Evaluation of the Antibacterial Effect of Biosynthesized Selenium Nanoparticles Conjugated with Different Antibiotics upon Bacterial Strains Isolated from Urinary Tract Infections

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Abstract

Background: The biosynthesis of nanoparticles utilizing entophytic bacteria has received increasing attention, due to its eco-friendly and cost-effective process. **Objective:** The aim of this study firstly is to isolate bacterial isolate from agricultural fields having the ability to generate biogenic selenium nanoparticles, to detect the antibacterial efficacy of biogenic selenium nanoparticles (SeNPs) lonely, and the antibacterial effect of the combination (biogenic SeNPs/antibiotics) separately against the bacterial isolates selected from urinary tract inflammation. **Materials and Methods:** The physical features of biosynthesized selenium nanoparticles (bio-SeNPs) including morphology, size, composition, stability, and external surface were analyzed by using different instruments. The antibacterial activity of bio-SeNPs was determined as minimum inhibitory concentration and minimum bactericidal concentration by applying microdilution assay toward bacterial strains causing urinary tract infections. **Results:** In this study, a selenium-producing bacterium (*Pantoea agglomerans.S10*) was detected by implying the 16S rRNA fragment amplification method. Depending on the result of the energy-dispersive X-ray, the chemical composition of obtained nanoparticles consisted of selenium, carbon, and oxygen atoms. In addition, the scanning electron microscope image illustrated the small and spherical nanoparticles at size 24 ± 2 nm. Dynamic light scattering and zeta-potential results exhibited polyscattred bio-SeNPs with an average size of 127 nm and a negative charge of 29.5 ± 1.9 mV, which demonstrated their stability in liquids. Fourier-transform infrared analysis revealed selenium metal coated with biomolecules including carbohydrates and proteins that are responsible for the stability of selenium nanoparticles. Combination of bio-SeNPs/antibiotics exposed a higher antibacterial effect compared to each of bio-SeNPs, antibiotics alone. **Conclusion:** Considering the biocompatibility of bio-SeNPs, it is suggested that bio-SeNPs be used in pharmaceutical drugs as a combination with antibiotics (amikacin, levofloxacin, and piperacillin). Consequently, this combination is required to increase the antibacterial effect of bio-SeNPs, leading to preventing the emergence of multidrug-resistant bacterial strains that cause urinary tract infections.

Keywords: Bio-SeNPs/antibiotics combination, microdilution assay, nanoparticles biosynthesis, *Pantoea agglomerans.S10*

Introduction

The emergence of antibiotic-resistant bacterial strains is the result of a decrease in global awareness of the dangers of misuse and overuse of antibiotics. It has led to a hidden pandemic that threatens public health.[1] To devastate the destructive effects of antibiotics, pathogens have developed many mechanisms including the production of hydrolysis enzymes, conversion of antibiotic targets, declined permeability to antibiotics by mutation of porins, and getting rid of antibiotics by membrane efflux pumps.[2] This conducted the adoption of various strategies to reduce the prescription of therapeutic antibiotics and select a type of therapy with low efficacy to emerge antibiotics-resistant bacterial strains.[3] In the last few decades, there has been significant interest in research related to the medical usage

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of nanotechnology.[4] Applying nanomaterial in medical treatments is considered an impactful strategy to curtail the proliferation of pathogens.[5] Nanoparticles have many aspects such as increasing their surface area to size proportion, owing unique to physicochemical properties compared to their bulk, which made them used as bioactive agents.[6] Nanoparticles bioactivity can be promoted by adding specified antibiotics to increase their antibacterial activity upon infectious disease.[7] Several bactericidal pathways of nanoparticles, such as disrupting the bacterial membrane and release of intracellular components, are contributed to reduce the probability of different bacteria to become resistant.[8] The bactericidal impact of most nanoparticles belongs to multiple pathways that destroy antibiotics-resistant bacteria[9] involved disrupting the permeability of cell membrane leading destruction of bacterial cells, obstruction of biofilm construction, and finding defects in the function of the efflux pump. Another pathway is related to formation-reactive oxygen species as a result of desperate ions from the surface nanoparticles inside bacterial cells.[10] Selenium (Se) is an essential micronutrient for human health involved in many biological functions such as immune defense, proper functioning of thyroid gland, and reproductive system.[11] According to the estimation of the European Food Safety Authority, the daily dose of Se is allowed at 60–70 µg/day for women and men, respectively.[12] Se can be found in three forms including inorganic and organic compounds and nanoparticles. Due to the direct or indirect antioxidant features of selenium nanoparticles (SeNPs), it has anticancer, antibacterial, and antiviral efficacy to combat cancer cells and infectious diseases.[13,14] There are many strategies for the synthesis SeNPs including physical, chemical, hybrid, and biological methods. Physical method depends on the heat and forces to produce the required size of nanoparticles from its bulk material. Chemical method includes the use of suitable reducing and stabilizing agents to obtain stabilized and small size of NPs.[15] In biological method, the biomolecules and micromolecules are applied to produce nanoparticles.[16] *Pantoea agglomerans* is considered one of those plant endophytic bacteria that have antimicrobial compounds against pathogenic bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Escherichia coli*, and *Candida albicans*.[17] *Pantoea agglomerans* is a member of the enterobacteriaceae existing in many various habitats such as plants, soil, water, animals, and humans. Furthermore, it is considered as biological control agent against the pathogens of apple and pear causing the fire blight.[18] Previous studies demonstrated that *P. agglomerans* produce many of antibiotics, including pantocins, herbicolins, microcins, and phenazines, which are eradicating the growth of *Erwinia amylovora* as well as pathogenic bacteria.[19] Also, it releases volatile organic compounds such as dimethyl disulfide which enhance the interaction between the plant and bacteria through induction of the growth of lateral roots of tomato as well as promotes the secretion of siderophores by *P. putida*.[20]

