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Evaluation of antibacterial activity of zinc oxide nanoparticles synthesized using phycobilins of *Anabaena variabilis* NTSS17

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ABSTRACT

Objective: To evaluate the antibacterial activity of zinc oxide nanoparticles synthesized using phycobilins of *Anabaena variabilis* NTSS17.

Methods: The cyanobacterial isolate was collected from paddy field and morphologically identified as *Anabaena variabilis* NTSS17, that produces a pigment *i.e.* phycobiliproteins. The biosynthesized zinc nanoparticles were characterized by different spectroscopic and analytical techniques such as UV-visible spectrophotometer, Fourier transform infrared spectroscopy and X-ray diffraction which confirmed the formation of zinc nanoparticles.

Results: Antibacterial activity of zinc oxide nanoparticles was examined against *Escherichia coli*, *Rhodococcus rhodochrous* and *Pseudomonas aeruginosa*. The maximum zone of inhibition occurred at 5 mg/1 000 mL concentration of zinc oxide nanoparticles.

Conclusions: Due to potent antimicrobial and intrinsic properties of zinc oxide, it can be actively used for biomedical applications.

1. Introduction

Now a days, human beings are often infected by microorganisms such as bacteria, molds, yeasts and viruses present in their living environments. Bacterial pathogens like *Escherichia coli* (*E. coli*), *Staphylococcus aureus* and *Pseudomonas aeruginosa* (*P. aeruginosa*) are responsible for diseases like mastitis, endocarditis and upper respiratory complications[1,2]. Enteric bacteria comprised of *Salmonella* sp., *Shigella* sp., *Proteus* sp., *Klebsiella* sp., *E. coli*, *Pseudomonas* sp., *Vibrio cholerae* and *Staphylococcus aureus* are the major etiological agents of sporadic and epidemic diarrhea both in children and adults[3]. The treatment of bacterial infection is increasingly complicated because of the ability of bacteria to develop resistance to antimicrobial agents. Multi-drug resistant organisms are bacteria resistant to the current antibiotic therapy and therefore difficult to treat. They result in serious local and systemic infections that can be seriously debilitating and even life threatening. The increasing antibiotic resistance is a major threat to our society. The development of new antibiotics and drug targets encourages the scientific community to look for other alternatives for antibiotic production.

Recent research revealed that nanoparticles have received prominence like antimicrobial, antioxidant, larvicidal, insecticidal and anticancer activities[4-10]. The recovery of the precious metals by microbial sources such as cyanobacteria, actinobacteria, bacteria, fungi with the formation of their nanoparticles is an eco-friendly alternative to the conventional methods[11]. To date, many researchers have reported the eco-friendly, reliable, energy-efficient and non-toxic methods for the synthesis of useful nanoparticles employing microbes[12-16]. A number of physical and chemical methods have been employed for the synthesis of zinc oxide nanoparticles (ZnO NPs) including the use of toxic chemicals[17,18]. It is felt that extensive usage of these metal oxide nanoparticles may lead to their potential introduction into the environment which could adversely affect human health. Their possible toxicity could induce cell membrane leakage, warranting the need to develop alternative methods of environmentally benign synthesis protocols[19]. Presently, much research emphasis is being laid upon the antimicrobial properties of ZnO NPs and their exploitation as antimicrobial agents for textile finishing against a wide spectrum of bacteria, fungi and yeast[20-22].

Our research group Mubarak *et al*.[23] has previously reported that fluorescent property of protein in phycobilins is solely due to tyrosine, phenylalanine, and tryptophan moieties and would be responsible for the very efficient formation of cadmium sulfide

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nanoparticles and may also be involved in preventing agglomeration. Cyanobacteria (blue-green algae), one of the largest and most primitive ancestral groups of photoautotrophic bacteria are a rich source of pigments/proteins. Like red algae and cryptomonads, blue-green algae also contain phycobiliproteins. They are large water soluble supra molecular protein aggregates involved in light harvesting in these organisms and may comprise as much as 40%–60% of the total soluble protein in these cells[24]. They are highly diverse in their structure and pigment composition in cyanobacteria, red algae and cryptomonads[25,26].

Literature evidenced that synthesis of silver nanoparticles has been achieved by using the whole cells of non-nitrogen-fixing cyanobacteria, namely, *Plectonema boryanum* and the marine *Oscillatoria willei*[27,28]. Jayaseelan *et al*.[29] reported the synthesis of ZnO NPs using the bacterium *Aeromonas hydrophila*. In the present study, the evaluation of antibacterial activity of ZnO NPs synthesized using phycobilins of *Anabaena variabilis* NTSS17 (*A. variabilis*) has been studied.

