Uttar Pradesh Journal of Zoology

Volume 45, Issue 13, Page 189-203, 2024; Article no.UPJOZ.3576
ISSN: 0256-971X (P)

Isolation and Identification of Bacterial Biosurfactant Producing Strain from Soil and Evaluation of Their Antimicrobial Activity Mediated Zinc Oxide Nanoparticles

Ashitha K Sanuj [a++*] and N. Vanitha [a#]

[a] Department of Microbiology, Hindusthan College of Arts and Science, Coimbatore, Tamil Nadu - 641 028, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i134147

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3576

Original Research Article

Received: 07/04/2024
Accepted: 10/06/2024
Published: 11/06/2024

ABSTRACT

Nanoparticle synthesis using biosurfactants is currently a popular and environmentally friendly method for improving both particle synthesis and stabilization. Zinc oxide nanoparticles (ZnO NPs) have found extensive applications in the field of biomedicine. The present study focused on isolating microorganisms that produce biosurfactants from soil contaminated with oil. The isolated microorganisms were identified through molecular identification of 16s r-RNA gene sequencing and characterization as *Bacillus pseudomycoides*. We determined biosurfactant production using

[++] Research Scholar, PG and Research;
[#] Professor;
**Corresponding author: Email: ashithascholarhicas@gmail.com;*

Cite as: *Sanuj , Ashitha K, and N. Vanitha. 2024. "Isolation and Identification of Bacterial Biosurfactant Producing Strain from Soil and Evaluation of Their Antimicrobial Activity Mediated Zinc Oxide Nanoparticles". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (13). https://doi.org/10.56557/upjoz/2024/v45i134147.*

various assays such as hemolysis assay, emulsification index, oil spreading tests, and drop-collapsing assays. This investigation confirms that isolated microorganisms possess biosurfactant properties. We utilized a biosurfactant extract from the *B. pseudomycoides* strain for the synthesis of ZnO NPs. Advanced techniques such as UV-vis spectroscopy, XRD, FTIR, SEM, and Edax were employed to ensure accurate characterization. Furthermore, the biosurfactant-mediated ZnO NPs effectively killed a few bacterial pathogens, such as *Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella pneumonia*. They were also effective against fungal pathogens, such as *Aspergillus niger, Aspergillus flavus*, and *Fusarium oxysporum*. This research highlights the impressive effectiveness of newly synthesized biosurfactant-mediated ZnO against clinical isolates. This research suggests that the biosurfactant extract from *B. pseudomycoides* could be used to make ZnO nanoparticles that could be useful for treating harmful infections.

Keywords: Bacillus pseudomycoides; Biosurfactant; zinc nanoparticles; antimicrobial activity.

1. INTRODUCTION

Biosurfactants, or microbial surfactants, are surface-active chemicals that are synthesized by microorganisms such as bacteria, yeast, and fungus as secondary metabolites during biotechnological processes [1]. These compounds are amphiphilic, meaning they have both hydrophobic and hydrophilic parts. This allows them to be present at the boundary between polar and nonpolar substances [2]. Biosurfactants are categorized based on their origin and the chemical structures they possess. These categories include lipopeptides, lipoproteins, glycolipids, phospholipids, and polymeric biosurfactants. Biosurfactants provide significant benefits to the strains that produce them. They improve the availability of hydrophobic pollutants, bind to heavy metals, exhibit antibacterial properties, and govern the attachment or detachment of microbes to surfaces [3]. Biosurfactants surpass chemical surfactants in terms of their uncomplicated chemical composition, enhanced durability, substantial foaming activity, minimal toxicity, pH balance, environmental friendliness, and ability to decompose naturally. Biosurfactants possess distinct functional groups that confer selectivity in the process of detoxifying contaminants, even in conditions of high salt, temperature, and pH levels [4]. The low toxicity and eco-friendly characteristics of biosurfactants make them suitable for use in various industries such as cosmetics, pharmaceuticals, oil, and food [5].

New developments in nanotechnology have revolutionized the field, allowing for the creation of meticulously structured nano-particulates with limitless possibilities in terms of size and shape. This breakthrough has paved the way for the development of innovative biocidal compounds. Nanoparticles (NPs) are often hailed as a remarkable advancement in the field of modern medicine. There have been exciting developments in the field of antimicrobial research, particularly around finding new treatments for bacteria that are resistant to multiple drugs. One such development is the discovery of nanoparticles that possess potent antimicrobial properties. These nanoparticles show great potential as alternative treatments for combating drug-resistant bacteria. Nanomaterials have garnered significant attention due to their exceptional properties, which encompass a multitude of advantageous characteristics for biological applications [6].

The Zinc oxide (ZnO) nanoparticle has garnered global interest for its unique properties that set it apart from its larger counterparts. It serves as a versatile semiconductor with piezoelectric properties. ZnO nanoparticles have been utilized in a wide range of applications, including pharmaceuticals, cosmetics, photocatalysis, and dietary supplements for animals. Various chemical methods have been suggested for the synthesis of ZnO nanoparticles, including the solvent gel process, solvent evaporation, and microemulsion precipitation [7]. However, due to the utilization of harsh chemicals in the reduction and stabilization processes, which form strong bonds with the ZnO nanoparticles and limit their potential for biological applications, these methods fail to meet environmentally friendly standards. In addition, these procedures are also contributing to the production of harmful by-products that can lead to secondary contamination. Consequently, researchers are actively working on the development of more sustainable methods for preparing ZnO nanoparticles. They are conducting extensive studies to explore alternatives to traditional approaches [8].

Biosurfactants can play a crucial role in promoting responsible and sustainable use of nanotechnology as it continues to advance. Additionally, they can act as stabilizing agents and are being recognized as an environmentally friendly option for synthesizing and stabilizing nanoparticles. Biosurfactants have shown great potential in the production of high-performance nanomaterials due to their ability to readily form various liquids in aqueous solutions [9,10]. As per some researchers, a biological approach involving the utilization of biosurfactants, specifically rhamnolipids derived from *Pseudomonas aeruginosa*, has been reported. Through their analysis, they discovered that biosurfactant-mediated Zinc nanoparticles possess impressive antioxidant properties, making them a highly promising option in the field. This method utilises a cost-effective and environmentally friendly approach to synthesise nanoparticles [11]. In the process of synthesising Zinc sulfide nanoparticles, biosurfactants (rhamnolipid) produced by *Pseudomonas aeruginosa* were utilized [12]. According to Mulligan [13], certain bacteria strains, like Bacillus, could endure harsh conditions for extended periods. These strains are commonly referred to as biosurfactant producers. Multiple studies have explored the extraction of Bacillus species, known for their ability to produce biosurfactants, from soil samples with high salt content [14,15,16].

The present study focuses mainly on the Biosurfactant Producing Bacterial Strain found in soil samples. Furthermore, biosurfactant was synthesized and characterized utilizing an environmentally benign process based on ZnO NPs. Antibacterial and antifungal activities were also investigated as biopotential applications.

