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ORIGINAL ARTICLE

Extracellular synthesis of silver nanoparticles by bioluminescent bacteria: characterization and evaluation of its antibacterial and antioxidant properties

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Abstract

In this study, the silver nanoparticles (AgNPs) were extracellularly synthesized using a bioluminescent bacterium, *Vibrio campbellii,* and characterized their functional properties and morphological nature by UV–Vis spectroscopy, X-ray diffraction (XRD), Fourier transformed infrared spectroscopy, scanning electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM–EDS), and atomic force microscopy (AFM). Further, the synthesized AgNPs were analyzed for their antibacterial and antioxidant activity (2,2-diphenyl-1-picrylhydrazyl (DPPH), and hydrogen peroxide) in in vitro method. The antibacterial activity of AgNPs was tested against pathogenic bacteria such as *Aeromonas hydrophila* MTCC 1739, *Klebsiella pneumoniae* MTCC 4030, *Klebsiella oxytoca* MTCC 3030, and *Pseudomonas aeruginosa* MTCC 1934. Characterization studies revealed that the synthesized AgNPs were poly-dispersed, spherical shaped with various size ranges, and exhibited as crystalline in nature. The assay of antibacterial activity showed the synthesized AgNPs strongly inhibited the tested pathogenic bacterial growth. Also, the AgNPs showed good antioxidant activity by strong scavenging actions on DPPH (61.88%) and hydrogen peroxide (53.48%) free radicals. Overall results demonstrated that AgNPs could be used in the pharmaceutical field due to their good antibacterial and antioxidant activity.

Keywords Bioluminescent bacteria · AgNPs · In vitro assay · Antibacterial · Antioxidant

Introduction

In recent years, nanotechnology has increased attention due to its attractive and diversified applications in various fields of medicine, environmental control, agriculture, cosmetics, solar cells, food, and the textile industry [1–4]. Presently, the materials of various types at the nanoscale level are produced by nanotechnology, in particular, materials with less than 100 nm of dimensions. Nano-sized materials have attracted a lot of attention in the fields of electronics and biotechnology because of their unique physicochemical and electrical properties. In medicine, metal nanoparticles are being used in the delivery system for drugs, proteins, DNA, and monoclonal antibodies [5]. Although the metal nanoparticles have several therapeutic benefits, some disadvantages have been noted i.e., they can be toxic to both diseased and healthy cells even at low concentrations. Some metal nanoparticles have toxicity at high doses over a long period [6]. However, metal nanoparticles synthesis is important due to its extensive use in various fields. Various methods such

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as physical, chemical, biological, and hybrid systems have been employed in nanotechnology to synthesize nanoparticles. The synthesis of nanoparticles by physical, chemical, and hybrid systems is costly and generates undesirable environmentally hazardous byproducts [7]. Biological-based metal nanoparticles synthesis has several unique advantages over the physical and chemical-based synthesis approach. The nanoparticles synthesized by the biological method are eco-friendly, less cost, and very efficient and also alternatives to physical and chemical methods [8]. Different metal nanoparticles (Au, Ag, Fe, Cu, Zn, Ti, Co, and Ni) are efficiently synthesized by biological methods in recent years. In the biological methods, the various parts of plants and microbes (bacteria, fungi, algae, and actinomycetes), as well as their derivatives, have been successfully employed to synthesize nanoparticles [9]. For instance, the plant parts include extracts of bark, leaves, fruits, stem, root, seed, and flower are used to syntheses of metallic nanoparticles [10]. For microbes, the cells and their derivatives are used for nanoparticle synthesis. In general, the synthesis of metal nanoparticles by microbes is performed extracellularly or intracellularly. Various species of bacteria and their derivatives have been studied for their metal nanoparticle synthesizing capability [11].

