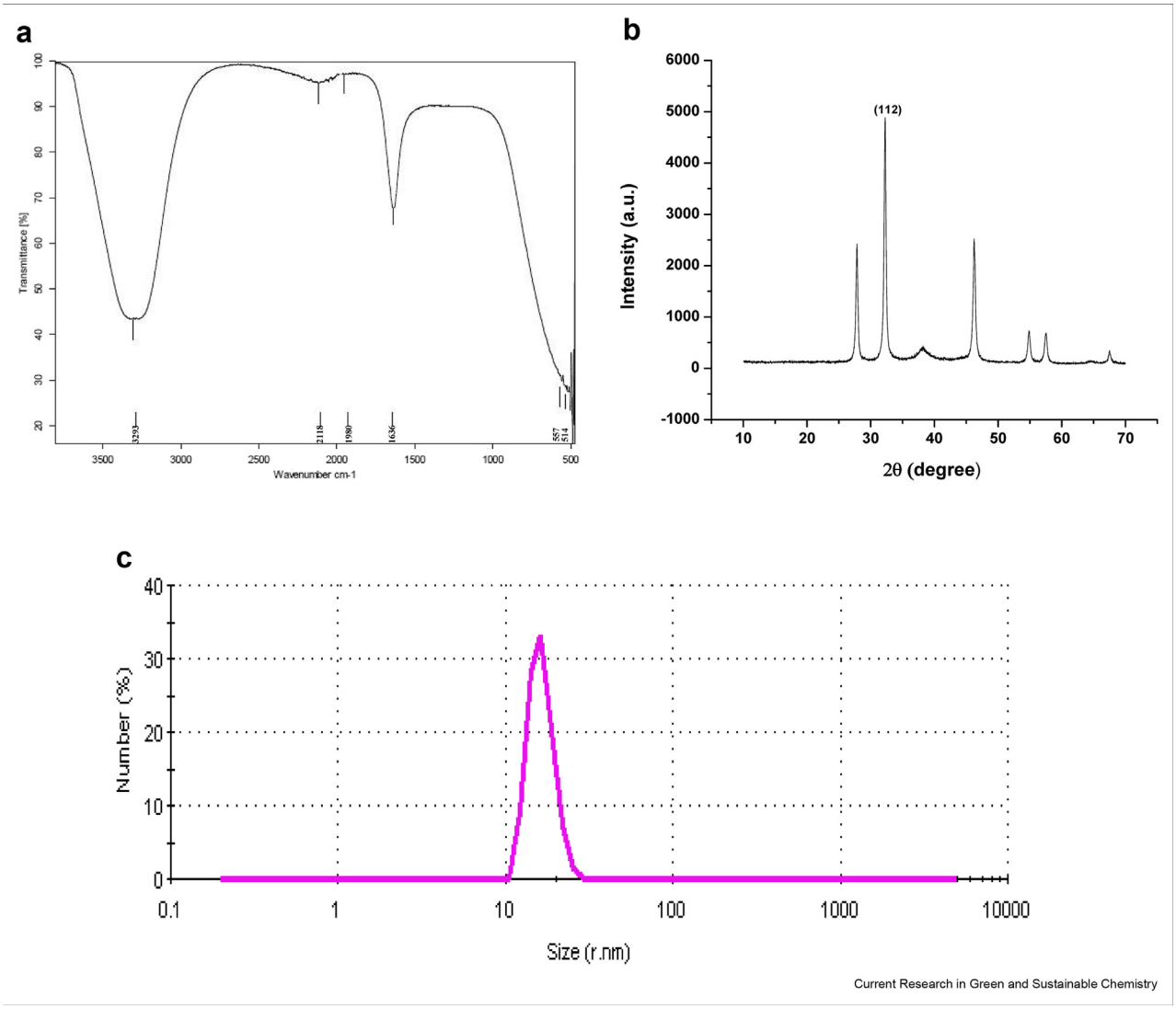

Fig. 2. (a). FT-IR spectra of mycosynthesized AgNPs; (b) X-ray diffraction pattern of mycosynthesized AgNPs from *S. thermophile* BJLRMDU7; (c) DLS graph showing mean average size of silver NPs.