2. Materials and methods

2.1. Chemicals

Zinc nitrate was purchased from Sigma–Aldrich. The media for bacterial growth and antimicrobial activity, Muller-Hinton agar, nutrient broth and nutrient agar were supplied by HiMedia, India.

2.2. Bacterial pathogens used in this study

Bacterial isolates of *E. coli*, *Rhodococcus rhodochrous* (*R. rhodochrous*) and *P. aeruginosa* (purchased from MTCC, Mumbai, India) were used in this study. These isolates were cultured in nutrient broth at 37 ˚C for 24 h.

2.3. Samples

The cyanobacteria samples were collected from paddy field in Suraiyur Village, Tiruchirappalli, Tamil Nadu, India.

2.4. Isolation, identification and growth condition of cyanobacteria

The collected samples were enriched initially in BG11 broth in erlenmeyer flask at (24 ± 2) ˚C and illuminated with cool white Sylvania 40 W T12 fluorescent lamps at an intensity of (14.4 ± 1.0) W/m^2 for a 16/8 h light/dark cycle. The culture broth was shaken manually for five to six times a day. The purity of the culture was monitored by regular observation under microscope. The isolated cyanobacteria were identified microscopically using light and confocal microscope with help of standard manual.

2.5. Mass cultivation

A. variabilis was mass cultured in 20 L tank at (24 ± 2) ˚C and illuminated with cool white Sylvania 40W T12 fluorescent lamps at an intensity of (14.4 ± 1.0) W/m^2 for a 16/8 h light/dark cycle for further experiments.

2.6. Harvesting the biomass of A. variabilis

The *A. variabilis* was harvested in their stationary growth phase by centrifugation at 4000 r/min for 15 min. The harvested biomass was used for phycobilin protein extraction for nanoparticle synthesis.

2.7. Extraction and estimation of phycobilins from A. variabilis

A total of 100 mL of homogenized log phase *A. variabilis* was centrifuged at 8000 r/min to obtain pellet. *A. variabilis* was suspended in sterile water and sonicated for 2 min at maximum output and duty cycles. The resulting extract was centrifuged at 8000 r/min for 30 min and filtered through Whatman No. 1 filter paper to remove cell debris. The amount of phycobili proteins was measured as described by Bennett and Bogorad[30].

2.8. Characterization of phycobilins of A. variabilis using UV-visible spectral analysis

One milliliter sample was withdrawn at different time intervals and surface plasmon resonance of phycobilins was characterized using a UV–vis spectrophotometer (Cary 60, Agilent Technologies, USA) at the resolution of from 200 to 800 nm.

2.9. Extracellular biosynthesis of ZnO NPs using phycobilins of A. variabilis

Ten grams of *Anabaena*sp. NTSS17 was suspended in 100 mL of sterile water and sonicated for 2 min at maximum output and duty cycles. The resulting extract was centrifuged at 8000 r/min for 30 min and filtered through Whatman No. 1 filter paper to remove cell debris. 50 mL *A. variabilis* cell free extracts of phycobilins were taken in a separate flask and zinc nitrate solution (1 mmol/L final concentration) was added for testing the synthesis of nanoparticles. Together with this, the cell free extracts without zinc nitrate and zinc nitrate solution alone were taken separately in two flasks to serve as positive and negative controls, respectively. The flasks were incubated in the dark at 25 ˚C in a shaker (150 r/min) for 3 days. All the experiments were performed in triplicate.

2.10. Characterization of biosynthesized ZnO NPs using phycobilins of A. variabilis

Biosynthesis of ZnO NPs using phycobilins of *A. variabilis* was visibly observed by change in the color of the medium and the aliquots were subjected to UV-visible spectroscopy to measure the peak, then the particles were purified by density gradient centrifugation method. The chemical composition of zinc nanoparticles were analyzed by Fourier transmission infrared (FTIR) spectroscopy. The structure was studied by X-ray diffraction (XRD) technique. Releases of zinc ions in the solution were also measured.

2.10.1. UV-visible spectral analysis

Sample of 1 mL was withdrawn at different time intervals and surface plasmon resonance of zinc nanoparticles was characterized using a UV-vis spectrophotometer (CARY 60, Agilent Technology, USA) at the resolution of from 200 to 500 nm.

2.10.2. FTIR spectroscopy measurements

The residual solution after reaction was centrifuged at 10000 r/min for 15 min and the resulting suspension was repeated for three times, after that the purified suspension was washed with deionized water to get pure form *i.e.*, free of proteins/enzymes which are not able to capping the zinc nanoparticles. The sample was completely dried at 60 ˚C.

Finally the dried nanoparticles were analyzed by FTIR (Perkin Elmer, USA).