2. MATERIALS AND METHODS

2.1 Collection of Soil Sample

Soil sample containing oil (petrol and diesel) was collected from a petrol pump station in Coimbatore, Tamil Nādu, India. The sample was carefully collected using a sterile spatula and placed in an air-tight container. It was then transported to the laboratory for further analysis, specifically to isolate bacteria that produce biosurfactants.

2.2 Isolation of Bacterial Strains

1 gram of soil sample was added to 20 ml of sterile normal saline and thoroughly mixed, 0.1 ml portion of the collected sample was carefully applied onto a blood agar plate. The culture plates were incubated at 37°C for 24 hours to identify potential isolates for biosurfactant production. The bacteria obtained were subcultured in nutrient broth (Himedia, Mumbai, India) following sterilization. The medium was prepared by dissolving 13g in 1000 ml of distilled water and sterilized under an autoclave at 121°C for 15 minutes. The media was cooled down to room temperature in a sterile environment a loop of culture was inoculated and incubated at 37°C for 24 hours. After confirming the growth based on turbidity, it was utilized for further study [17].

2.3 Identification of Bacterial Isolates

The 16S rRNA gene was successfully amplified from bacterial genomic DNA using the widely used primers 27F and 1492R. Following a 5-minute PCR run at 94°C, a total of 35 cycles of denaturation, annealing, and extension were performed. The sample was stored at 4°C. A 50µL solution for PCR amplification was prepared, and the molecular masses of a specific product were determined using 1% agarose electrophoresis. The amplified products were analysed using a biosystem 3500 Genetic analyzer for sequencing. The resulting sequence was then compared to the GenBank database to identify the organism of origin [18]. The primary analysis of the nucleotide sequence was determined using NCBI Blast in this study. The BioEdit Sequence Alignment Editor and MEGA11 software are utilized for the verification and reorganization of the sequences. The bacterial phylogenetic analysis was performed using MEGA 11 software. MEGA's latest version, Version 11, has incorporated a wide range of methods and tools to cater to the ever-growing needs of researchers. It is worth mentioning that the integration of evolutionary dating methods in MEGA streamlines the process of estimating species and strain divergence times. This is achieved by considering the accuracy of node calibrations and optimizing sampling times to enhance the quality of information obtained [19].

2.4 Screening of Biosurfactant (BS) Producers

The bacterial strains were screened for using blood hemolysis techniques as described by Rajesh et al. [3]. The ability of the strain to produce biosurfactants was analyzed using various methods, including the emulsification index, oil spreading test, and drop collapsing test.

These tests were conducted on oil-coated agar plates, as described in the respective studies [20,21].

2.5 Biosurfactant (BS) Extraction

Discovering bacterial species, the culture broth was collected and then subjected to centrifugation to obtain a cell-free supernatant that contains a biosurfactant. The supernatant was acidified to pH 2 using HCl and then allowed undisturbed overnight at 4°C. The supernatant was centrifuged at 10,000 rpm at 4°C to separate the acid-precipitated biosurfactant (pellet). The crude biosurfactant have been treated with a mixture of methanol and chloroform in a specific ratio. After this treatment, the biosurfactant was centrifuged again to obtain a purified biosurfactant precipitate [22,23,24]. The biosurfactant was dried and stored in a phosphate buffer solution (PBS) [21].

2.6 Synthesis of Zinc Oxide Nanoparticles Using Biosurfactant

A two-step method was used to synthesize ZnO NPs. Concisely, a bacterial biosurfactant with a concentration of 0.1% (w/v) was combined with 100 ml of a 0.05 mol aqueous solution of Zinc acetate. The mixture was continuously stirred for 10 minutes at room temperature and subsequently autoclaved at 121°C under 15 lbs pressure for 30 minutes. The transition from a transparent state to a light white particle signifies the creation of ZnO NPs. This was achieved by subjecting the mixture to centrifugation at 10,000 rpm for 10 minutes at ambient temperature, and subsequently drying it in an oven at 60°C.

2.7 Biosynthesized n-ZnO Characterization

The analysis of reaction mixture confirmed the morphological and physicochemical attributes of the biosurfactant-synthesized ZnO NPs using UV-visible spectra. Further analysis was conducted using techniques like FESEM and EDAX. X-ray diffraction (XRD) analysis was used to determine the phase purity of the ZnO nanoparticles synthesized using essential oil. In addition, the researchers in a previous study utilized FTIR spectroscopy to analyse the particle size and the functional groups present [25].

2.8 Antimicrobial effect of ZnO NPs

To evaluate the antibacterial properties of the essential oil derived from biosurfactant-mediated ZnO NPs. The agar well-diffusion method was employed to test the effectiveness of the oil against various strains of bacteria, including *E. coli* (MTCC 1304), *K. pneumonia* (MTCC 109), and *Salmonella typhi* (MTCC 733). For the antibacterial assay, we used axenic cultures of each bacterium that were grown in a nutrient broth medium. These cultures were then extracted and carefully spread onto different agar plates using a sterile glass L-shaped rod. Small wells measuring 5 mm in diameter were carefully created in nutrient agar plates using a borer. Zinc oxide nanoparticles (ZnO NPs) were added into the wells of all media plates carried out using a biological synthesis method, with a concentration of 20 mg/ml. The evaluation of the bacterial growth inhibition zone was observed after 24 hours of incubation at a temperature of 28±2°C [26]. For comparison purposes, we used a disc containing Amoxyclav antibiotic as a positive control and Zinc acetate as a negative control alongside our synthesized nanomaterials.

2.9 Antifungal Activity

The evaluation of the antifungal study involved the use of the well-diffusion method, where a Petriplate was prepared by pouring malt extract agar into it. Following the solidification process, a total of 80 µl of fungal spores from *Aspergillus niger* (MTCC 282*), Aspergillus flavus* (MTCC 277) and *Fusarium oxysporum* (MTCC 9913) were evenly spread using a sterile cotton swab. Wells were created using a cork borer, and the ZnO NPs, which were enhanced by the biosurfactant, were added. A positive control of 5 mg/ml of antibiotic (F-Fluconazole -antibiotic) was utilized, while a negative control of 20 mg/ml of Zinc acetate was employed. The plates were placed in an incubator at 30°C for 3-5 days. The measurement of the inhibition zone in millimetre was taken after the incubation period.