In the present work, the *P. agglomerans*.*S10* synthesizing biogenic SeNPs was determined by PCR, and the characteristic properties of biogenic SeNPs were reported by applying UV–vis spectrophotometer, scanning electron microscopy (SEM), energy-dispersive spectroscopy (EDS), and Fourier-transform infrared (FTIR) analysis. The goal of this work was to estimate the antibacterial potency of biogenic SeNPs lonely, and the antibacterial effect of the combination (biogenic SeNPs/antibiotics) separately against the bacterial strains isolated from urinary tract inflammation.

Materials and Methods

The selection of bacterial isolates forming selenium nanoparticles

Soil sample was collected from the agricultural fields of Babylon Governorate, then placed in a sterilized container and transferred to the laboratory. First, 1 g of homogenized soil sample was added to 10 mL normal saline, then shacked at 150 rpm/5 min. The suspension was diluted serially to the fifth dilution. An amount of 0.1 mL of diluted tubes was swabbed on trypticase soy agar (HiMEDIA, India) and incubated at 26°C for 2 days. To investigate the ability of bacteria to generate Se nanoparticles, the developing bacterial colonies with a smooth surface were inoculated once on nutrient agar containing sodium selenite (0.5 mM) (HiMedia Laboratories Pvt. Ltd., Thane, Maharashtra, India) and other on nutrient agar, then cultivated aerobically at 26°C for 2 days. The isolated bacterial colony that turned to red color was cultivated on agar slants and kept at 4°C for the following experiments.

Bacterial identification

After performing Gram-staining, cultivation on blood agar and MacConkey agar, and biochemical testes, the identity of bacterial strain growing on nutrient agar containing sodium selenite (0.5 mM) was confirmed by applying the PCR technique. First, the bacterial DNA was extracted by using the boiling method.[21] Then, the PCR Promega kit was used to amplify universal 16 rRNA genes, as the following: 20 pmol of each forward (AGAGTTTGATCCTGGCTCA) and reverse primers (GGTTACCTTGTTACGACTT) were mixed with 25 µm of reaction solution consisting PCR buffer (10 mM), $MgCl_2$ (1.5 mM), deoxynucleotide (200 µL), Taq DNA polymerase (1.25 U), bacterial DNA (2 µL), and distilled water. After electrophoresis processes, the regions containing bands of amplified DNA were removed from 1% agarose. By utilizing the forward and reverse primers in PCR, the amplified DNA was sent to Macrogene/Korea for Sanger sequencing sequenced, and the resulted 16S rRNA sequence was compared by applying BLAST system with nucleotide databases found in gene bank of the National

Center for Biotechnology Information. The Mega5 software was carried out to compare the sequence of bacterial strain with the closed similarity sequences. A phylogenetic tree was drawn using the neighbor-joining method.

Biosynthesis of selenium nanoparticles by bacterial strain (*Pantoea agglomerans* strain S10)

First, the fresh bacterial growth (100 mL) with 0.5 McFarland turbidity was centrifuged for 10 min at 6000 rpm and suspended in 10 mL normal saline. After addition, the bacterial suspension to 1000 mL Luria–Bertani (LB) broth (HiMEDIA, India), 10 mL of filtered sodium selenite solution (1 M) was transferred to LB broth to get the final concentration (10 mM). The flask was covered with foil and incubated in an orbital shaker (150 rpm) at 30°C for 48 h. The conversion color of broth from yellow to red is referring to the production SeNPs. The broth was concentrated to 100 mL, and then 10 mL of Tris was added and kept at 4°C for one night. The obtained broth was sonicated three times and passed out through Whitman paper to get free—cell suspension that was concentrated by centrifugation (13.000 rpm/15 min) to collect the SeNPs.

Evaluation of the antibacterial efficacy of biosynthesized selenium nanoparticles

The antibacterial impacts of bio-SeNPs, amikacin, levofloxacin, and piperacillin were estimated separately against four pure cultures of bacterial strains that were isolated from urinary tract infection including *S. aureus*, *Enterococcus ficalis*, *E. coli*, and *Pseudomonas aerugiosa.* The 96-well plate technique is the most accurate procedure to detect minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of biosynthesized SeNPs. At first, two-fold dilution of SeNPs in 50 µL was carried out in the well of 96-microtiter plate to obtain the following concentrations: 4000, 2000, 1000, 500, 250, 125, 62.5, 31.25, 15.6, 7.8, and 3.75 mg/L. A volume of 50 µL of bacterial growth at 0.5×10^5 cells/mL was incolated, all the walls of microtiter plate except the walls of negative control. Finally, the inoculated micotiter plates were incubated at 37°C for 24 h. After incubation, the MIC detected the first well not having bacterial growth, and then 10 µL from each well having no bacterial growth which were plated on UTI chromogenic agar and kept at 37°C for 24 h to know MBC values representing the decline of 3 log of bacterial growth.