2.10.3. XRD analysis

The XRD technique was used to analyze the metallic nature of particles. After bioreduction, zinc nanoparticles solution thus obtained was purified by repeated centrifugation at 5 000 r/min for 20 min followed by redispersion of the pellet of zinc nanoparticles into 10 mL of sterile deionized water. After freeze drying of the purified zinc particles, the structure and composition were analyzed by XRD.

2.11. Antibacterial activity of zinc nanoparticles

Antibacterial activity was carried out using the well diffusion method (Kirby Beyer method). The Petri plates were prepared with 20 mL of sterile Mueller Hinton agar (HiMedia) and the bacterial cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. Four wells were made using a sterile cork borer. Well diffusion method was used to observe the dose dependent antibacterial activity of ZnO NPs on bacterial species. Bacterial inoculums were prepared by growing a single colony overnight in nutrient broth and adjusting the turbidity to 0.5 McFarland standards. And 50 μL of biosynthesized ZnO NPs from *A. variabilis* were used to study the antibacterial activity against Gram-negative bacteria *E. coli* MTCC B948, *R. rhodochrous* MTCC 265 and *P. aeruginosa* MTCC 2453 with well diffusion method by culturing the microorganism in Muller-Hinton agar. Each strain was individually swabbed uniformly onto the surface of Mueller-Hinton agar plate using sterile cotton swab (Himedia Labs, Mumbai, India). These plates were incubated at 37 ˚C for 24 h. The zone of inhibition was measured by subtracting the well diameter from the total inhibition zone diameter.

3. Results

3.1. Morphological identification of cyanobacteria

The sample appeared to be dark brown in colour when observed with the naked eye. Cyanobacteria identification was done with light microscope observation (Micros Austria-MCX500) with the morphological properties like slightly constricted, cross walls, conical and obture end cells, oval shaped heterocyst. Finally, it was identified as *Anabaena variabilis* (Figure 1). This strain was deposited in the germplasm of Department of Microbiology, Bharathidasan University with strain named as *A. variabilis*.

3.2. Determination of phycobiliproteins of A. variabilis

A. variabilis extract was analysed by UV-vis specrophotometer for phycobiliproteins (Figures 2A and 3). *A. variabilis* extracts having (λ_{max} ~569 nm) phycoerythrin, (λ_{max} ~616 nm) phycocyanin, (λ_{max} ~676 nm) allophycocyanin B.

3.3. Biosynthesized ZnO NPs characterized by UV, FTIR and XRD

3.3.1. UV-visible spectral analysis of ZnO NPs

Reduction will occur and nano will form as it was monitored by sampling the reaction mixture at regular intervals and the absorption maxima was monitored by UV-vis spectra, at the wavelength of 200–500 nm in Cary spectrophotometer operated at a resolution of 1 nm (Figures 2B and 4). Zinc nanoparticles exhibit unique and tunable optical properties on account of their surface plasmon resonance, dependent on shape, size and size distribution of the nanoparticles. The reduction of Zn^{+} ions was monitored by measuring the UV-visible spectra of the solutions after diluting a small aliquot (0.2 mL) of the sample 20 times.

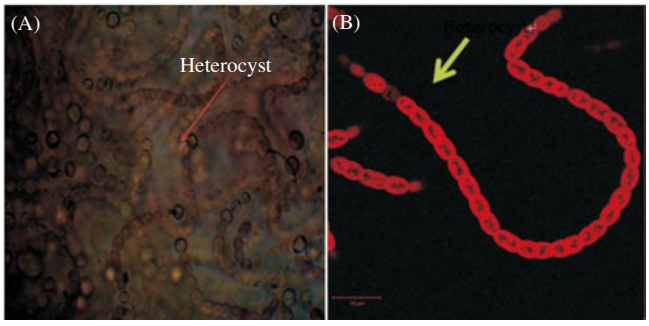

Figure 1. Microphotograph (A) and confocal image (B) of *A. variabilis*.

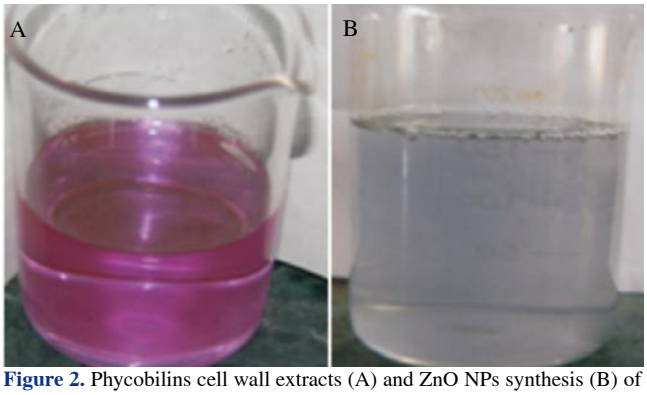

Figure 2. Phycobilins cell wall extracts (A) and ZnO NPs synthesis (B) of *A. variabilis*.