Multidrug-resistant bacteria have become a serious public health problem and the treatment of infection caused by those bacteria is very difficult using conventional antibiotics. Widespread overuse and abuse of conventional antibiotics can lead to the emergence of multidrug-resistant bacteria in the environment. Therefore, the development of bioactive metal nanoparticles could be a new strategy to combat multi-drug-resistant bacteria [12]. AgNPs have been experimentally proved as effective bioactive materials against multidrug-resistance bacteria and have been vastly used as antimicrobial, anticancer, and anti-inflammatory agents in medicine [2, 13]. In addition, the AgNPs have potential larvicidal, and nematicidal properties [14, 15]. Bioluminescent bacteria are light-emitting organisms and they are found as free-living organisms or symbionts which diversely exist in both terrestrial and marine environment, surface, and gut of marine animals [16]. They are classified into four major genera such as *Vibrio*, *Photobacterium*, *Alteromonas*, and *Xenorhabdus* [17] and are used in various applications include biosensors for contaminants detection, and pollutant toxicity measurement [18, 19]. Amongst, the *Photobacterium* and *Vibrio* species predominantly exist in the marine environment. Some of the luminescent bacteria cause diseases in the aquaculture industry [20]. Further, the research on bioactive compounds isolation from bioluminescent bacterial species has already shown their promising antibiotic characteristics. Bioactive substances produced by bioluminescent bacteria include polysaccharides, proteins (bacteriocins), enzymes (proteinase, L-asparaginase), and organic acids, which may be responsible for metal ions reduction [21]. To our knowledge, this is the first study to investigate the AgNPs synthesizing ability of bioluminescent bacteria isolated from coastal water samples of Thondi, Palk Strait region, India. The study aimed to investigate the AgNPs synthesizing capability of bioluminescent bacteria, in particular, belongs to *Vibrio* species which were isolated in the earlier study [22, 23]. Further, the structural characterization and investigation of the antibacterial and antioxidant potential of AgNPs synthesized by *Vibrio campbellii* were performed.

Materials and methods

Bacterial strains and screening of AgNPs synthesis ability

In this study, the bioluminescent bacteria, in particular, belongs to *Vibrio* species were screened for their AgNPs synthesizing ability. In total, 8 *Vibrio* species (*Vibrio* sp. GSAU-14, *Vibrio campbellii* GSAU-15, *Vibrio* sp. GSAU-17, *Vibrio owensii*, *Vibrio harveyi*, *Vibrio campbellii*, *Vibrio* sp. GSAU-22, and *Vibrio rotiferianus*) were screened in this study. The *Vibrio* species and their GenBank Accession number are shown in Table 1. For screening, all bacterial strains were inoculated separately in 20 ml of prepared luminescence (LM) medium and incubated on a shaker (200 rpm) at room temperature for 24 h. The LM medium contained 3 g yeast extract; 3 g glycerol; 1 g $CaCO_3$; 3 g trypton; dissolved in aged seawater (1000 ml); pH 7.2 [24]. After 24 h, the cultures were centrifuged (10,000 rpm for 10 min) to collect the supernatant for AgNPs synthesis and the rest of the biomass was discarded. The collected supernatant was mixed with 100 ml of freshly prepared 1 mM $AgNO_3$ (1:4) solution and incubated at room temperature and 200 rpm for 72 h. The synthesis of $AgNO_3$ in the reaction mixture was

Table 1 The strain name with GenBank accession number and AgNPs synthesizing ability of *Vibrio* species used in the study

S. no.	Strain name	Gene bank accession number	AgNPs synthesizing ability
1	*Vibrio* sp. GSAU-14	JQ757166	M
2	*V. campbellii* GSAU-15	JQ801440	M
3	*Vibrio* sp. GSAU-17	JX280417	S
4	*V. owensii*	JX280419	M
5	*V. harveyi*	JX280420	S
6	*V. campbellii*	JX280421	S
7	*Vibrio* sp. GSAU-22	JX280422	M
8	*V. rotiferianus*	JX280423	S

M moderate, *S* strong

checked periodically by monitoring the color changes and spectral analysis in the range of 200–800 nm by UV–Vis spectrophotometer.