Evaluation of the antibacterial impact of the combination SeNPs/antibiotics

The antibacterial impact of the combination SeNPs/antibiotics at ratio (10:1) was also determined against *S. aureus*, *E. ficalis*, *E. coli*, and *P. aerugiosa.* The antibiotics used in the combination of bio-SeNPs were chosen from three groups owing a different mode of action, including amikacin (aminoglycoside), levofloxacin (quinolone), and piperacilin (β-lactam). As remembered previously in microdilution procedure, two-fold dilution of (bio-SeNPs + amikacin), (bio-SeNPs + levofloxacin), and (bio-SeNPs + piperacillin) in 50 µL was carried out in the well of 96-microtiter plate to obtain the following concentrations: 2000 + 200, 1000 + 100, 500 + 50, 250 + 25, 125 + 12.5, 62.5 + 6.25, 31.25 + 3.125, 15.6 + 1.56, 7.8 + 0.78, 3.75 + 0.375 mg/L). A volume of 50 µL of bacterial growth at 0.5×10^5 cells/mL was incolated, all the walls of microtiter plate except the walls of negative control. Finally, the inoculated microtiter plates were incubated at 37°C for 24 h. After incubation, the MIC was detected as the first well not having bacterial growth, then 10 µL from each well having no bacterial growth were plated on UTI chromogenic agar and kept at 37°C for 24 h to know MBC values representing a decline of 3 log of bacterial growth.

Physical properties of biogenic selenium nanoparticles

The characteristic features of biosynthesized produced by *P. agglomerans.S10* were performed by the following means involved: UV–visible spectroscopy (Lamda 365, PerkinElmer, USA), SEM, energy-dispersive X-ray (EDAX) spectrometer (FESEM, Tescan Mira3), FTIR spectrophotometer (Perkin Elmer, USA), dynamic light scattering (DLS), and zeta-potential analyzer (Zetasizer Nano ZS, Worcestershire, UK). First, the absorbance of biosynthesized SeNPs suspension is measured by UV–visible spectroscopy in the wavelength between 220 and 400 nm. The SEM was used to characterize the morphology and size of biosynthesized SeNPs, and energy-dispersive X-ray spectroscopy was applied to detect the elemental component of obtained biosynthesized nanoparticles. The functional groups of dried biosynthesized SeNPs were determined by applying FTIR spectrophotometer at the wavelength ranging from 500 to 4000 cm^{-1}. Additionally, the hydrodynamic diameter and the charge of biosynthesized SeNPs were done by applying DLS and zeta-potential analyzer, respectively.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before the sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to document number M230101 on January 8, 2023.

Results

Identification of the microorganism

First, a bacterium that has the ability to generate Se nanoparticles was identified according to their appearance on the surface of nutrient agar and selenite salt provided nutrient agar as depicted in Figure 1. The colonies with

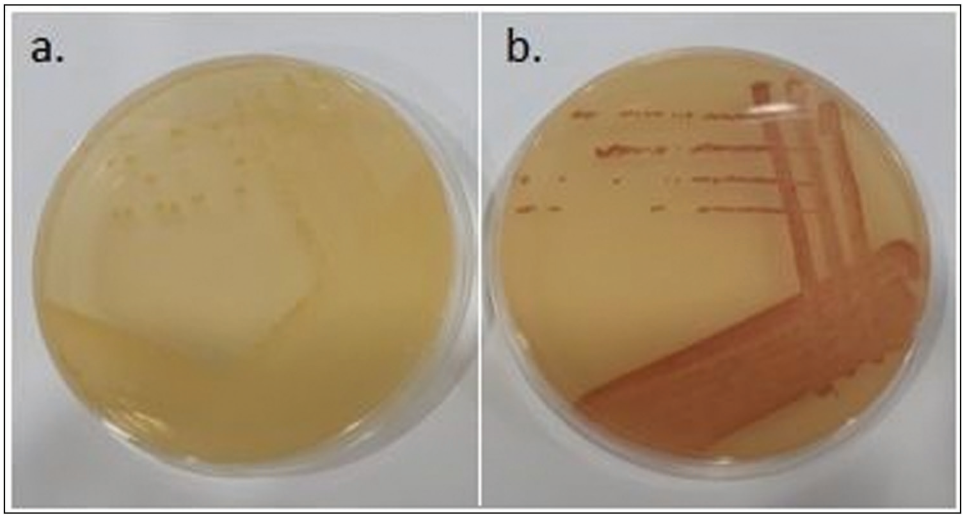

Figure 1: (A) White appearance of *P. agglomerans* colonies in nutrient agar. (B) Red appearance of *P. agglomerans* of colonies in nutrient agar containing sodium selenite (0.5 mM)