CO stretch of the linkage between amides. The bands found around the 690-515 cm^{-1} are assigned for alkyl halides. The listed observations of bands gave the confirmation of proteins and amino acids in the capping agents of the AgNPs. Thus, the proteins around the $AgNO_3$ act as reducing, capping and stabilizing agents during the synthesis of the AgNPs [14,22] which can be attached to the AgNPs with either the cysteine residues of the proteins or free amino groups. These results are in consistence with the observations made by other researchers on the mycosynthesized AgNPs [23,24].

The X-ray diffraction (XRD) pattern clearly showed the crystalline nature of silver nanoparticles [25]. Total five intense bands were observed and the main band of Ag^o was seen at 112 (Fig. 2 b). The average AgNPs particle size was calculated to be 40 nm by the Debey Scherrer formula:

$$d = k\lambda/\beta\cos\theta$$

where d is the mean crystallite size (nm), k is the shape factor (here k = 0.89), λ is the X-ray wavelength (for a Ag Kα source wavelength is 1.54 Å), θ is the Bragg angle (2θ = 29.8), and β is the full width at the half maxima (0.2 × 3.14/180 radian).

The results are consistent with the similar peaks with earlier studies reporting equivalent diffraction peaks for silver nanoparticles [24–26]. The Dynamic Light Scattering (DLS) revealed the size of AgNPs as 73 nm (Fig. 2c).

3.2. Optimization of process of silver nanoparticles synthesis

Medium composition effects the microbial growth and metabolite production, which are key factors for the biological synthesis of silver nanoparticles [14]. In the present study, Medium A containing sucrose supported the good fungal growth and also promote the extracellular enhanced synthesis of AgNPs (Fig. 3 a). Similar type of study was carried out by Birla et al. [8], they used around ten different medium source in the production of AgNPs from *F. oxysporum*. Zomorodian et al. [6], used glucose as a carbon source for the synthesis of silver nanoparticles from *Aspergillus* species. Saxena et al. [13], also checked on different media like Glucose Yeast Extract Peptone (GYP), Potato dextrose broth (PDB), Richard medium (RM), Sabouraud's dextrose (SB), Czapek Dox (CZA-PEK) and Protease production media (PP) for the better production of silver nanoparticles with the induction of $AgNO_3$. A similar type of study shows the optimum change of silver ions to AgNPs, which might be due to the production of nitrate reductase, which reduces silver nitrate to AgNPs [14].

Medium pH has a significant role in the synthesis and maintenance of microbial metabolites. In our study, the optimum pH for synthesis of