Synthesis and characterization of AgNPs

From the screening assay, Four *Vibrio* species such as *Vibrio* sp. GSAU-17, *V. harveyi*, *V. campbellii*, and *V. rotiferianus* showed strong AgNPs synthesizing capability. Amongst, *V. campbellii* were selected for further studies due to their strong AgNPs synthesizing capability within the shorter period of incubation. The AgNPs synthesis by *V. campbellii* was done as the method described above and collected after 48 h. To collect the synthesized AgNPs, the reaction mixture was centrifuged (10,000 rpm for 10 min) and the collected pellet was purified by washing with 50 mM Tris buffer (pH 7.0). The purified pellet was dried (80 °C for 12 h) and powdered for characterization studies [9]. The characterization of powdered AgNPs was characterized using Fourier transformed infrared (FT-IR), X-ray diffraction (XRD), scanning electron microscope coupled with energy dispersive X-ray spectroscopy (SEM–EDS), and atomic force microscopy (AFM) analysis. The functional groups of AgNPs were examined through FT-IR (Thermo scientific Nicolet iS5) in the range between 4000 and 400 cm^{-1}. XRD analysis was performed to determine the crystallinity nature of AgNPs. For this, the spectrum for AgNPs was scanned at 15 kV and 25 mA in the range between 10 and 80º counts (2θ) using PANalytical X'PERT-PRO powder X-ray diffractometer. The AgNPs size and morphology were studied using SEM–EDS analysis (Joel JSM-56010 with INSA-EDS) and surface topography was analyzed by AFM analysis (A100 SGS, A.P.E. Research-Italy).

Antibacterial activity assay

The antibacterial activity of AgNPs was evaluated by the agar well diffusion method against pathogenic bacteria. The pathogenic bacterial strains such as *A. hydrophila* MTCC 1739, *K. oxytoca* MTCC 3030, *K. pneumoniae* MTCC-4030, and *P. aeruginosa* MTCC 1934 were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh, India. Briefly, 12 h grown pathogenic bacterial cultures were swabbed on previously well punched Mueller Hinton agar plates, and then, AgNPs solution at different concentrations (10, 25, 50, and 100 µl) were loaded in each well and incubated at 37 °C for 24 h. After incubation, the zone of inhibition was measured and expressed as mm in diameter. The experiment was conducted in triplicate.

Antioxidant activity assay

The antioxidant activity of AgNPs was determined by evaluating their potential free radical scavenging properties. The scavenging of free radicals by different concentrations (10, 20, 40, 60, 80, 100 µg/ml) of AgNPs solution was investigated using two in vitro assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl) and hydrogen peroxide scavenging assay. The DPPH and hydrogen peroxide scavenging assay was conducted according to the method [25]. For each assay, L-ascorbic acid and double distilled water were used as the reference and blank, respectively. Each assay was conducted in triplicate.

Statistical analysis

All the experiments were done in triplicate. The experimental data were analyzed by calculating mean ± SD and analysis of variance (ANOVA) with Bonferroni test using GraphPad Prism 5.0 for windows.

Results and discussion

Screening of AgNPs synthesizing bacteria

The photograph of AgNPs synthesis by selected eight *Vibrio* species is shown in Fig. S1. Amongst, four strains such as *Vibrio* sp. GSAU-17, *V. harveyi*, *V. campbellii*, and *V. rotiferianus* synthesized the AgNPs effectively in the reaction mixture after 48 h incubation. The rest of the four strains (*Vibrio* sp. GSAU-22, *V. campbellii* GSAU-15, *V. owensii,* and *Vibrio* sp. GSAU-14) did synthesize the AgNPs moderately even after 48 h. Generally, the colour changes of the reaction mixture from yellow into brown colour might be due to Ag^{+} reduction [26]. The UV spectra for the reaction mixture of *Vibrio* sp. GSAU-17, *V. harveyi*, *V. campbellii*, and *V. rotiferianus* are shown in Fig. 1a–d. A strong absorption peak in the UV spectra of *Vibrio* sp. GSAU-17, *V. harveyi*, *V. campbellii*, and *V. rotiferianus* were observed in 400 nm, 420 nm, 430 nm, and 423 nm, respectively. The UV spectral results confirmed the strains such as *Vibrio* sp. GSAU-17, *V. harveyi*, *V. campbellii*, and *V. rotiferianus* were able to synthesize AgNPs extracellularly. Amongst, the strain *V. campbellii* showed a strong AgNPs synthesizing ability within the short period of incubation. In general, the AgNPs characteristics peak can be detected between 400 and 450 nm in the UV–Vis region [27]. Also, the existence of absorption peaks at various positions between 400 and 450 nm in the UV–Vis absorption spectra for AgNPs is generally determined by nanoparticle size. Hong and Li [28] reported that the absorption peak for gold nanoparticles was found to increase between 510 and 550 nm in the UV-spectra