3.2.2. Identification of functional groups ZnO NPs

The synthesized zinc nanoparticles were subjected to FTIR analysis to detect the various characteristic of functional groups associated with the synthesized nanoparticles (Table 1 and Figure 5A). The peaks indicated the characteristics functional group presented in the synthesized ZnO NPs. The spectrum showed the presence of bands at 3 274.95, 1 626.01, 1 537.61, and 1 033.59 cm^{-1}. The peaks at 1 537.61 cm^{-1} and 1 626.01 cm^{-1} were ascribed to the vibrational modes of aromatic nitrocompounds and alkyl C=C stretch, amide, open chain amino. The positions of these bands were close to those reported for native proteins. The FTIR results indicated that the secondary structures of proteins were not affected as a consequence of reaction with zinc ions or binding with zinc nanoparticles. This evidence suggested that the release of extracellular protein molecules could possibly perform the function for the formation and stabilization of zinc nanoparticles in aqueous medium.

Table 1
Frequency values of FTIR peaks for biosynthesized ZnO NPs corresponding to their functional groups.

Wave number (cm^{-1})	Functional groups
3 274.95	Alcohol, O-H (stretch H-bounded)
1 626.01	Carbonyl C=O, N-H bending for primary and secondary amides
1 537.61	C=O/C=N group shifted
1 033.59	C-H

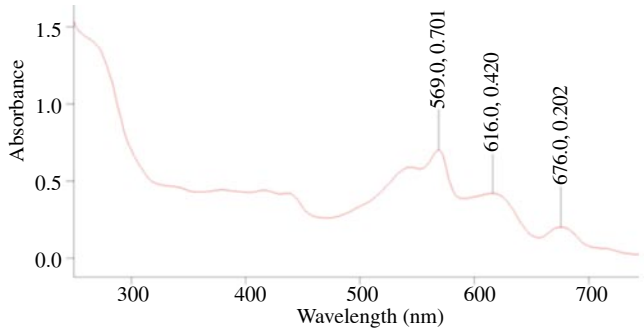

Figure 3. UV-vis spectrum of phycobiliproteins of *A. variabilis* NTSS17.

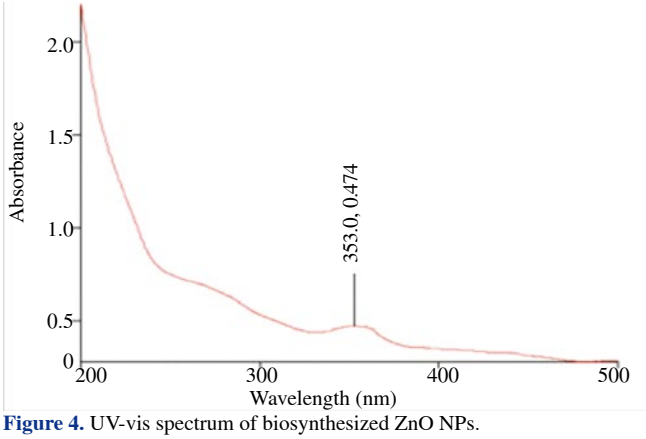

Figure 4. UV-vis spectrum of biosynthesized ZnO NPs.

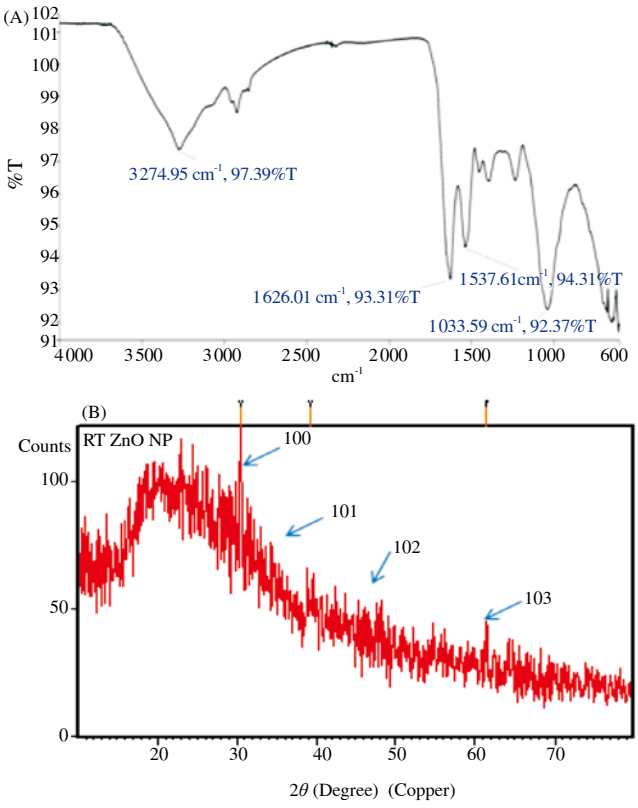

Figure 5. FTIR spectrum (A) and XRD pattern (B) of biosynthesized ZnO NPs.