3. RESULTS AND DISCUSSION

3.1 Isolation, Screening and Identification Studies for Selected Strains

The results showed that we successfully isolated a pure strain from oil-polluted soil (Fig. 1). The biosurfactant production capacity of the isolated bacterial strains was assessed via established screening procedures, including hemolysis techniques, emulsification index, oil spreading test, and drop collapsing test. The chosen bacterial strains demonstrated good results for all the abovementioned tests, as shown in Table 1

Table 1. Preliminary test for screening of isolated bacteria for biosurfactant production ability

Strain	Hemolysis activity	Emulsification index (EI)	Oil spreading test (CM)	Drop collapsing test
Bacterial strain	+	6.25	3	+

Hemolytic activity measurement is commonly used as a primary method for screening biosurfactant production [27]. In a similar manner, Abouseoud [28] conducted a study on the biosurfactant activity of *Pseudomonas fluorescens*. The emulsification assay, emulsification index, and hydrocarbon layered assay test provide an indication of the presence of biosurfactants. However, it is important to note that these tests may not always correlate with surface activity. The collapse and spreading assay depend on the destabilization of droplets caused by a decrease in interfacial tension when surfactants and surfactant activity are present [29]. Sharma et al. [30] also observed similar results, where they isolated *P. aeruginosa* DSVP20, a biosurfactant-producing bacteria, from soil samples contaminated with petroleum.

Fig. 1. Cultivation of bacterial strain on nutrient Agar Plates

3.2 Molecular Identification of Isolation Bacteria

16s rRNA sequencing is a very practical and effective technology for determining phylogenetic connections and identifying organisms. Both conserved and changeable regions are present in the 16s rRNA molecule. The variable parts are more intriguing than the conserved areas for identification reasons, since they are used to ascertain the evolutionary history of the species. Bacteria possess a distinct and species-specific small subunit ribosomal ribonucleic acid (ribosomal SSU) that is unique to each species. By doing a comparative analysis of the SSU with publicly available databases, it is possible to determine the specific organism [31]. The bacteria were identified as *Bacillus Pseudomycoides* strain AVMB1 via sequencing. The strain *B. pseudomycoides* AVMB1 was identified as the most efficient producer of biosurfactants among the strains tested. The evolutionary connections of *B. pseudomycoides* strain AVMB1 were determined using the neighbour joining technique in MEGA11 (Fig. 2). The study conducted by Sohail et al. [21] revealed four promising strains, namely *Bacillus subtilis* (MH142143), *Pseudomonas aeruginosa* (MH142144), *Bacillus tequilensis* (MH142145), and *Bacillus safensis* (MH142146), as the sources of biosurfactants. This identification was done by 16S rRNA gene sequencing.

3.3 Extraction of Biosurfactant Using *Bacillus pseudomycoides*

Biosurfactant Production using *B. pseudomycoides*: In order to produce biosurfactants, the bacterial strain *B. pseudomycoides* was introduced into broth that contained 0.5% crude oil as the only carbon source. The soup was then incubated on a rotatory shaker. Following centrifugation, the supernatant was collected, and to test for surface activity, the supernatant's pH was adjusted to 2.0. Next, an equal amount of a 2:1 ratio of chloroform to methanol was added to the mixture. In Fig. 3, the coloured biosurfactant was visible. The biosurfactant that was so produced was then dried and kept for further use.

3.4 Synthesis of Zinc oxide nanoparticles Using biosurfactant Characterization

The extraction of biosurfactant-synthesised ZnO NPs was characterized using various techniques such as UV-vis FTIR, XRD, SEM, and EDAX

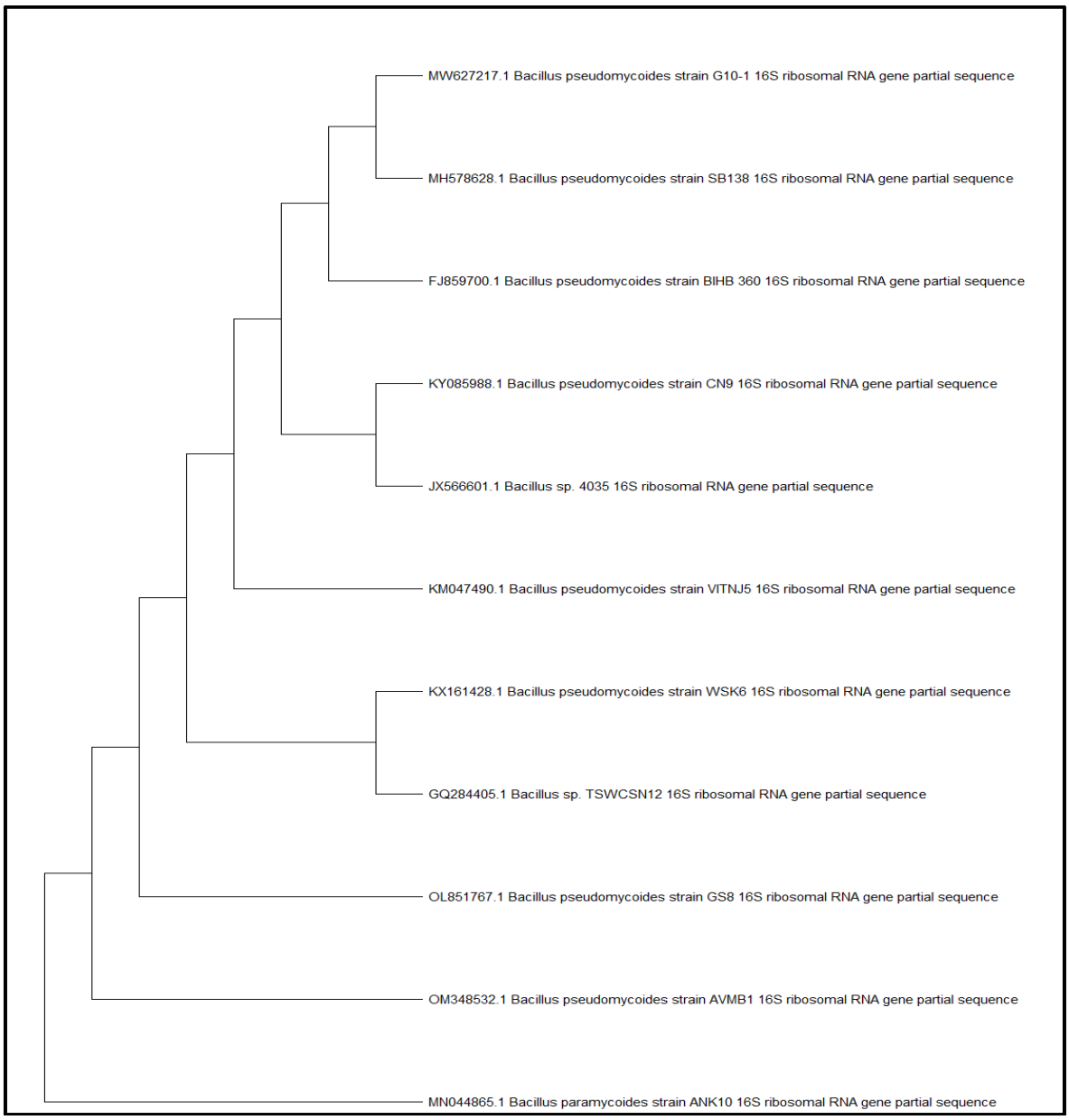

Fig. 2. The phylogenetic tree of *Bacillus pseudomycoides* and related strains based on 16S rRNA sequences, was constructed by the neighbor-joining method using MEGA11

analysis. The process involved bio-reduction of Zinc acetate with biosurfactant from the *B. pseudomycoides* bacterial strain. This changed the color of the substance from its original color to a pale-yellow white (Fig. 4). This color change served as a clear visual indication of ZnO NP formation, effectively demonstrating the biogenesis of these nanoparticles.