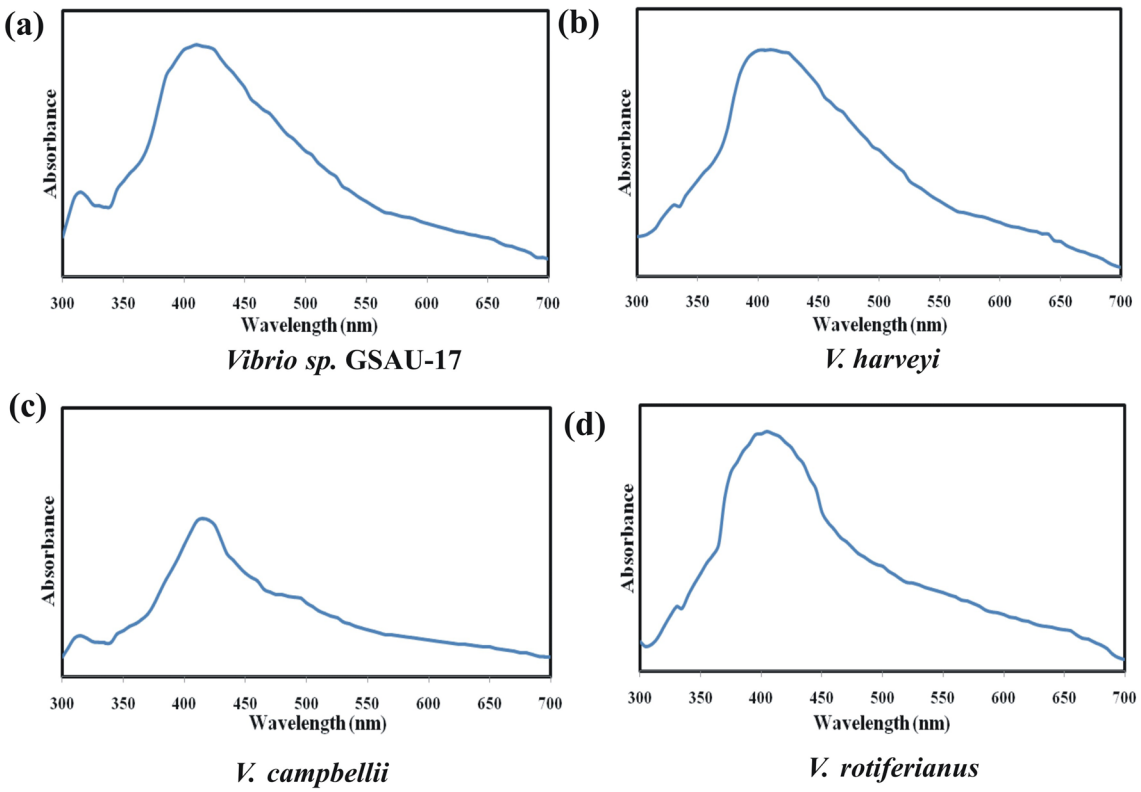

Fig. 1 UV spectra of the AgNPs synthesized by selected *Vibrio* species **a** *Vibrio* sp. GSAU-14, **b** *V. harveyi*, **c** *V. campbellii*, and **d** *V. rotiferianus*

when analyzed various sizes of nanoparticles (17–80 nm in size). When 17 nm, 30 nm, 40 nm, 50 nm, 60 nm, and 80 nm size gold nanoparticles were examined, the absorption peak was observed at 510 nm, 525 nm, 530 nm, 536 nm, 540 nm, and 550 nm in the UV spectra, respectively.

Characterization studies

FTIR analysis

The FTIR spectrum obtained for AgNPs synthesized by *V. campbellii* is illustrated in Fig. 2. The wavenumber of peaks detected in the IR spectra and their corresponding functional groups are shown in Table 2. As shown in Table 2, the functional groups such as O–H, N–H, C–H, C=O, and C–O groups had existed in the IR spectra of AgNPs and these functional groups containing biomolecules (polysaccharides, proteins, and other constituents) might be responsible for Ag^+ reduction [29]. Sayed Ahmed et al. [30] stated that functional groups such as amide, hydroxyl, and carboxylate can be involved in the reduction of Ag^+ to Ag^0. Whereas, the functional groups such as amine and carbonyl groups belong to protein molecules present in the culture supernatant of *Bacillus* sp. possessed silver ions reducing capability [31]. Sing et al.