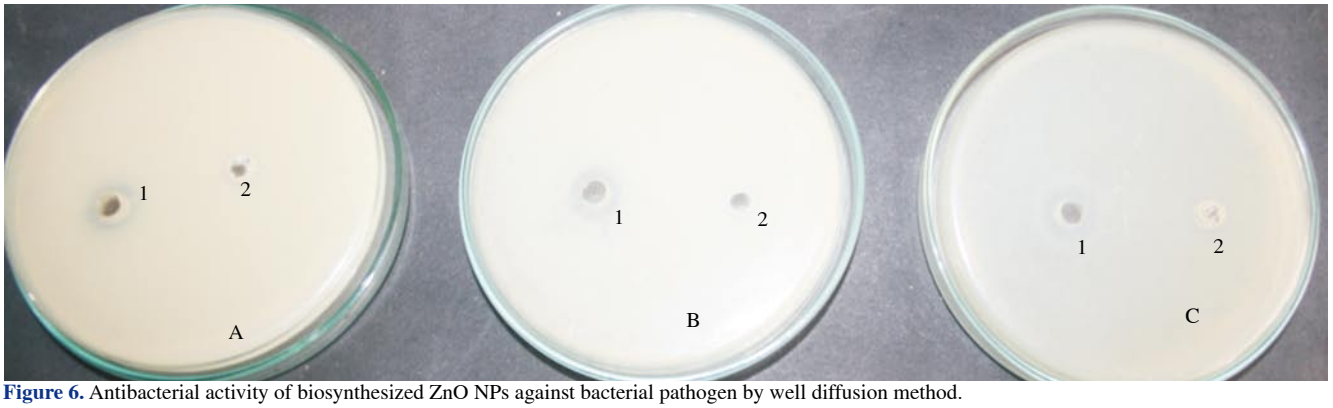

Figure 6. Antibacterial activity of biosynthesized ZnO NPs against bacterial pathogen by well diffusion method.
1: ZnO NPs from *A. variabilis*; 2: H_2O control; A: *R. rhodochrous* MTCC 265; B: *P. aeruginosa* MTCC 2453; C: *E. coli* MTCC B948.

3.3.3. XRD analysis

The crystalline natures of zinc nanoparticles were confirmed from the XRD analysis. The XRD pattern of freeze-dried nanoparticles exhibited peaks at 31.6°, 36.2°, 47.5° and 62.8° which correspond to the (100), (101), (102) and (103) reflection of fullerene containing carbon, respectively. The XRD results showed that the zinc nanoparticles formed were crystalline in nature (Figure 5B).

3.4. Antibacterial activity

The antibacterial activity of metal nanoparticles of zinc oxide exhibited strong activity against *E. coli*, *R. rhodochrous* and *P. aeruginosa*, which was studied in Kirby Beyer method and Muller-Hinton agar. The formation of zone was clearly observed around the disc containing ZnO NPs. The maximum inhibition zones of zinc oxide for nanoparticles *E. coli* MTCC B948, *R. rhodochrous* MTCC 265 and *P. aeruginosa* MTCC 2453 were 16, 13 and 12 mm, respectively, with a concentration of 5 mg/1000 mL (Table 2 and Figure 6).

Table 2
Zone of inhibition of antibacterial activity of biosynthesized ZnO NPs against bacterial pathogen. mm.

Bacterial pathogens	Zone of inhibition
R. rhodochrous MTCC 265	13
P. aeruginosa MTCC 2453	12
E. coli MTCC B948	16

4. Discussion

In the present study, the *A. variabilis* used for synthesis of ZnO NPs was isolated from a paddy field. It produced pink pigment phycoerythrin, then due to the absorption of light it changed to blue colour *i.e.* phycocyanin. These brilliantly colored proteins can be broadly divided into three classes based on their spectral properties: phycoerythrin (λ_{max} ~565 nm), phycocyanin (λ_{max} ~620 nm), and

allophycocyanin (λ_{max} ~650 nm). A fourth phycobiliprotein known as allophycocyanin B (λ_{max} ~670 nm) has also been shown to be present in cyanobacteria in low amounts[31].

In the present study *A. variabilis* extract was analysed by UV-vis for phycobiliproteins. It showed characteristic peaks at λ_{max} ~569 nm, λ_{max} ~616 nm and λ_{max} ~676 nm having phycoerythirin, phycocyanin and allophycocyanin B respectively. Eriksen[32] reported that *Anabaena* has been mostly exploited for phycocyanin which is another important cyanobacterial accessory pigment having a number of industrial applications. But exploitation of *Anabaena* for phycoerythrin production is very limited according to the literatures available till date. Only few reports were available from India regarding the phycoerythrins production from cyanobacterial and red algae[33-35].