3.5 UV–Visible Spectroscopy

The analysis of biosurfactant-synthesized ZnO nanoparticles using a UV-vis spectrophotometer showed a peak at about 324 nm. The band's symmetrical structure indicated that the particles were evenly distributed, thereby proving their existence and stability (Fig. 5). This is consistent with the findings of earlier studies conducted by Dutta et al. [32] and Kundu et al. [33]. Previously, Narayanan et al. [34] reported a distinct and concentrated absorption peak at a wavelength of 340 nm for ZnS particles that were coated with a biosurfactant. The absorption peak was found exhibit a change in color in comparison to the bulk ZnO, where the absorption maximum is at 373 nm [35].

Fig. 3. Extraction of Biosurfactant using *Bacillus Pseudomycoides*

Fig. 4. Extraction of bio-surfactant synthesized ZnO NPs

3.6 FTIR Studies

Fig. 6 displays the FTIR transmittance spectra of ZnO NPs synthesized using biosurfactant. The exhibit displays distinct bands within the range of 600-3600 cm^{-1}, with specific peaks at 3302.13 cm^{-1}, 2985.81 cm^{-1}, 1877.33 cm^{-1}, 1644.79 cm^{-1} ,1574.30 cm^{-1}, 1381.03 cm^{-1}, 1249.87 cm^{-1}, 1049.87 cm^{-1}, 685.66 cm^{-1}, and 601.79 cm-1. The FTIR spectrum of synthesized ZnO NPs exhibits bands ranging from 4000-600 cm^{-1}, which are indicative of the functional groups present in ZnO NPs [36,37]. For instance, there is a broad band observed at 3302.13 cm^{-1} and a small absorption peak around 1381.03 cm^{-1}. These correspond to O–H stretching, which indicates the presence of alcohol and phenol compounds (O–H bending). The peaks at 2985.81 cm^{-1} are attributed to the stretching of alkane C-H bonds. The absorption peak at the 1877.33 cm^{-1} is associated with the C-H modes of aromatic compounds. The peaks observed at 1644.79 cm^{-1} and 1574.30 cm^{-1} are indicative of C=C stretching vibrations commonly found in alkene compounds. At 1249.87 cm^{-1}, the C-O stretch in amine groups can be detected. The peaks at 1049.87 cm-1 correspond to C-N stretching. The Halo compound obtained in biosurfactant synthesized ZnO NPs exhibits a peak at 685.66 cm^{-1} and 601.79 cm^{-1}, indicating

the presence of C–Br stretch. The fabrication of inorganic nanoparticles on biological scaffolds, both metallic and semiconducting, has been achieved through the selective reduction of metal ions to a metallic form in the presence of biomolecules [38]. Our findings align with the other reports on green synthesis of ZnO NPs [39].

3.7 X-ray Diffraction Studies

The phase and crystalline structure of biosurfactant synthesized ZnO NPs were explored using X-ray diffraction analysis, resulting in the identification of a unique diffraction pattern (Fig. 7). The peaks at 31.73°, 34.32°, 36.21°, 47.45°, 56.48°, 62.86°, 67.92° were assigned to specific reflections, as mentioned in the references [39,33]. These reflections exhibited the combined characteristics of biosurfactant from bacterial strain with ZnO. The peaks observed in the data align perfectly with the pattern found in the (JCPDS card No: 36-1451), providing solid evidence for the presence of a hexagonal phase structure in the synthesized ZnO nanoparticles [40]. The XRD pattern clearly showed strong diffraction line peaks, indicating the high-quality crystallinity of the biosynthesized ZnO NPs. The structure of ZnO NPs described in this study adheres to the well-established hexagonal wurtzite configuration [41].

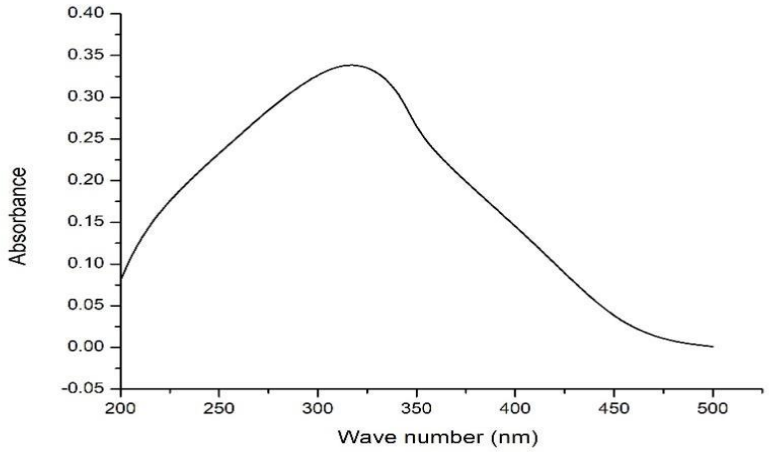

Fig. 5. UV-visible spectrum of biosurfactant synthesized ZnO nanoparticles

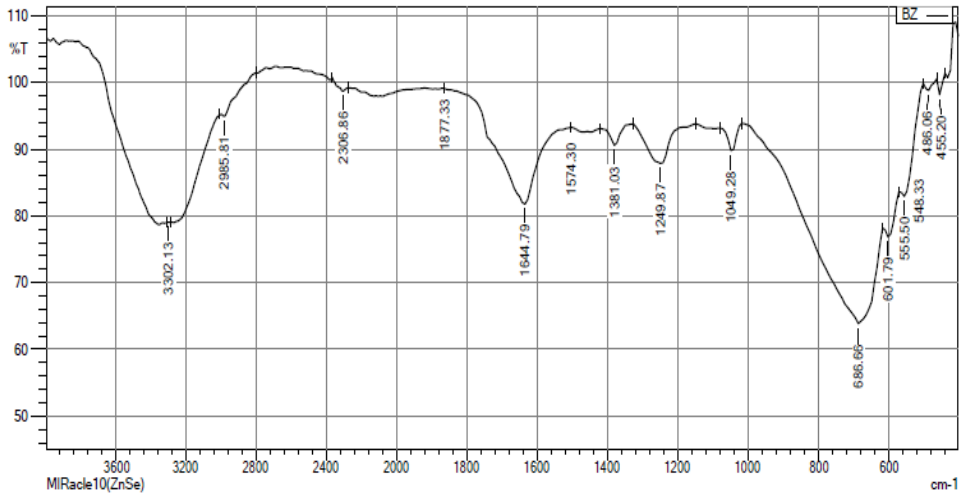

Fig. 6. Fourier transform infrared spectroscopy analysis of biosurfactant synthesized ZnO nanoparticles