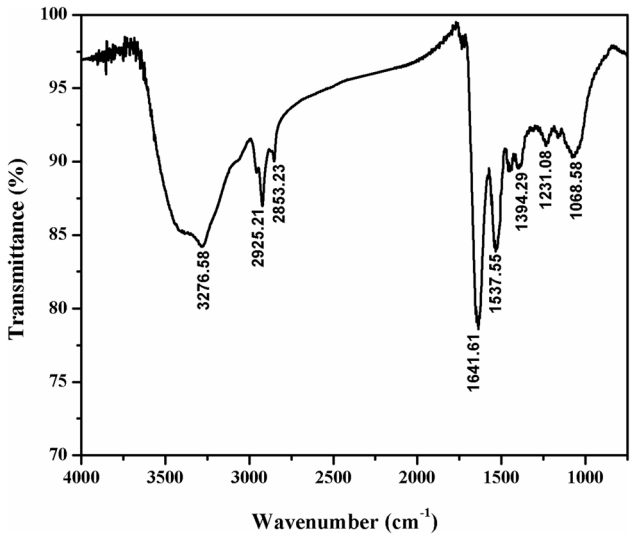

Fig. 2 FT–IR spectrum of AgNPs synthesized by selected *V. campbellii*

[32] stated that hydroxyl groups present in the polysaccharides produced by brown macroalga *Padina gymnospora* took part in the reduction of gold ions. From this, the biomolecules having functional groups such as O–H,

Table 2 Wave numbers (cm^{-1}) of peaks obtained from FTIR spectrum of AgNPs synthesized by *V. campbellii*

Wavenumbers (cm^{-1})	Functional groups	References
3276.58	OH/NH	[33]
2925.21	CH_2	[34]
1641.61	Amide I, C=O	[34]
1537.55	Amide II	[34]
1394.29	C–O	[29]
1068.58	C–O–C	[29]

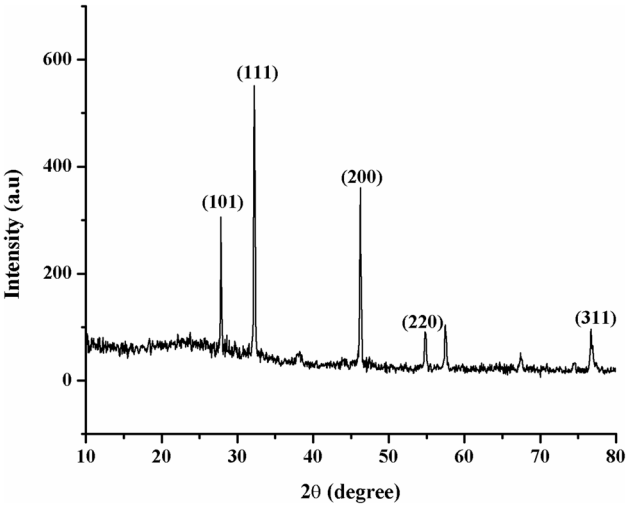

Fig. 3 X-ray diffraction spectrum of AgNPs synthesized by selected *V. campbellii*

N–H, C–H, C=O, and C–O are involved in metal ions reduction and also acted as stabilization agents. Mathivanan et al. [9] stated that the biomolecules present in the reaction mixture might have acted as a capping/stabilization agent for AgNPs.

XRD spectral analysis

XRD spectrum of AgNPs synthesized by *V. campbellii* is shown in Fig. 3. The spectrum showed the diffraction peaks at 2θ values of 27.75°, 32.15°, 46.15°, 54.75°,, and 76.65°, corresponded to plans of (101), (111), (200), (220), and (311), respectively (Fig. 3). Further, the diffraction data of AgNPs were compared with the powder diffraction data files of known compounds (ICDD/JCPDS, PDF Nos. 04-0783 and 84-0713). The XRD spectral analysis confirmed the presence of AgNPs in the sample and revealed the AgNPs were face-centered, cubic, and crystalline in nature [34].

SEM–EDS and AFM analysis

The structure, size, and morphology of the synthesized AgNPs were studied by scanning electron microscope (SEM) are shown in Fig. 4a. SEM analysis showed that the synthesized AgNPs by *V. campbellii* predominantly were polydispersed, spherical in shape, and existed uniformly. The size of AgNPs was varied and observed in the range between 10 and 250 nm. The EDS analysis showed strong peaks at 2–3 keV regions, which confirms the presence of elemental silver. Further, the EDS results revealed the formation of Ag^0 by reduction of Ag^+ through biomolecules present in the culture supernatant (Fig. 4b). The AFM images of AgNPs synthesized *V. campbellii* is shown in Fig. 5a, b. Results showed that the synthesized AgNPs existed as aggregates with various heights and roughness. The roughness and height of the AgNPs are 0.43 nm and 16 nm.