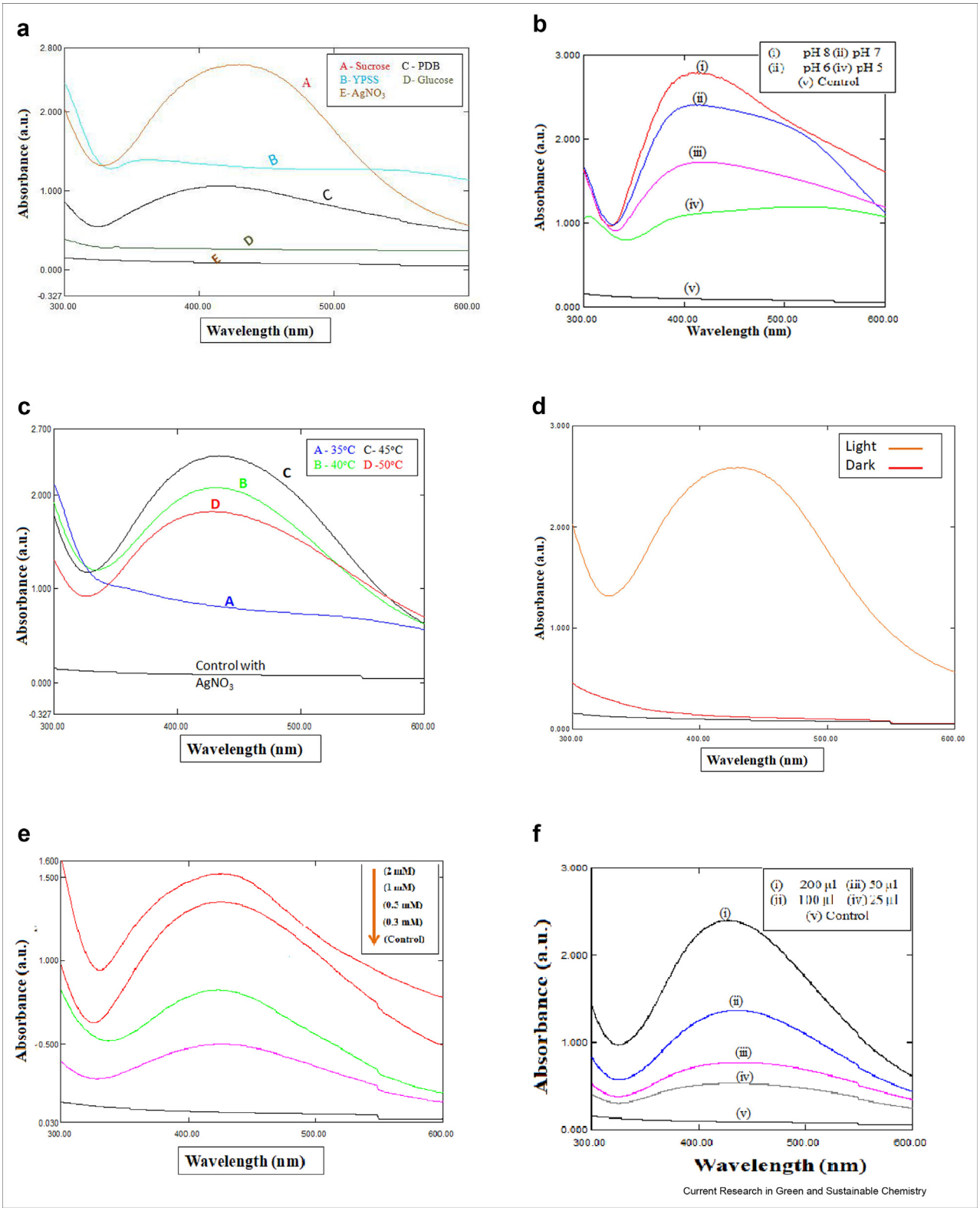

Fig. 3. (a). UV–vis spectroscopy of SNPs synthesized by growing fungus on different culture media— A-sucrose, B– YPSS, C- PDB, D- Glucose and E– Control of $AgNO_3$; (b) Effect of medium pH on the silver NPs synthesis by MCFF of *Sporotrichum thermophile* BJLR MDU7; (c) Effect of temperature on the synthesis of Ag NPs; (d) Effect of light and dark in mycosynthesis of AgNPs; (e) Effect of different concentrations of culture filtrate; (f) Effect of different concentrations of $AgNO_3$ on AgNPs synthesis.

AgNPs was found as pH 8.0 (Fig. 3 b). Similar results have been observed for the mycosynthesis of AgNPs by *Aspergillus* clavatus [27] and *Penicillium* sp. [4]. Omran et al. [28] recorded high NPs synthesis at pH 7.0. The change in pH might be responsible for the denaturation of the enzymes which in turn will decrease the reduction of Ag^+ [29]. It has been reported that increase in pH resulted in more contest between hydrogen ion and metal ions for negatively charged binding sites; therefore, it shows that a higher synthesis of AgNPs under alkaline conditions as compared

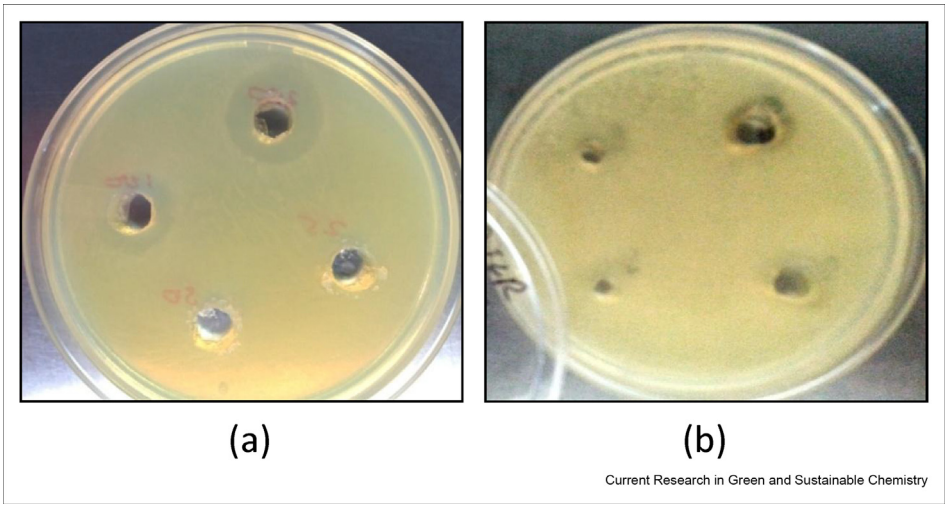

Fig. 4. Anti-bacterial activity of AgNPs against Gram -ve and Gram + ve bacteria.