However, very few reports are available dealing with the use of cell extracts of bacteria and cyanobacteria in synthesis of nanoparticles[13]. Most probably cell extract moieties involved in the formation of nanoparticles probably render stability to the synthesized particles. While the exact mechanisms of reactions involved in ZnO NPs synthesis could not be established, and previous studies have indicated that proteins can bind to nanoparticles through free amine groups or cysteine residues or negatively charged carboxylate groups of enzymes[13,23,29]. As the cell extract contains bulk of phycobiliproteins, their role in interaction seems more important. Role of phycobiliprotein in the synthesis of metal nanoparticles has been well documented[23]. However, further work is needed to ascertain the exact mechanisms of ZnO NPs synthesis.

In the present study, ZnO NPs revealed peaks at 358 nm. These surface plasmon resonance bands undergo a red or blue shift[36], depending on the quantum size effects. The bioreduction of zinc ions were visually suggested by color change from white to pale brown for ZnO NPs during the exposure of alga extract while no color change was noticed with zinc nitrate solutions without alga extract. It is reported that the color change may be due to the excitation of surface plasmon resonance effect and decrease of $AgNO_3$[37]. Bioreduction of the aqueous Zn^{2+} ions after exposure to the macroalgae extract was monitored by UV-vis spectroscopy which is an important technique to determine the production and constancy of metal nanoparticles in an aqueous solution. The algae, when reacted with Zn^{2+} ions, reduces the precursor resolution and leads to the formation of extracellular nanoparticles as monitored by UV-vis spectroscopy. It is generally recognized that the UV-vis spectroscopy could be used to examine size- and shape-controlled nanoparticles in aqueous suspensions[36]. Singh *et al*.[38] clearly demonstrated that the cell extract of the cyanobacterium *Anabaena* isolate L31 efficiently reacts with $ZnNO_3$ to form ZnO NPs. As such UV-vis absorption spectra are known to be quite sensitive for testing the formation of various metal nanoparticles including ZnO NPs and thus could be used as one of the simplest confirmatory tools.

In the present study, FTIR spectrum revealed the presence of bands at 3 274.95, 1 626.01, 1 537.61, and 1 033.59 cm^{-1}. The peaks at 1 537.61 cm^{-1} and 1626.01 cm^{-1} were ascribed to the vibrational modes of aromatic nitrocompounds and alkyl C=C stretch of amide group respectively. The positions of these bands were close to those reported for native proteins. The FTIR results indicated that the secondary structures of proteins were not affected as a consequence of reaction with zinc ions or binding with zinc nanoparticles. Infrared study confirms the presence of amide groups and aliphatic residues of proteins have a strong ability to bind to metal, so that the protein is most possibly covered by the metal nanoparticle. It has been earlier reported that protein can bind nanoparticles either through amide or aliphatic groups residues[39]. This evidence suggested that the release of extracellular protein phycobiliproteins molecules could possibly perform the function for the formation and stabilization of zinc nanoparticles in aqueous medium. In the present study, the XRD results showed that the zinc nanoparticles formed were crystalline in nature. It was quite common, the broadening of the peaks in the XRD patterns of solids was attributed to particle size effects. This study clearly indicated that antibacterial activity of ZnO NPs exhibited strong activity against *E. coli*, *R. rhodochrous* and *P. aeruginosa* in Muller-Hinton agar. The antibiogram study of biosynthesized zinc oxide nanoparticles has been previously reported by various researchers against pathogenic microorganisms[40]. Reports of Zhang *et al*.[41] supported the finding that antibacterial behavior of ZnO NPs could be due to chemical interactions between hydrogen peroxide and membrane proteins, or between other chemical species produced in the presence of ZnO NPs and the outer lipid bilayer of bacteria. The hydrogen peroxide produced enters the cell membrane of bacteria and kills them. It was shown in the study that zinc oxide particles are responsible for inhibiting bacterial growth.

Interestingly, in this study cell extract contains bulk of phycobiliproteins, their role in interaction seems more important. They may be a suitable candidate for green synthesis of metal nanoparticles. The antimicrobial activity was tested against three different human pathogens. Thus the application of such eco-friendly zinc nanoparticles in bactericidal, anticancer and other medical and electronic applications, make this method potentially exciting for the large scale synthesis of other inorganic nano material. Further researches are required to gain insight into the molecular mechanism involved in the ZnO NPs, its toxicity, immunity and mode of action, which is necessary for safe and effective exploitation of zinc nanoparticles in nanotechnology based industries.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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References

[1] Leven MM. *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic and enteroadherent. *J Infect Dis* 1987; **155**: 377-89.