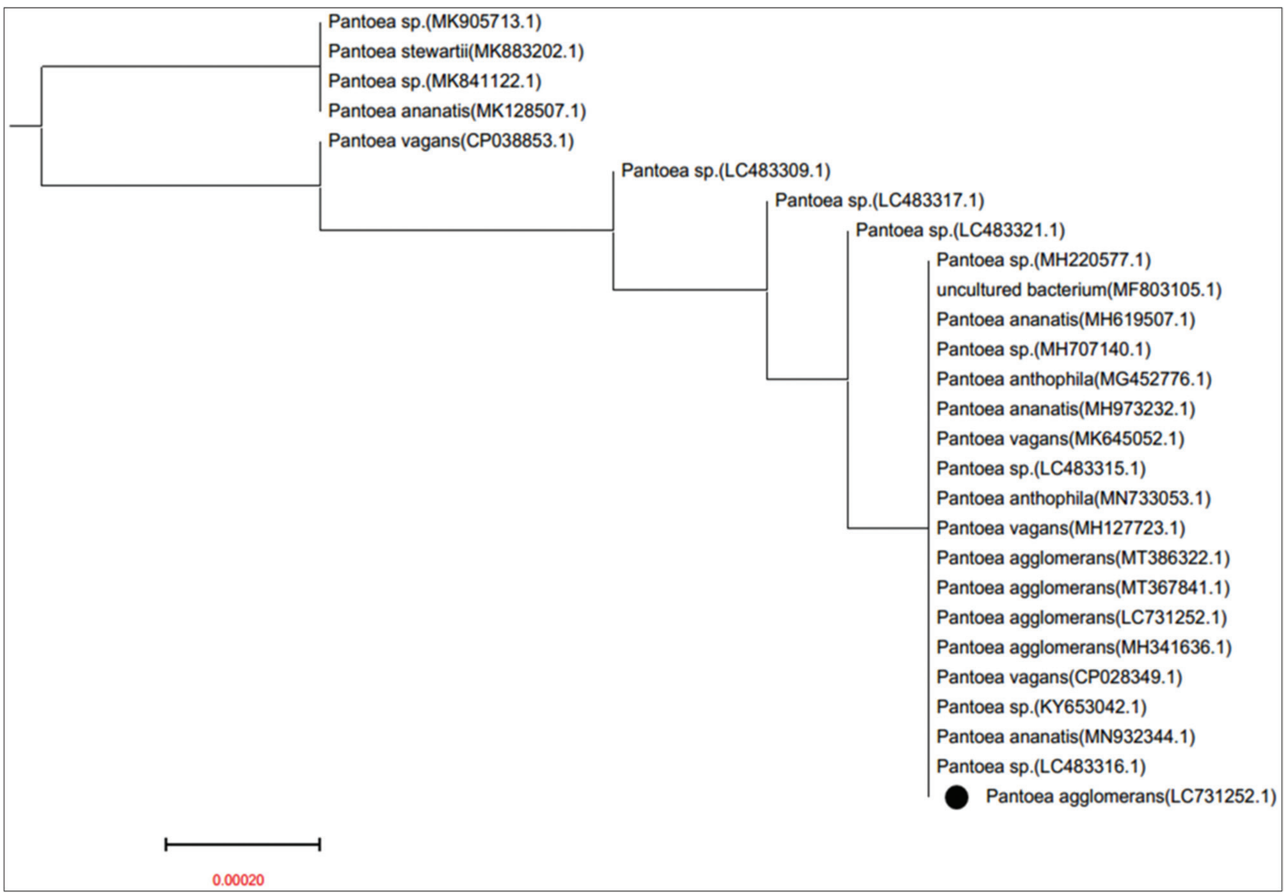

Figure 2: Phylogenetic tree depending on the maximum similarities referred to the genetic relationship of strain OsEp_A&N_15A8 with *P. agglomerans*

red appearance on the media containing selinate salt and white appearance on the media without selinate salt were considered owing the ability to convert selenite ions to Se^0. Many studies informed NADH-dependent nitrate reductase enzyme in microorganism is one of the mechanisms involved in the biological creation of metal nanoparticles. To confirm the identity of strain producing selenium nanoparticles, 16S rRNA was amplified using PCR. The obtained sequence of 16 sRNA gene was compared with sequences in GenBank and the result of alignment exhibits to the presence of similarity reached 100% with the strain of *P. agglomerans*. The result of phylogenetic analysis depends on the maximum similarities referred to genetic relationship of strain (S10) with *P. agglomerans*, which enhances the

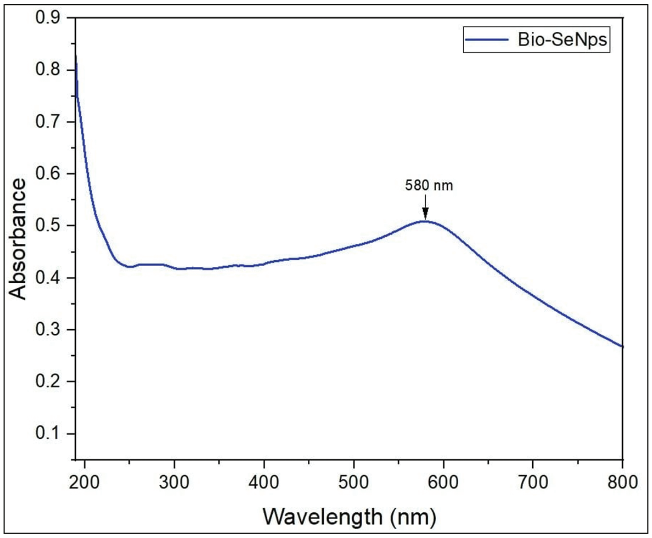

Figure 3: UV–visible spectrum of bio-SeNPs

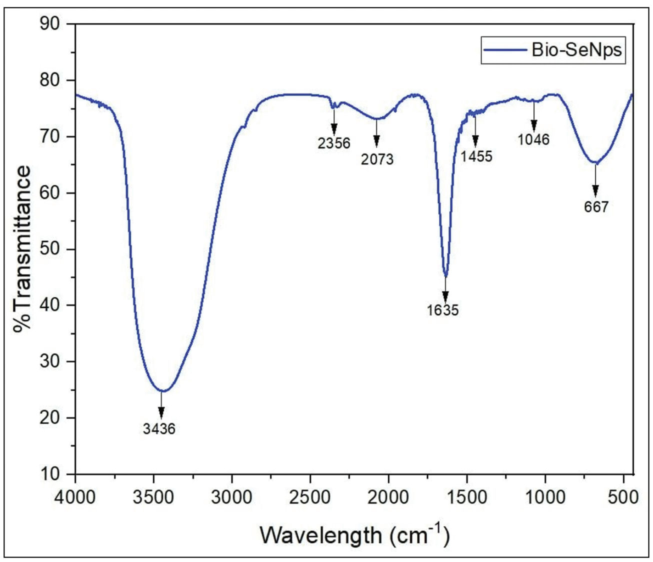

Figure 4: Functional groups presented in the Fourier-transform infrared (FTIR) spectrum of bio-SeNPs