to acidic pH [30]. Enhanced synthesis of gadolinium oxide nanoparticles by *Humicola* sp. was recorded at pH 8.0 [16].

Like pH, temperature also affects the growth and metabolite synthesis by the filamentous fungi. Mycosynthesis of AgNPs was increased with increase in temperature up to followed by decline afterwards (Fig. 3c). Similarly, Syed et al. [15], reported AgNPs synthesized at 50 °C by a thermophilic mould *Humicola* sp. and same as Khan et al. [16], observed the synthesis of gadolinium oxide nanoparticles by *Humicola* sp. at 50 °C

The concentration of the mycosynthesized AgNPs was high under the light conditions (Fig. 3d). This might be due to light have the greater number of photons, which catalyzed the reducing action [31]. Similarly, presence of light supported enhanced synthesis of AgNPs by *Klebsiella pneumoniae* [32]. Neethu et al. [10], first time reported that the endophytic fungus *P. polonicum* ARA10 synthesize extracellular silver nanoparticles in the presence of light. Also similar type of study was done by Zomorodian et al. [6], that AgNPs formations were confirmed by the presence of laser light. In contrast, Khan et al. [16], reported the synthesis of gadolinium oxide nanoparticles by *Humicola* sp. under dark conditions. At high time of incubation period, fungal culture of *Aspergillus oryzae* synthesizes silver nanoparticles at higher concentration of $AgNO_3$ (>2 mM $AgNO_3$) in dark condition [33].

Different concentrations of $AgNO_3$ are also responsible for the varying synthesis of AgNPs. In this study, AgNPs synthesis rise as similarly increase in the concentration of $AgNO_3$ from 0.3 mM to 2 mM. However, further increase (>2 mM $AgNO_3$), gives negative result and decrease in the production of AgNPs (Fig. 3e). Similarly, synthesis of AgNPs at 2 mM $AgNO_3$ resulted in sharper peak at 426 nm, which showing the tiny size of the mycosynthesized AgNPs [34]. Phanjom and Ahmed [33] reproduced that fungal culture of *A. oryzae* synthesizes AgNPs at different concentration of 1–10 mM $AgNO_3$ for 12 h incubation periods. Similar type of experiment was checked by Omran et al. [28] on different concentration of $AgNO_3$ (0.5–5 mM) and optimum concentration was found at 2 mM $AgNO_3$. Khan and Jameel [34] observed that optimal concentration of silver nitrate is important factor affecting the synthesis of AgNPs.

In the better of reaction optima, the amount of culture filtrate is major critical factors for affecting the yield of AgNPs. Our result indicates that the synthesis of AgNPs increases with increase in the volume of culture filtrate from 25 μl to 200 μl (Fig. 3f). This is due to increase in the amount of protein/enzyme required to reduce silver nitrate into silver nanoparticles [8,28]. The higher concentration of enzymes "reducing agents" in the broth medium, synthesize higher concentration of AgNPs [28]. Similar work was done by the Saxena et al. [13], and Omran et al. [18], to checked by different concentration of dry biomass of culture with optimized concentration of $AgNO_3$ and found the synthesis of AgNPs was increased with increasing the amount of dry biomass of culture.