[2] Jawetz E, Mellnick JL, Adelberg EA. *Review of medical microbiology.* Norwalk: Applellation Lange Norwalk; 1995. p. 139-218.

[3] Feachem RG, Hogan RC, Merson MH. Diarrhoeal disease control: reviews of potential interventions. *Bull World Health Organ* 1983; **61**: 637-40.

[4] Chauhan R, Reddy A, Abraham J. Biosynthesis of silver and zinc oxide nanoparticles using *Pichia fermentans* JA2 and their antimicrobial property. *Appl Nanosci* 2015; **5**: 63-71.

[5] Singh BN, Rawat AK, Khan W, Naqvi AH, Singh BR. Biosynthesis of stable antioxidant ZnO nanoparticles by *Pseudomonas aeruginosa*

rhamnolipids. *PLoS One* 2014; **9**(9): e106937.

[6] Dhanasekaran D, Thangaraj R. Evaluation of larvicidal activity of biogenic nanoparticles against filariasis causing *Culex* mosquito vector. *Asian Pac J Trop Dis* 2013; **3**(3): 174-9.

[7] Dhanasekaran D, Thangaraj R. Microbial secondary metabolites are an alternative approaches against insect vector to prevent zoonotic diseases. *Asian Pac J Trop Dis* 2014: **4**(4): 253-61.

[8] Dhandapani P, Siddarth AS, Kamalasekaran S, Maruthamuthu S, Rajagopal G. Bio-approach: ureolytic bacteria mediated synthesis of ZnO nanocrystals on cotton fabric and evaluation of their antibacterial properties. *Carbohydr Polym* 2014; **103**: 448-55.

[9] Thangaraju N, Venkatalakshmi RP, Chinnasamy A, Kannaiyan P. Synthesis of silver nanoparticles and the antibacterial and anticancer activities of the crude extract of *Sargassum polycystum* C. Agardh. *Nano Biomed Eng* 2012; **4**(2): 89-94.

[10] Suresh A, Praveenkumar R, Thangaraj R, Oscar FL, Baldev E, Dhanasekaran D, et al. Microalgal fatty acid methyl ester a new source of bioactive compounds with antimicrobial activity. *Asian Pac J Trop Dis* 2014: **4**(Suppl 2); S979-84.

[11] Li WJ, Shi EW, Zheng YQ, Yin ZW. Hydrothermal preparation of nanometer ZnO powders. *J Mater Sci Lett* 2001; **20**(15): 1381-3.

[12] Kumar SA, Peter YA, Nadeau JL. Facile biosynthesis, separation and conjugation of gold nanoparticles to doxorubicin. *Nanotechnology* 2008; **19**: 495101.

[13] Sharma VK, Yngard RA, Lin Y. Silver nanoparticles: green synthesis and their antimicrobial activities. *Adv Colloid Interface Sci* 2009; **145**: 83-96.

[14] Zhang X, Yan S, Tyagi RD, Surampalli RY. Synthesis of nanoparticles by microorganisms and their application in enhancing microbiological reaction rates. *Chemosphere* 2011; **82**: 489-94.

[15] Otari SV, Patil RM, Nadaf NH, Ghosh SJ, Pawar SH. Green synthesis of silver nanoparticles by microorganism using organic pollutant: its antimicrobial and catalytic application. *Environ Sci Pollut Res Int* 2014; **21**: 1503-13.

[16] Liu L, Shao Z, Ang HM, Tadé MO, Liu S. Are microorganisms indispensable in green microbial nanomaterial synthesis? *RSC Adv* 2014; **4**: 14564-8.

[17] Hu XL, Zhu YJ, Wang SW. Sonochemical and microwave-assisted synthesis of linked single-crystalline ZnO rods. *Mater Chem Phys* 2004; **88**: 421-6.

[18] Zhai HJ, Wu WH, Lu F, Wang HS, Wang C. Effects of ammonia and cetyltrimethylammonium bromide (CTAB) on morphologies of ZnO nano- and micromaterials under solvothermal process. *Mater Chem Phys* 2008; **112**: 1024-8.

[19] Limbach LK, Wick P, Manser P, Grass RN, Bruinink A, Stark WJ. Exposure of engineered nanoparticles to human lung epithelial cells: influence of chemical composition and catalytic activity on oxidative stress. *Environ Sci Technol* 2007; **41**: 4158-63.

[20] Ates ES, Unalan HE. Zinc oxide nanowire enhanced multifunctional coatings for cotton fabrics. *Thin Solid Films* 2012; **520**: 4658-61.