identity of strain producing selenium nanoparticles, as illustrated in Figure 2. The sequence of 16s RNA of determined strain was recorded in NCBI under the name of *P. agglomerans*.S10 with accession number LC731252.1.

P. agglomerans is an endophytic bacteria inhabiting some of their life cycle inside internal tissue of plants.[22] It is able to produce many bioactive compound having antimicrobial and antitumor impacts.[23] In addition, many studies demonstrated that *P. agglomerans* can generate intracellular selenium nanoparticles.[24,25]

Physical properties of biogenic selenium nanoparticles

The shifting color of the culture from yellow to red indicates the ability of *P. agglomerans.S10* to reduce selinate ions to SeNPs. It was confirmed by using a UV–visible spectroscopy in the absorption spectrum between 200 and 600 nm. As observed in Figure 3, an UV–vis spectrum demonstrates a maximum peak at 580 nm, implying the presence of biogenic SeNPs as a result of plasmon resonance excitation.

FTIR was performed to detect the functional groups concerned in the biocreation of SeNPs. As illustrated in Figure 4, the FTIR spectrum of the biosynthesized SeNPs was obtained at 450–4000, which exhibits eight strong absorption peaks at 3436, 2356, 2073, 1635, 1558, 1046, and 667 cm^{-1}. The band at 3436 cm^{-1} corresponds to O–H starching involved in the reduction of Se ions.[22] The sharp band at 1635 cm^{-1} in the spectra corresponds to C–N and C–C bonds indicating the presence of proteins.[23] The peak at 1558 and 1046 cm^{-1} confirms the existence of amide II group. IR spectrum also indicates to the existence of carbohydrates involved in the reduction of Se ion due to the peaks of C–H and C–O–C stretching at 1046 cm^{-1}.[24] The peak around 667 cm^{-1} corresponds to Se metal coated with biomolecules including carbohydrates and proteins that are responsible of the stability of Se nanoparticles.

SEM and EDS analyses were performed to identify the morphological characteristics and chemical composition of bio-SeNPs. SEM images in Figure 5A–D displayed that the bio-SeNPs are spherical in shape with small nanoparticle aggregates as a result of sample preparation. Their size is between 20 and 29 nm with an average size of 24 ± 2 nm. As depicted in Figure 5E, EDS analysis demonstrated that the main component of obtained nanoparticles was Se with the presence of carbon and oxygen which confirms the synthesis of bio-SeNPs by bacterial strain.

The hydrodynamic size and distribution of bio-SeNPs in liquid were determined by applying DLS analysis. As shown in Figure 6A, the size of most biosynthesized nanoparticles is estimated between 100 and 300 nm with an average size of 172 ± 2 nm. The reason for the increase of the average size of bio-SeNPs is probably related to the presence of a few large-sized nanoparticles as well as an agglomeration of small bio-SeNPs. The polydispersity index is low at 0.211, which demonstrates a uniform distribution and homogenecity of bio-SeNPs.[25] The stability and the surface charge of bio-SeNPs were measured by zeta-potential. As observed in Figure 6B, the result of this analysis displayed that bio-SeNPs have a negative zeta-potentials value of 29.5 ± 1.9 mV in deionized distilled water. The negative charge surface of bio-SeNPs belongs to the presence of reductive N–H groups due to their production by bacteria, which confers stability and dispersion in liquid solutions.[26]

Discussion

Pantoea agglomerans is endophytic bacteria inhabiting some of their life cycle inside internal tissue of plants.[27] It is able to produce many bioactive compounds having antimicrobial and antitumor impacts.[28] In addition, many studies demonstrated that *P. agglomerans* can