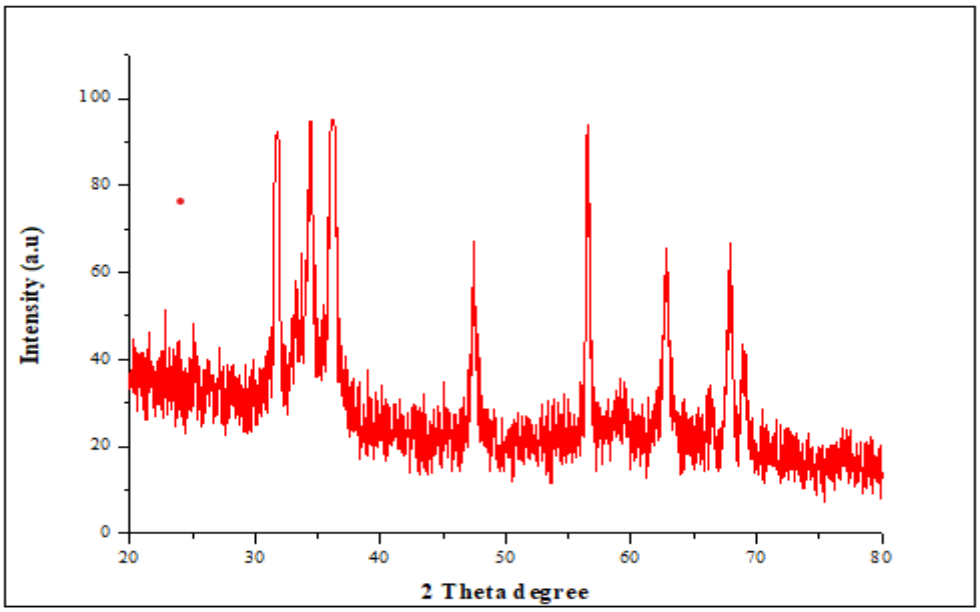

Fig. 7. XRD analysis of biosurfactant synthesized ZnO nanoparticles

3.8 SEM Analysis

The morphological traits of biosurfactant synthesized ZnO NPs were examined using Field Emission Scanning Electron Microscopy (FESEM) to determine the shape of the ZnO NPs. The SEM analysis shows that the synthesized nanoparticles have a range of shapes, ranging from 25-50 nm. They appear to be agglomerated and have structures that range from quasi-spherical to pentagonal, as shown in Fig. 8. The protein-capped NPs have a quasi-spherical shape, which is consistent with previous observations. It has been suggested that the presence of sulfur-containing cysteine residues in different parts of the protein plays a crucial role in determining the quasi-spherical shape of the NPs [42]. In a study conducted by Kundu et al. [33], they also noted the presence of a quasi-spherical to hexagonal morphology in the extracellular biosynthesis of Zinc oxide nanoparticles using *Rhodococcus pyridinivorans.*

3.9 Energy Dispersive X-ray Analysis (EDX) Spectrum of ZnO NPs

The elemental composition of ZnO nanoparticles synthesized using biosurfactant was determined through Energy Dispersive X-ray Spectroscopy (EDS). The EDS analysis revealed that the composition of ZnO consisted solely of Zn and O atoms (Fig. 9). The Energy-dispersive X-ray Spectroscopy (EDS) analyses in our study consistently demonstrate the findings for synthesized nanoparticles. The EDS spectra displayed prominent peaks at 0.5 keV, 1.1 keV, 8.6 keV, and 9.5 keV, corresponding to O Kα, Zn Lα, Zn Kα, and Zn Kα, respectively. This aligns with earlier studies [43,35,44].

3.10 Antibacterial Assay

ZnO nanoparticles synthesized using biosurfactant were tested against *E. coli, K. pneumoniae,* and *S. typhi* on a nutritive agar medium at specific doses. The screening of 20 mg/ml concentrations led to more potent antibacterial effects. Fig. 10 demonstrates the investigation's findings of a zone of inhibition against *E. coli, K. pneumoniae* and *S. typhi*. The inhibitory zones of *E. coli, S. typhi,* and *K. pneumoniae* were measured as 26 mm, 25 mm, and 24 mm, respectively (Table 2).

Similarly, bio-surfactant-based Ag/ZnO nanoparticles shown greater bacterial inhibitory efficacy against Gram (+ve) and Gram (-ve) microbial pathogens [45]. Other related study on ZnO-based materials with excellent antibacterial outcomes was presented in earlier works (Li et al., 2017; Xiang et al., 2017) [46,47]. Furthermore, comparable studies showed that production of ZnO nanoparticles increased antibacterial properties against *S. aureus, S. epidermidis, E. coli,* and *K. pneumoniae* bacteria [48].

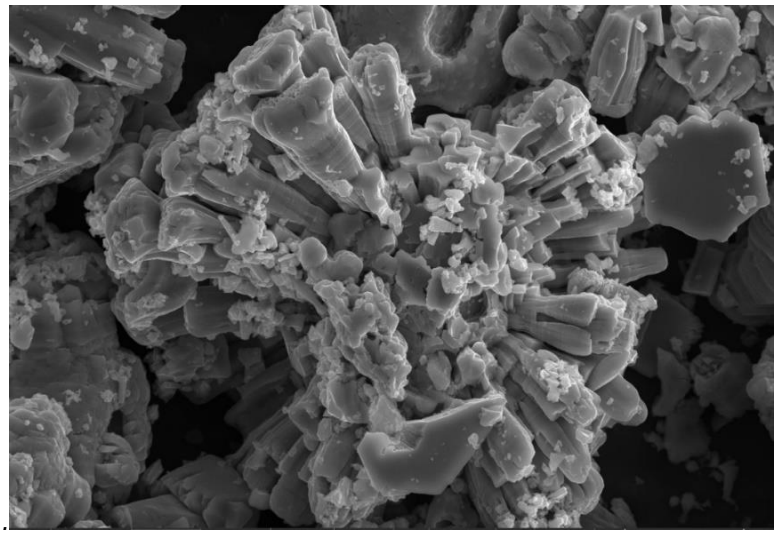

Fig. 8. Scanning electron microscopy of biosurfactant synthesized ZnO nanoparticles