Antibacterial activity of AgNPs

The growth inhibition activity of AgNPs synthesized by *V. campbellii* against pathogenic bacteria such as *A. hydrophila* MTCC 1739, *K. pneumoniae* MTCC 4030, *K. oxytoca* MTCC 3030, and *P. aeruginosa* MTCC 1934 are shown in Table 3 and Supplementary Fig. S2. The agar well diffusion assay results showed that AgNPs strongly inhibited the growth of tested pathogenic bacteria. The maximum inhibition activity of AgNPs against tested pathogenic bacteria was observed at a higher concentration (100 µl). The inhibitory zone of AgNPs (100 µl) observed for tested pathogenic bacteria is as follows; 8 ± 0.1 mm for *A. hydrophila* MTCC 1739; 6.8 ± 0.4 mm for *K. pneumoniae* MTCC-4030; 9.4 ± 0.5 mm for *K. oxytoca* MTCC 3030; 7.2 ± 0.3 mm for *P. aeruginosa* MTCC 1934. Loo et al. [12] reported that the AgNPs synthesized using pu-erh tea leaves extracts inhibited the growth of *K. pneumoniae* (10 mm) in the disk diffusion method. Singh et al. [35] reported that the AgNPs synthesized using culture supernatant of *Pseudomonas* sp. THG-LS1.4 had good growth inhibition against pathogenic bacteria such as *Bacillus cereus*, *Staphylococcus aureus*, *Candida tropicalis*, *Vibrio parahaemolyticus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella enterica.*

Antioxidant activity of AgNPs by *V. campbellii*

The antioxidant activity of AgNPs synthesized by *V. campbellii* was evaluated through their scavenging potential of DPPH and nitric oxide free radicals. Results showed that the scavenging of DPPH free radicals by AgNPs increased with an increasing concentration from 10 to 100 µg/ml. The scavenging free radicals of DPPH by AgNPs of *V. campbellii* was 61.88% at 100 µg/ml concentration (Fig. 6a). Keshari et al. [36] reported that the free