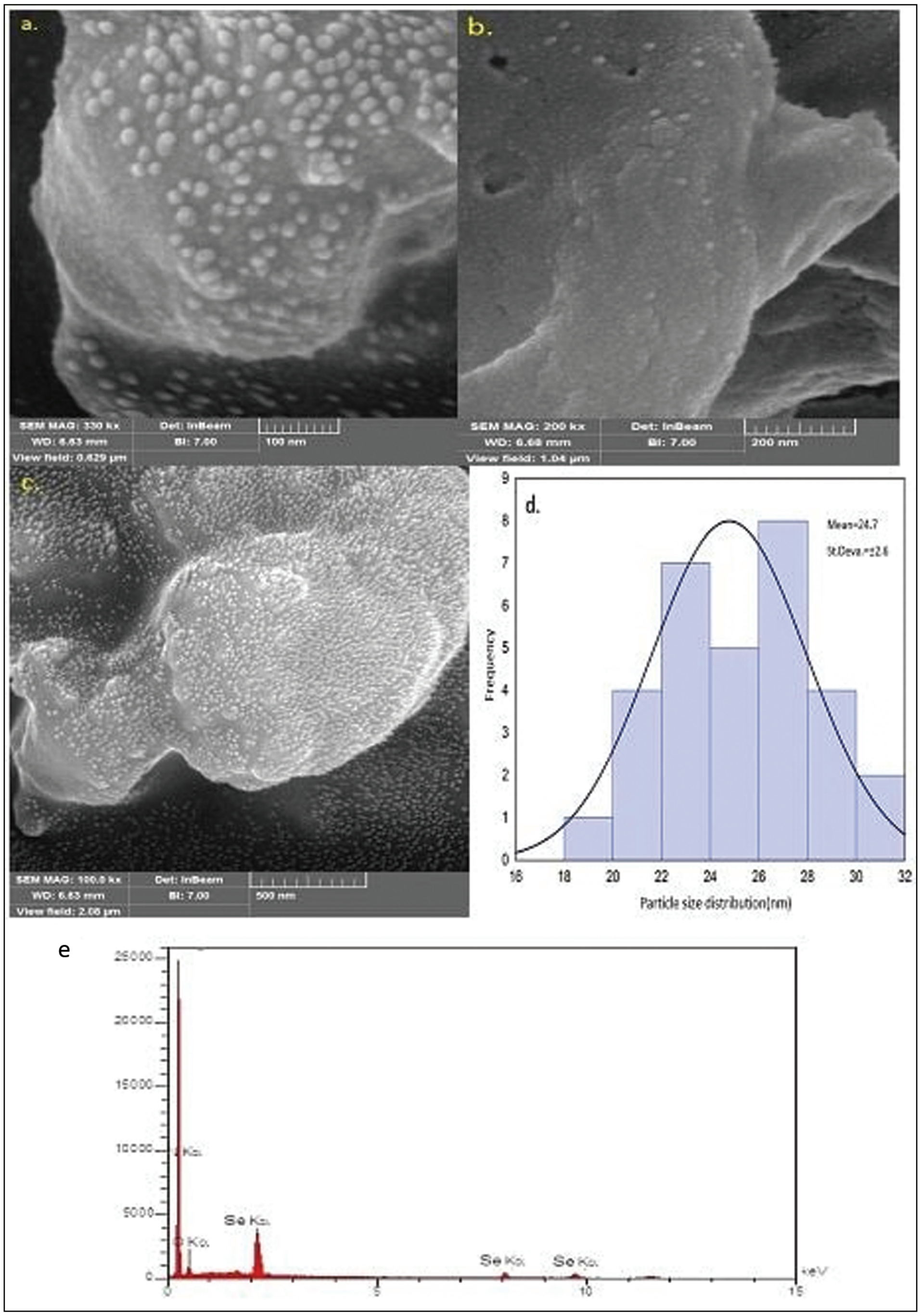

Figure 5: Scanning electron microscope (SEM) images of bio-SeNPs in different magnifications. (A) 100 nm, (B) 200 nm, (C) 500 nm, (D) histogram of bio-SeNPs size distribution showing average size of 24.7 ± 2.6, (E) energy-dispersive X-ray (EDX) spectrum showing the presence of selenium, nitrogen, and oxygen

generate intracellular Se nanoparticles.[29,30] It reduces the metal ions and may also work as a stabilizing agent preventing the aggregation of the created nanoparticles.[31] The study worked by Lampis *et al.*[32] proposed the synthesis bio-SeNPs by *Bacillus mycoides* occurred as result of the reduction of SeO_3^{2-} to Se^0 by the protein released by bacterial cells or thiol groups found on extracellular proteins of the surface of plasma membrane or cell wall.

This study was mostly focused on an evaluation of the MIC and MBC values of bio-SeNPs, amikacin, levofloxacin, piperacillin, and their combinations for the treatment of urinary tract infections. As shown in Table 1, the MIC of bio-SeNPs, amikacin, levofloxacin, and piperacilin were in the range between 250 and 2,000, 15.6 and 31.25, 7.8 and 62.5, 7.8 and 500 mg/mL, respectively. The lowest MIC values of bio-SeNPs, amikacin, levofloxacin, and piperacillin were