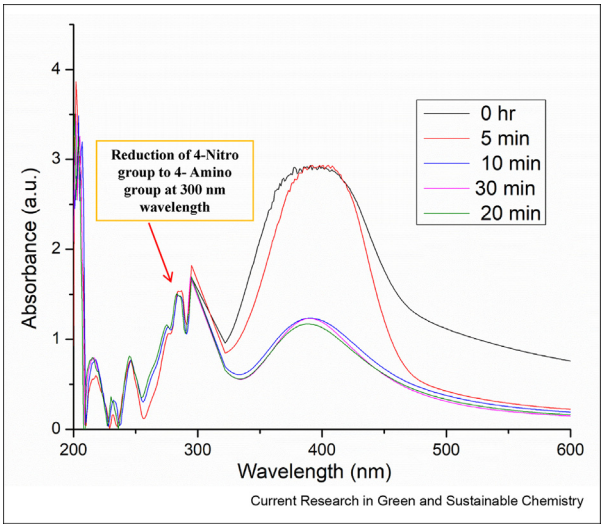

Fig. 5. Catalytic reduction of *p*-nitrophenol into *p*-aminophenol using AgNPs.

3.3. Anti-bacterial activity

Anti-bacterial potential of synthesized NPs was explored by the agar well diffusion assay method. Both Gram-positive and Gram-negative bacterial cultures were inhibited by the added mycosynthesized AgNPs with differential zone of inhibition (Fig. 4 a). It has been found that the effect of inhibition was higher in Gram -ve bacteria (*E. coli* DH5α) than Gram-positive bacteria (*B. subtilis*, *M. luteus*) (Fig. 4b).

The probable reason of the antibacterial activity is associated with interaction of NPs with cell wall of bacterial strains. The cell wall of Gram -ve bacteria are made up of lipopolysaccharides at the exterior. It is not rigid as peptidoglycan because of the covalent linkage between the lipid and polysaccharides. But in the case of Gram + ve bacteria, composed of thick layer of peptidoglycan, due to its thick cell wall silver ions has hard to penetrate, thus silver NPs are more toxic to *E. coli* than *Bacillus subtillis*. Maliszewska and Sadowski [35] reported that inhibition of bacterial growth (*Bacillus cereus, Staphylococcus aureus, E. coli* and *Pseudomonas aeruginosa*) by silver NPs. The silver NPs of *Aspergillus clavatus* has the antimicrobial activity against *Candida albicans*, *Pseudomanas fluorescens* and *E. coli* [27]. Ahluwalia et al. [36], observed that large surface area and strong reactive sites of the AgNPs showed high interaction with microbial cell that resulted in cell rupture with concomitant disturbance in cell function.

3.4. Catalytic activity

In order to explore the catalytic activity of synthesized silver nanoparticles, *p*-nitrophenol (most common pollutant in industrial waste) was treated with $NaBH_4$ in presence of AgNPs. It has been observed that *p*-nitrophenol is converted into *p*-aminophenol successfully. The reaction was monitored by UV–vis spectroscopy. A continuous reduction in peak at 405 nm (due to *p*-nitrophenol) was observed revealing the reduction in the amount of *p*-nitrophenol in the reaction mixture (Fig. 5). However, simultaneously, there was increase in absorbance at 300 nm which showed the reduction of *p*-nitrophenol into *p*-aminophenol. It has also been observed that addition of 50 μl AgNPs at room temperature to the reaction mixture caused a major decreased in the absorbance peak at 405 nm with time, corresponding to the disappearance of the *p*-nitrophenolate ion and in similar time, the absorption peak at 300 nm increased in intensity, due to increasing *p*-aminophenol. Similar observations have been made for catalytic reduction of p-nitrophenol to p-aminophenol by NaBH4 by the reaction of Au and platinum nanoparticles [37,38].

4. Conclusion

An eco-friendly method for the synthesis of silver nanoparticles has been developed by using culture-extract of a thermophilic mould *S. thermophile*. Further, anti-bacterialand catalytic activities of the synthesized AgNPs have been explored. AgNPs successfully reduced *p*-nitrophenol, a major component of environmental pollution, to *p*-aminophenol. In conclusion, silver NPs may be used as therapeutic agents, as food preservatives and in the mitigation of environmental pollution.

CRediT authorship contribution statement

Akshay Shankar: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - original draft, Writing - review & editing. **Vinod Kumar:** Conceptualization, Formal analysis, Software, Writing - review & editing. **Naveen Kumar Kaushik:** Conceptualization, Formal analysis, Writing - review & editing. **Anil Kumar:** Formal analysis, Methodology, Writing - review & editing. **Vinay Malik:** Conceptualization, Methodology, Software, Writing - review & editing. **Davender Singh:** Conceptualization, Data curation, Writing - review & editing. **Bijender Singh:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

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