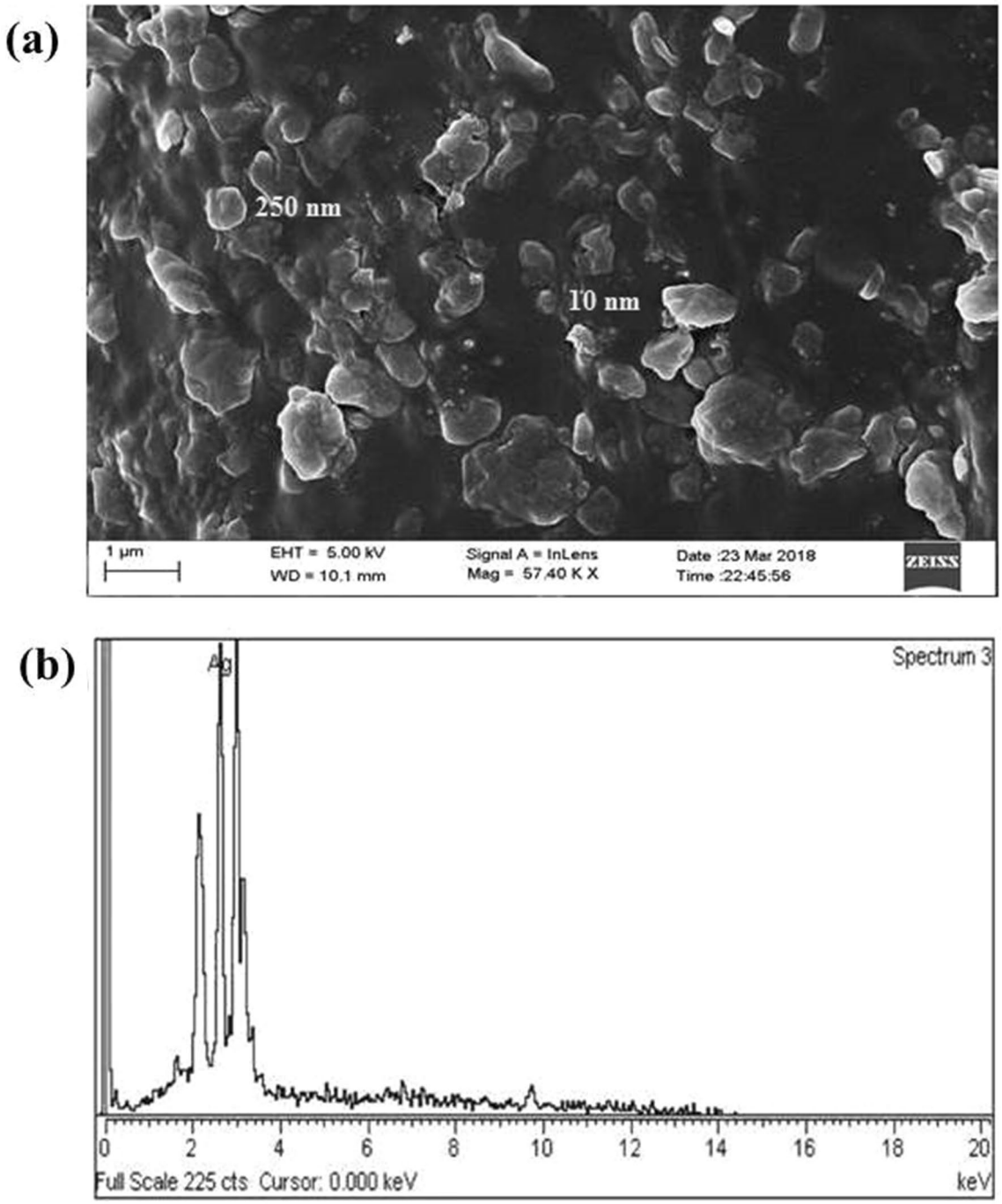

Fig. 4 **a**, **b** Scanning Electron Microscopic images of AgNPs synthesized by *V. campbellii* with two different magnifications and energy dispersive X-ray spectral analysis of AgNPs synthesized by *V. campbellii*

radicals of DPPH were scavenged by AgNPs synthesized by *Cestrum nocturnum* was 29.55% at 100 µg/ml concentration. Saravanakumar et al. [37] stated the scavenging of DPPH free radicals by AgNPs synthesized by *Prunus japonica* (Rosaceae) leaf extract was 55% at 200 µg/ml concentration. A similar trend of DPPH free radicals scavenging activity was reported for AgNPs synthesized by *Streptomyces olivaceus* MSU3 [37] and EPS of *Streptomyces violaceus* MM72 [25].

The hydrogen peroxide scavenging activity of AgNPs increased (%) with increasing the concentration of AgNPs from 10 to 100 µg/ml (Fig. 6b). The maximum scavenging of hydrogen peroxide by AgNPs of *V. campbellii* was observed as 53.48% at 100 µg/ml concentration. Keshari et al. [36] reported

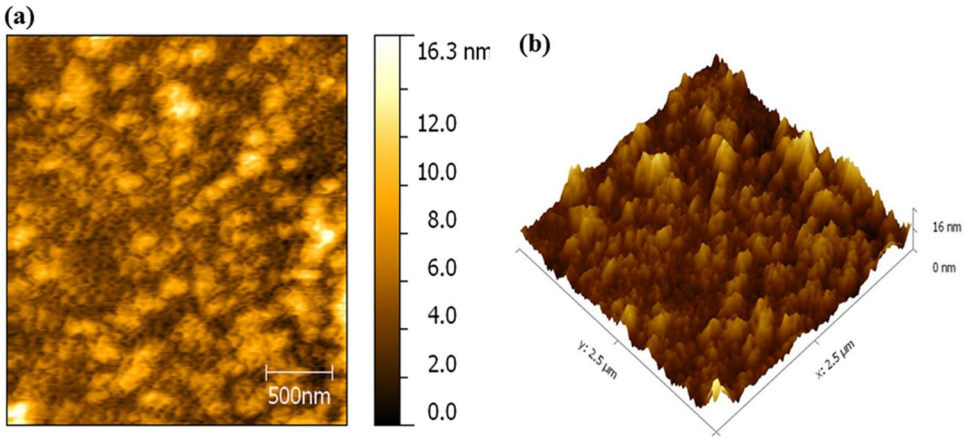

Fig. 5 Atomic force microscopic image of AgNPs synthesized by *V. campbellii* **a** typical, **b** depth