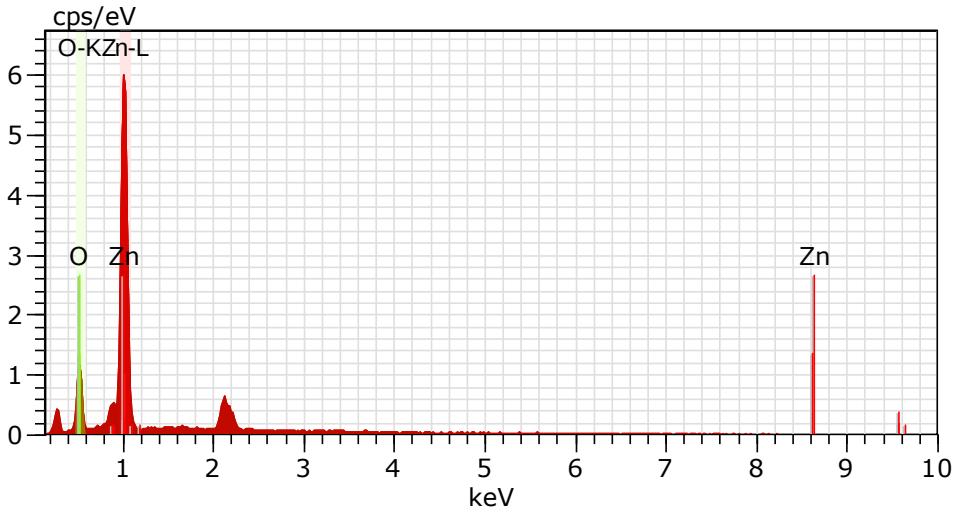

Fig. 9. Energy dispersive X-ray analysis of biosurfactant synthesized ZnO NPs

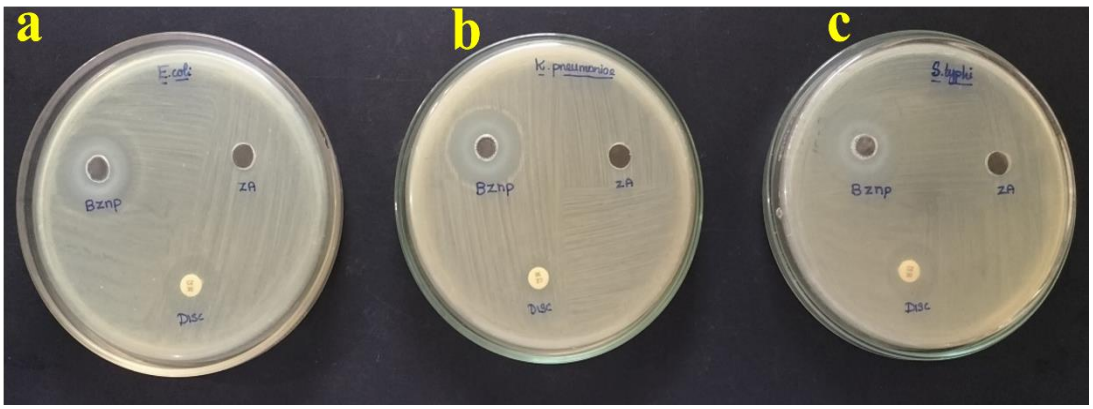

Fig. 10. Antibacterial activity of Biosurfactant synthesized ZnO nanoparticles against the tested microbial species (a*) E. coli*, (b) *K. pneumoniae*, (c) S. *typhi*

Table 2. Inhibition zone generated by Biosurfactant synthesized ZnO nanoparticles against the bacteria *E. coli, P. aeruginosa,* and *K. pneumoniae.*

Name of the Organism	Zone of Inhibition (mm)		
	B+ ZnONPs	ZA	DISC
Escherichia coli	26	Nil	13
Klebseilla pneumoniae	24	Nil	8
Salmonella typhi	25	Nil	15

3.11 Antifungal Assay

Biosurfactant-derived ZnO nanoparticles shown strong antifungal activity against strains of *A. niger, A. flavus,* and *F. oxysporum*. According to the *in vitro* examination completed for this study, disturbing the membrane's integrity allows the test sample to be employed as antifungal agents. As a result, the test samples' effectiveness against *A. niger, A. flavus,* and *F. oxysporum* was shown in Fig. 11. The results showed that biosurfactant synthesized ZnO nanoparticles (20 mg/ml) have potential antifungal activity against fungi that disrupt membrane integrity such as *A. niger, A. flavus,* and *F. oxysporum* with inhibition zones of 17 mm, 12 mm, and 13 mm, respectively (Table 3). Jayaseelan et al. [49] developed a low-cost, basic microbiological strategy for producing ZnO-NPs employing *Aeromonas hydrophila* as an ecologically friendly reducing and capping agent, and they tested their efficiency against dangerous bacteria and fungus. Previous investigations have proven that specifically designed metal nanoparticles have strong antibacterial action. Nanoparticle-based antimicrobial formulations might be efficient bactericidal and fungicidal materials [50]. Jamdagni et al. [51] tested ZnO NPs against *A. alternata, A. niger, B. cinerea, F. oxysporum,* and *P. expansum*. ZnO NPs were effective against all tested fungi, with *A. niger* having the lowest MIC value of 16 µg/mL. Thus, the findings of this research suggest that ZnO NPs might serve as a possible antifungal agent alternative for traditional fungicides, perhaps preventing the development of resistance.

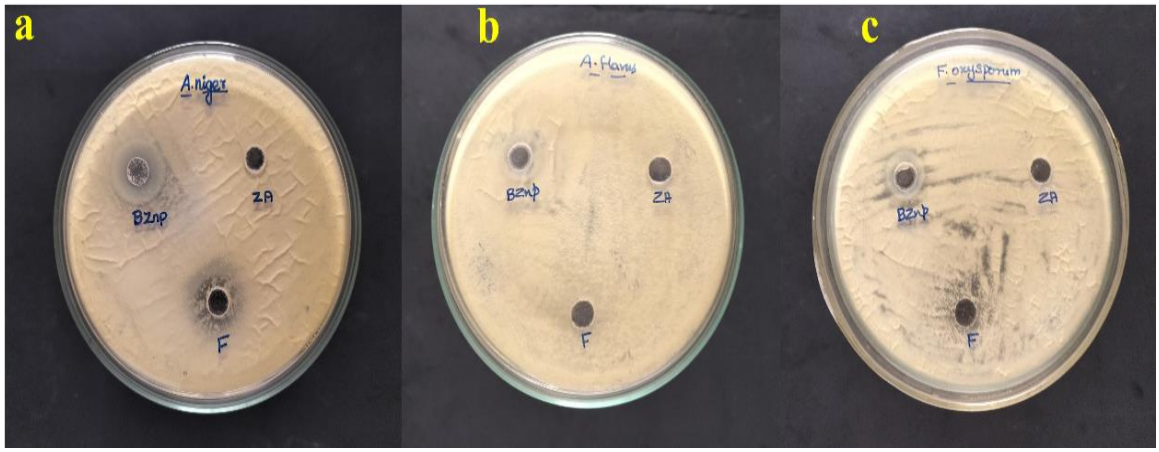

Fig. 11. Anti-fungal activity against Test Pathogens. (A) *Aspergillus niger*, (B) *Aspergillus flavus*, and (C) *Fusarium oxysporum*

Table 3. Antifungal activity of Biosurfactant synthesized ZnO nanoparticles against fungal pathogens (Zone of Inhibition)

Name of the Organism	Zone of Inhibition (mm)		
	B+ ZnONPs	ZA	Fluconazole
Aspergillus niger	17	Nil	Nil
Aspergillus flavus	12	Nil	Nil
Fusarium oxysporum	13	Nil	Nil