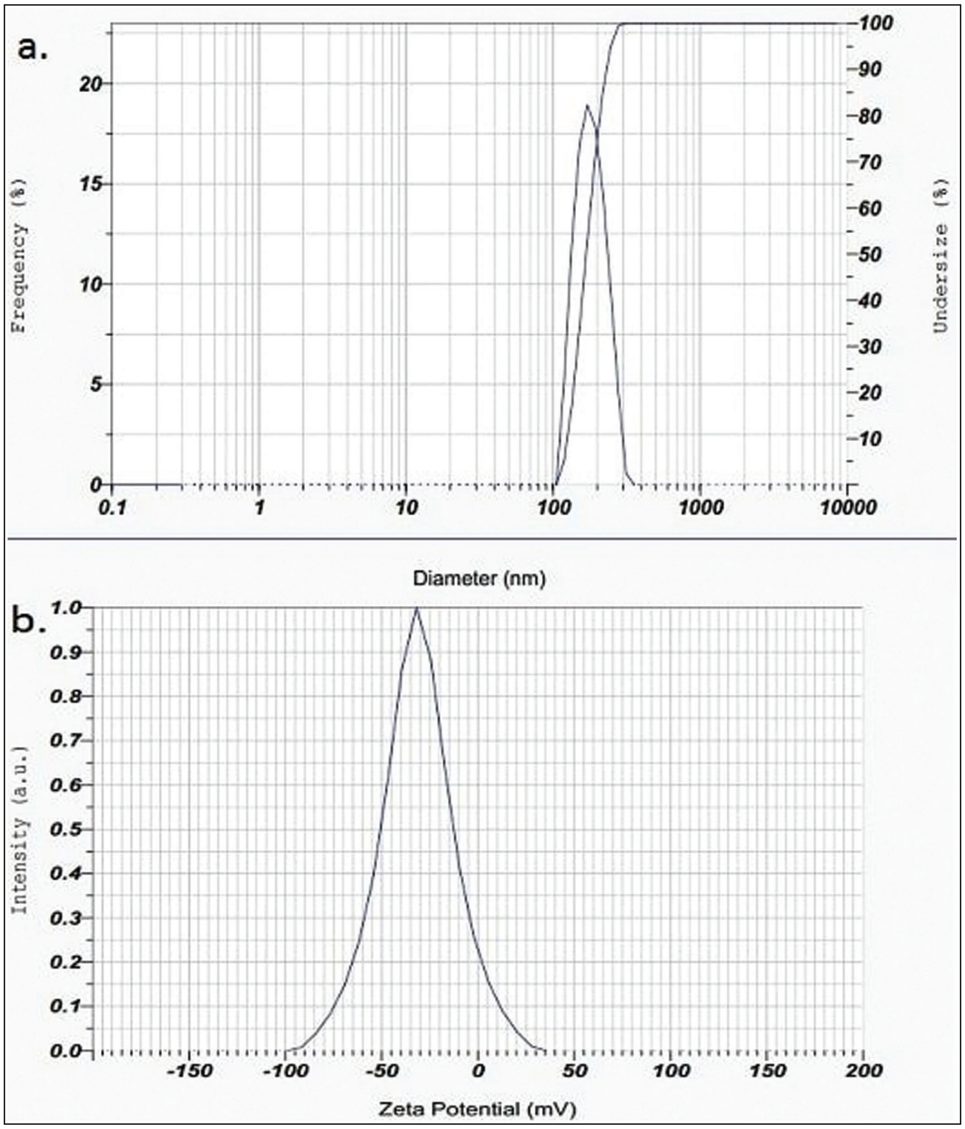

Figure 6: (A) Hydrodynamic of bio-SeNPs measured by dynamic light scattering (DLS). (B) Surface charge of bio-SeNPs by zeta-potentials

Table 1: Minimum inhibition concentration of bio-SeNPs toward different pathogenic bacteria isolated from urinary tract infections

	Minimum inhibition concentration (MIC) (mg/mL)			
	Bacterial strains			
	E. coli	***P. aeruginosa***	***S. aureus***	***E. feacalis***
Bio-SeNPs	250	2000	500	500
Amikacin	31.5	62.5	15.6	15.6
Bio-SeNPs + amikacin	15.6 + 1.56	>500 + 50	3.9 + 0.39	31.25 + 3.12
Levofloxacin	7.8	62.5	7.8	7.8
Bio-SeNPs + levofloxacin	7.8 + 0.78	>500 + 50	125 + 12.5	15.6 + 1.56
Piperacilin	7.8 ± 0	500	–	–
Bio-SeNPs + piperacilin	7.8 + 0.78	>500 + 50	–	–

–: Not tested on Gram-positive bacteria

correspondingly noticed as 250, 15.6, 7.8, and 7.8 mg/mL against *E. coli* (*E. coli*, *S. aureus*), (*S. aureus*, *E. feacalis*), and *E. feacalis*, while highest MIC values of bio-SeNPs, amikacin, levofloxacin, and piperacilin were equaled to 2,000, 62.5, 62.5, and 500 mg/mL against *P. aeruginosa.*

Table 2: Minimum bactericidal concentration of bio-SeNPs toward different pathogenic bacteria isolated from urinary tract infections

	Minimum bactericidal concentration (mg/mL)			
	Bacterial strains			
	E. coli	*P. aeruginosa*	*S. aureus*	*E. feacalis*
Bio-SeNPs	500	>2000	1000	500
Amikacin	31.25	125	31.25	31.25
Bio-SeNPs + amikacin	15.6 + 1.56	500 + 50	7.8 + 0.78	31.25 + 3.12
Levofloxacin	31.25	125	15.6	15.6
Bio-SeNPs + levofloxacin	7.8 + 0.78	>500 + 50	125 + 12.5	15.6 + 1.56
Piperacillin	31.25	500	–	–
Bio-SeNPs + piperacillin	15.6 + 1.56	>500 + 50	–	–