[21] Espitia PJP, de Fátima Ferreira Soares N, dos Reis Coimbr JS, de Andrade NJ, Cruz RS, Medeiros EAA. Zinc oxide nanoparticles: synthesis, antimicrobial activity and food packaging applications. *Food Bioproc Tech* 2012; **5**: 1447-64.

[22] Ma H, Williams PL, Diamond SA. Ecotoxicity of manufactured ZnO nanoparticles--a review. *Environ Pollut* 2013; **172**: 76-85.

[23] Mubarak Ali D, Gopinath V, Rameshbabu N, Thajuddin N. Synthesis and characterization of CdS nanoparticles using C-phycoerythrin from the marine cyanobacteria. *Mater Lett* 2012; **74**: 8-11.

[24] Bogorad L. Phycobiliproteins and complementary chromatic adaptation. *Ann Rev Plant Physiol* 1975; **26**: 369-401.

[25] Grossman AR, Bhaya D, Apt KE, Kohoe DM. Light-harvesting complexes in oxygenic photosynthesis: diversity, control and evolution. *Ann Rev Genet* 1995; **29**: 231-88.

[26] Guan X, Qin S, Zhao F, Zhang X, Tang X. Phycobilisomes linker family in cyanobacterial genomes: divergence and evolution. *Int J Biol Sci* 2007; **3**: 434-45.

[27] Ali DM, Sasikala M, Gunasekaran M, Thajuddin N. Biosynthesis and characterization of silver nanoparticles using marine cyanobacterium *Oscillatoria willei* NTDM01. *Dig J Nanomater Biostruct* 2011; **6**: 385-90.

[28] Lengke MF, Fleet ME, Southam G. Biosynthesis of silver nanoparticles by filamentous cyanobacteria from silver(I) nitrate complex. *Langmuir* 2007; **23**: 2694-9.

[29] Jayaseelan C, Rahuman AA, Kirthi AV, Marimuthu S, Santhoshkumar T, Bagavan A, et al. Novel microbial route to synthesize ZnO nanoparticles using *Aeromonas hydrophila* and their activity against pathogenic bacteria and fungi. *Spectrochim Acta A Mol Biomol Spectrosc* 2012; **90**: 78-84.

[30] Bennett A, Bogorad L. Comparative chromatic adaptation in a filamentous blue-green alga. *J Cell Biol* 1973; **58**: 419-35.

[31] Glazer AN, Bryant DA. Allophycocyanin B (lambdamax 671, 618 nm): a new cyanobacterial phycobiliprotein. *Arch Microbiol* 1975; **104**: 15-22.

[32] Eriksen NT. Production of phycocyanin--a pigment with applications in biology, biotechnology, foods and medicine. *Appl Microbiol Biotechnol* 2008; **80**: 1-14.

[33] Ranjitha K, Kaushik BD. Purification of phycobiliproteins from *Nostoc muscorum*. *J Sci Ind Res* 2005; **64**: 372-5.

[34] Tripathi SN, Kapoor S, Shrivastave A. Extraction and purification of an unusual phycoerythrin in a terrestrial desiccation tolerant cyanobacterium *Lyngbya arboricola*. *J Appl Phycol* 2007; **19**: 441-7.

[35] Parmar A, Singh NK, Kaushal A, Sonawala S, Madamwar D. Purification, characterization and comparison of phycoerythrins from three different marine cyanobacterial cultures. *Bioresour Technol* 2011; **102**: 1795-802.

[36] Mulvaney P. Surface plasmon spectroscopy of nanosized metal particles. *Langmuir* 1996; **12**: 788-800.

[37] Singh A, Jain D, Upadhyay MK, Khandelwal N, Verma HN. Green synthesis of silver nanoparticles using *Argemone mexicana* leaf extract and evaluation of their antimicrobial activities. *Dig J Nanomater Biostruct* 2010; **5**: 483-9.

[38] Singh G, Babele PK, Kumar A, Srivastava A, Sinha RP, Tyagi MB. Synthesis of ZnO nanoparticles using the cell extract of the cyanobacterium, *Anabaena* strain L31 and its conjugation with UV-B absorbing compound shinorine. *J Photochem Photobiol B* 2014; **138**: 55-62.

[39] Basavaraja S, Balaji SD, Lagashetty A, Rajasab AH, Venkataraman A. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium semitectum*. *Mater Res Bull* 2008; **43**: 1164-70

[40] Oberdörster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, et al. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. *Part Fibre Toxicol* 2005; **6**(2): 8.

[41] Zhang LL, Jiang YH, Ding YL, Daskalakis N, Jeuken L, Povey M, et al. Mechanistic investigation into antibacterial behaviour of suspensions of ZnO nanoparticles against *E. coli*. *J Nanopart Res* 2010; **12**: 1625-36.