4. CONCLUSION

The study identified *Bacillus Pseudomycoides* as producers of biosurfactants, which have potential applications in nanotechnology. Specifically, they can be used in the development of reliable and eco-friendly processes for synthesizing Zinc nanoparticles. In summary, our goal is to find a more environmentally friendly and affordable method for producing biosurfactant mediated ZnO NPs that can be used in various industries such as medicine, textile, detergent, and drug delivery. The results obtained in our study were promising, indicating the successful synthesis of ZnO NPs using a biosurfactant extract from *B. pseudomycoides* strain as a capping agent. This allowed to produce small-sized ZnO NPs with enhanced stability. The synthesized nanoparticles were characterized and confirmed through various analytical techniques, including UV–vis spectroscopy, XRD, FTIR, SEM, and Edax analysis. Moreover, ZnO nanoparticles synthesized using biosurfactant were discovered to highly inhibited activity in *E coli* when compared to the other bacterial pathogens. It is evident that these nanoparticles could spread throughout the growth medium. In addition, the research found that the biosurfactant-synthesized ZnO NPs showed significant antifungal activity against strains of *A. niger, A. flavus,* and *F. oxysporum*. Based on our findings, it is clear that the biosurfactant extract from *Bacillus Pseudomycoides* synthesized ZnO NPs has significant potential as antimicrobial agents.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Vecino X, Rodríguez-López L, Rincón-Fontán M, Cruz JM, Moldes AB. Nanomaterials synthesized by biosurfactants. In: Comprehensive Analytical Chemistry. 2021:94:267-301.
2. Kulkarni P, Chakraborty R, Chakraborty S. Biosurfactant mediated synthesis of silver nanoparticles using *Lactobacillus brevis* (MTCC 4463) and their antimicrobial studies. Int J Pharm Sci Res. 2019:10(4):1753-1759.
3. Rajesh M, Samundeeswari M, Archana B. Isolation of biosurfactant producing bacteria from garbage soil. J Appl Environ Microbiol. 2017:5(2):74-78.
4. Sneha-Chakrabarti. Bacterial Biosurfactant: Characterization, Antimicrobial and metal remediation properties; 2013.
5. Silva RCFS, Almeida DG, Rufino RD, Luna JM, Santos VA, Sarubbo LA. Applications of Biosurfactants in petroleum industry and the remediation of oil spills. Int J Mol Sci. 2014:15 (6):1254-1267.
6. Ahamad Khan M, Lone SA, Shahid M, Zeyad MT, Syed A, Ehtram A, Elgorban AM, Verma M, Danish M. Phytogenically Synthesized Zinc Oxide Nanoparticles (ZnO-NPs) Potentially Inhibit the Bacterial Pathogens: *In Vitro* Studies. Toxics. 2023:11(5):452. DOI: 10.3390/toxics11050452.
7. Mohd Yusof H, Mohamad R, Zaidan UH, Rahman NA. Sustainable microbial cell nanofactory for zinc oxide nanoparticles production by zinc-tolerant probiotic *Lactobacillus plantarum* strain TA4. Microb Cell Fact. 2020:19 (1):1-7.
8. Suba S, Vijayakumar S, Vidhya E, Punitha VN, Nilavukkarasi M. Microbial mediated synthesis of ZnO nanoparticles derived from *Lactobacillus spp*: Characterizations, antimicrobial and biocompatibility efficiencies. Sensors Int. 2021:2:100104.
9. Kamalesh T. Advances in stabilization of metallic nanoparticle with biosurfactants-A review on current trends. Heliyon. 2024: 10(4):e01419.
10. Chandana VK, Hemalatha V, Kalyani DP, Kumar M, Hemalatha KPJ. Biological synthesis and characterization of silver nanoparticles using biosurfactant producing *Bacillus tequilensis*. Int J Curr Adv Res. 2017:6(12):8405-8409.
11. 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):106937.

12. Hazra C, Kundu D, Chaudhari A, Jana T. Biogenic synthesis, characterization, toxicity and photocatalysis of zinc sulfide nanoparticles using rhamnolipids from *Pseudomonas aeruginosa* BS01 as capping and stabilizing agent. J Chem Technol Biotechnol. 2013:88(6):1039-1048.
13. Mulligan CN. Environmental applications for biosurfactants. Environ Pollut. 2005:133(2):183-188.
14. Wu T, Xu J, Liu J, Guo WH, Li XB, Xia JB, Xie WJ, Yao ZG, Zhang YM, Wang RQ. Characterization and Initial Application of Endophytic *Bacillus safensis* Strain ZY16 for Improving Phytoremediation of Oil-Contaminated Saline Soils. Front Microbiol. 2019:10:991.
15. Hentati D, Chebbi A, Hadrich F, Frikha I, Rabanal F, Sayadi S, Manresa A, Chamkha M. Production, characterization and biotechnological potential of lipopeptide biosurfactants from a novel marine *Bacillus stratosphericus* strain FLU5. Ecotoxicol Environ Saf. 2019:167: 441-449.
16. Bezza FA, Tichapondwa SM, Chirwa EM. Synthesis of biosurfactant stabilized silver nanoparticles, characterization and their potential application for bactericidal purposes. Journal of Hazardous materials. 2020; 393:122319.
17. Dehghan-Noudeh G, Housaindokht M, Bazzaz BSF. Isolation, characterization, and investigation of surface and hemolytic activities of alipopeptide biosurfactant produced by *Bacillus subtilis* ATCC 6633. J Microbiol. 2005:43 (3):272-276.
18. De las Rivas B, Marcobal A, Muñoz R. Gene organization of the ornithine decarboxylase-encoding region in *Morganella morganii*. J Appl Microbiol. 2007:102(6):1551-60.
19. Tamura K, Stecher G, Kumar S. MEGA11: Molecular evolutionary genetics analysis version 11. Mol Biol Evol. 2021:38(7):3022-7.
20. Bami MS, Khazaeli P, Forootanfar H, Dehghannoudeh G, Ohadi M. Isolation and Identification of Biosurfactant Producing Bacterial Strain from Saline Soil Samples in Iran: Evaluation of Factors on Biosurfactant Production. Jundishapur J Nat Pharm Prod. 2020:15(4).
21. Sohail R, Jamil N. Isolation of biosurfactant producing bacteria from Potwar oil fields: Effect of non-fossil fuel based carbon sources. Green Process Synth. 2019:9(1):77-86.
22. Tabatabaee A, Assadi MM, Noohi A, Sajadian V. Isolation of biosurfactant producing bacteria from oil reservoirs. J Environ Health Sci. 2005:2(1):6-12.
23. Batool R, Ayub S, Akbar I. Isolation of biosurfactant producing bacteria from petroleum contaminated sites and their characterization. Soil Environ. 2017:36(1): 1-10.
24. Abu-Ruwaida A, Banat I, Haditirto S, Salem A, Kadri M. Isolation of biosurfactant-producing bacteria, product characterization, and evaluation. Eng Life Sci. 1991:11(4):315-324.
25. Ramimoghadam D, Bin Hussein MZ, Taufiq-Yap YH. Hydrothermal synthesis of zinc oxide nanoparticles using rice as soft biotemplate. Chem Cent J. 2013:7:1-0.
26. Azam A, Ahmed AS, Oves M, Khan MS, Memic A. Size-dependent antimicrobial properties of CuO nanoparticles against Gram-positive and-negative bacterial strains. Int J Nanomedicine. 2012:7: 3527-35.
27. Nogueira DR, Mitjans M, Infante MR, Vinardell MP. The role of counterions in the membrane-disruptive properties of pH-sensitive lysine-based surfactants. Acta Biomater. 2011:7(7):2846-2856.
28. Abouseoud M, Maachi R, Amrane A, Boudergua S, Nabi A. Evaluation of different carbon and nitrogen sources in production of biosurfactant by *Pseudomonas fluorescens.* Desalination. 2008:223(1-3):143-51.
29. Walter V, Syldatk C, Hausmann R. Screening concepts for the isolation of biosurfactant producing microorganisms. In: Sen R, editor. Biosurfactants. Springer: 2010:1-13.
30. Sharma D, Ansari MJ, Al-Ghamdi A, Adgaba N, Khan KA, Pruthi V, Al-Waili N. Biosurfactant production by *Pseudomonas aeruginosa* DSVP20 isolated from petroleum hydrocarbon-contaminated soil and its physicochemical characterization. Environ Sci Pollut Res Int. 2015:22(22): 17636-43.
31. Bilska-Zając E, Marucci G, Piróg-Komorowska A, Cichocka M, Różycki M,