–: Not tested on Gram-positive

In addition, the bio-SeNPs were integrated with various antibiotics (amikacin, levofloxacin, and piperacilin) at a ratio of 10:1 to determine their synergistic effect in arresting tested bacteria. The combination of bio-SeNPs + amikacin, bio-SeNPs + levofloxacin, and bio-SeNPs + piperacilin showed potent antibacterial activity upon infectious bacteria compared to the use of bio-SeNPs, amikacin, levofloxacin, and piperacilin alone. The MIC of bio-SeNPs + amikacin, bio-SeNPs + levofloxacin, and bio-SeNPs + piperacillin on tested bacteria were observed in the range of 3.9 + 0.39–>500 + 50, 7.8 + 0.78–>500 + 50, and 7.8 + 0.78–>500 + 50 mg/mL, respectively. The least MIC of bio-SeNPs + amikacin, bio-SeNPs + levofloxacin, and bio-SeNPs + piperacilin were correspondingly observed as 3.9 + 0.39, 7.8 + 0.78, 7.8 + 0.78 mg/mL against *S. aureus*, *E. coli*, and *E. coli*. The highest MIC of bio-SeNPs + amikacin, bio-SeNPs + levofloxacin, and bio-SeNPs + piperacillin were equaled to >500 + 50 mg/mL against *P. aeruginosa*. As shown in Table 2, the MBC of bio-SeNPs, amikacin, levofloxacin, and piperacilin were in the range of 500–>2,000, 15.6–500, 15.6–125, and 7.8–500 mg/mL, respectively. However, the lowest MBC values of bio-SeNPs, amikacin, levofloxacin, and piperacilin were correspondingly noticed as 500, 31.25, 15.6, and 15.6 mg/mL against (*E. coli*, *E. feacalis*), (*E. coli*, *S. aureus*, and *E. feacalis*), (*S. aureus*, *E. feacalis*), and (*E. coli*). The highest MIC values of bio-SeNPs, amikacin, levofloxacin, and piperacilin were >2000, 62.5, 6.25, 500 mg/mL against *P. aeruginosa.* Generally, the obtained results revealed that bio-SeNPs, amikacin, levofloxacin, and piperacilin have intense lethal effect toward Gram-positive bacteria (*S. aureus*, *E. faecalis*) compared to Gram-negative bacteria (*E. coli*, *P. aeruginosa*). When comparing the antimicrobial activity of tested antibiotics with bio-SeNPs separately. It was obvious that bio-SeNPs owing mild bactericidal effects ranging from 500 to >2000 mg/mL against tested bacteria.

The MBC of bio-SeNPs + amikacin, bio-SeNPs + levofloxacin, and bio-SeNPs + piperacillin on tested bacteria were observed in the range of 7.8 + 0.78–>500 + 50, 7.8 + 0.78–>500 + 50, 15.6 + 1.56–>500 + 50 mg/mL, respectively. The lowest MBC of bio-SeNPs + amikacin, bio-SeNPs + levofloxacin, and bio-SeNPs + piperacillin were orderly arranged at 7.8 + 0.78, 7.8 + 0.78, 15.6 + 1.56 mg/mL against *S. aureus*, *E. coli*, and *E. coli*. The highest MBC of bio-SeNPs + amikacin, bio-SeNPs + levofloxacin, and bio-SeNPs + piperacilin against *P. aeruginosa* was >500 + 50 mg/mL.

The synergistic effect of bio-SeNPs and antibiotics including amikacin, levofloxacin, and piperacillin was clearly demonstrated against *E. coli*, *P. aeruginosa*, *S. aureus*, and *E. feacalis*. The recent data illustrated in Table 1 reported the antibacterial influences of amikacin, levofloxacin, and piperacillin improved in the existence of bio-SeNPs. The obtained result displayed the most synergistic impacts of bio-SeNPs is observed with amikacin compared to levofloxacin and piperacillin. The lower concentrations of bio-SeNPs + amikacin depicted higher antibacterial activities on *S. aureus* (3.9 + 0.39 mg/mL), *E. feacalis* (15.6 + 1.56 mg/mL), *E. coli* (15.6 + 1.56 mg/mL), and *P. aeruginosa* (>500 + 50 mg/mL). As observed in results, the combinations of bio-SeNPs mixed with amikacin, levofloxacin, and piperacilin exhibited the antibacterial efficacy in low concentration against tested bacteria compared to each of them when exposed alone. The study performed by Zonaro *et al.*[33] demonstrated that SeNPs created by two microbial isolates (*Stenotrophomonas maltophilia* and *Ochrobactrum* sp.) exhibited an obvious antibacterial and antibiofilm impact against *E. coli*, *P. aeruginosa*, and *S. aureus*. Cremonini *et al.*[34] reported the antibacterial activity of SeNPs generated by *S. maltophilia* and *B. mycoides* is more active than synthetic Se nanoparticles coated with chitosan. Also, the authors found that biogenic SeNPs have more antibacterial potency upon clinical isolates of *P. aeruginosa* compared to *C. albicanis*. In previous works, researchers have confirmed that synthesized nanoparticles possess antimicrobial efficacy depending on different approaches. Interestingly, the antibacterial activity of synthesized SeNPs is attributed to different surface chemistry and size, which are influenced by materials such as reducing and stabilizing agents used in the synthesis process of nanoparticles.[35]

Conclusion

The endophytic bacterium (*P. agglomerans*) produces bioactive compounds, which effect on pathogenic bacteria. This work assured the isolated bacteria from soil owing to the ability to uptake Se ions and converted it to Se nanoparticles. The nano-Se generated by *P. agglomerans.S10* was appeared as the orange solution with maximum absorbance at 580 nm. The obtained nanoparticles were spherical in shape with a size 24 ± 4 nm as exhibited by SEM image. The bio-SeNPs presented by *P. agglomerans.S10* exhibits antimicrobial impacts toward some pathogenic strains isolated from urinary tract infections including *E. coli*, *P. aeruginosa*, *S. aureus*, and *E. feacalis*. The result exhibited that there is a significant difference in bacterial inhibition activity between bio-SeNPs and bio-SeNPs/antibiotics. Considering the biocompatibility of bio-SeNPs, it is suggested that bio-SeNPs be used in pharmaceutical drugs as a combination with antibiotics (amikacin, levofloxacin, and piperacillin) to increase the antibacterial effect, thus preventing the appearance of multidrug-resistant bacterial strains that cause urinary tract infections.

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Conflicts of interest

There are no conflicts of interest.

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