Table 3 Antibacterial activity of AgNPs synthesized by *V. campbellii*

Pathogenic bacteria	Zone of inhibition (mm) ± SD			
	10 µl	25 µl	50 µl	100 µl
Aeromonas hydrophila MTCC-1739	NZ	NZ	6.9 ± 0.4	8 ± 0.1
Klebsiella pneumoniae MTCC-4030	NZ	NZ	5.8 ± 0.2	6.8 ± 0.4
Klebsiella oxytoca MTCC-3030	NZ	4 ± 0.2	8.5 ± 0.5	9.4 ± 0.5
Pseudomonas aeruginosa MTCC-1934	NZ	NZ	5.2 ± 0.3	7.2 ± 0.3

The results are represented as mean ± SD of the three independent data

NZ no zone of inhibition

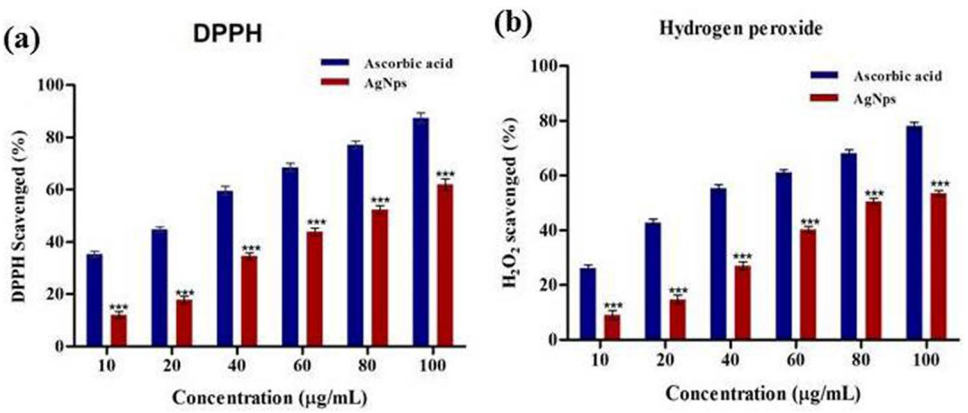

Fig. 6 Antioxidant activity of AgNPs synthesized by *V. campbellii* **a** DPPH, **b** hydrogen peroxide activity. The results are represented as mean ± SD of the three independent data

the AgNPs of *Cestrum nocturnum* scavenged 45.41% of hydrogen peroxide radicals at 100 µg/ml concentration. Sanjivkumar et al. [38] reported that AgNPs synthesized by *Streptomyces olivaceus* MSU3 have strong antioxidant activity. Further, the DPPH and hydrogen peroxide scavenging activity observed for AgNPs of *V. campbellii* was considerably lower than that of the reference standard ascorbic acid.

Conclusions

In the present study, the AgNPs synthesizing capability of bioluminescent bacteria, in particular, belongs to *Vibrio* species were screened. Amongst, four strains such as *Vibrio* sp. GSAU-17, *V. harveyi*, *V. campbellii*, and *V.*

rotiferianus showed strong AgNPs synthesizing capability. The characterization studies revealed that AgNp synthesized by *V. campbellii* were polydispersed, spherical shaped with sizes ranging from 10 to 250 nm. Further, the synthesized AgNPs were crystalline in nature and the bioactive compounds present in the solution were acted as a capping/stabilizing agent. The antibacterial activity assay showed the synthesized AgNPs by *V. campbellii* strongly inhibited the growth of tested pathogenic bacteria and showed a maximum inhibition on *K. oxytoca* MTCC 3030. Also, the AgNPs synthesized by *V. campbellii* effectively scavenged the DPPH (61.88%) and hydrogen peroxide (53.48%) free radicals. Overall experimental results suggested that AgNPs could be used in the pharmaceutical field due to their strong antibacterial and good antioxidant activity. Also, future work will be focused on understanding the inhibitory action of AgNPs on pathogenic bacteria.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40089-021-00360-y.

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Declarations

Ethical approval and consent to participate Not applicable.

Consent for publication Not applicable.

Availability of supporting data Not applicable.

Conflict of interest The authors declare that they have no competing interests.

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