Karamon J, Sroka J, Bełcik A, Mizak I, Cencek T. Occurrence of *Alaria alata* in wild boars (Sus scrofa) in Poland and detection of genetic variability between isolates. Parasitol Res. 2021:120:83-91.

32. Dutta RK, Nenavathu BP, Gangishetty MK. Correlation between defects in capped ZnO nanoparticles and their antibacterial activity. J Photochem Photobiol B. 2013:126:105-11.
33. Kundu D, Hazra C, Chatterjee A, Chaudhari A, Mishra S. Extracellular biosynthesis of zinc oxide nanoparticles using *Rhodococcus pyridinivorans* NT2: multifunctional textile finishing, biosafety evaluation and in vitro drug delivery in colon carcinoma. J Photochem Photobiol B. 2014:140:194-204.
34. Narayanan J, Ramji R, Sahu H, Gautam P. Synthesis, stabilisation and characterization of rhamnolipid-capped ZnS nanoparticles in aqueous medium. IET Nanobiotechnol. 2010:4:29-34.
35. Vimala K, Sundarraj S, Paulpandi M, Vengatesan S, Kannan S. Green synthesized doxorubicin loaded zinc oxide nanoparticles regulates the Bax and Bcl-2 expression in breast and colon carcinoma. Process Biochem. 2014:49(1): 160-72.
36. Rajiv P, Rajeshwari S, Venckatesh R. Bio-Fabrication of zinc oxide nanoparticles using leaf extract of *Parthenium hysterophorus* L. and its size-dependent antifungal activity against plant fungal pathogens. Spectrochim Acta A Mol Biomol Spectrosc. 2013:112: 384-7.
37. Das SK, Khan MM, Guha AK, Das AR, Mandal AB. Silver-nano biohybride material: synthesis, characterization, and application in water purification. Bioresour Technol. 2012: 124:495-9.
38. Fang X, Zhai T, Gautam UK, Li L, Wua L, Bando Y, Golberg D. ZnS nanostructures: from synthesis to applications. Prog Mater Sci. 2011:56:175-287.
39. Kumar RV, Vinoth S, Baskar V, Arun M, Gurusaravanan P. Synthesis of zinc oxide nanoparticles mediated by *Dictyota dichotoma* endophytic fungi and its photocatalytic degradation of fast green dye and antibacterial applications. S Afr J Bot. 2022:151:337-44.
40. Faisal S, Jan H, Shah SA, Shah S, Khan A, Akbar MT, Rizwan M, Jan F, Wajidullah AN, Akhtar N, Khattak A, Syed S. Green synthesis of zinc oxide (ZnO) nanoparticles using aqueous fruit extracts of *Myristica fragrans*: their characterizations and biological and environmental applications. ACS Omega. 2021:6(14):9709-22.
41. Arakha M, Saleem M, Mallick BC, Jha S. The effects of interfacial potential on antimicrobial propensity of ZnO nanoparticle. Sci Rep. 2015: 5:9578. doi: 10.1038/srep09578.
42. Jain N, Bhargava A, Panwar J. Enhanced photocatalytic degradation of methylene blue using biologically synthesized “protein-capped” ZnO nanoparticles. Chem Eng J. 2014:243:549-55.
43. Zhang J, Sun, Yin, Su H, Liao, Yan. Control of ZnO morphology via a simple solution route. Chem Mater. 2002:14(10):4172-7.
44. Dutta RK, Nenavathu BP, Gangishetty MK, Reddy AV. Studies on antibacterial activity of ZnO nanoparticles by ROS induced lipid peroxidation. Colloids Surf B Biointerfaces. 2012: 94:143-50.
45. Rajaboopathi S, Thambidurai S. Synthesis of bio-surfactant based Ag/ZnO nanoparticles for better thermal, photocatalytic and antibacterial activity. Mater Chem Phys. 2019:223: 512-22.
46. Li J, Tan L, Liu X, Cui Z, Yang X, Yeung KWK, Chu PK, Wu S. Balancing Bacteria–Osteoblast Competition through Selective Physical Puncture and Biofunctionalization of ZnO/Polydopamine/Arginine-Glycine-Aspartic Acid-Cysteine Nanorods. ACS Nano. 2017:11(11):11250-63.
47. Xiang Y, Li J, Liu X, Cui Z, Yang X, Yeung KWK, Pan H, Wu S. Construction of poly (lactic-co-glycolic acid)/ZnO nanorods/Ag nanoparticles hybrid coating on Ti implants for enhanced antibacterial activity and biocompatibility. Mater Sci Eng C. 2017:79:629-37.
48. Ghdeeb NJ, Hussain NA. Antimicrobial activity of ZnO Nanoparticles prepared using a green synthesis approach. Nano Biomed Eng. 2023:15(1):14-20.
49. Jayaseelan C, Rahuman AA, Kirthi AV, Marimuthu S, Santhoshkumar T, Bagavan A, Gaurav K, Karthik L, Rao KB. 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.

50. Płaza GA, Chojniak J, Banat IM. Biosurfactant mediated biosynthesis of selected metallic nanoparticles. Int J Mol Sci. 2014:15(8):13720-37.

51. Jamdagni P, Khatri P, Rana JS. Green synthesis of zinc oxide nanoparticles using flower extract of *Nyctanthes arbor*-tristis and their antifungal activity. J King Saud Univ Sci. 2018:30:168-75.

Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/3576