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Biosynthesis and recovery of rod-shaped tellurium nanoparticles and their bactericidal activities

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ABSTRACT

In this study, a tellurium-transforming *Bacillus* sp. BZ was isolated from the Caspian Sea in northern Iran. The isolate was identified by various tests and 16S rDNA analysis, and then used to prepare elemental tellurium nanoparticles. The isolate was subsequently used for the intracellular biosynthesis of elemental tellurium nanoparticles. The biogenic nanoparticles were released by liquid nitrogen and purified by an n-octyl alcohol water extraction system. The shape, size, and composition of the extracted nanoparticles were characterized. The transmission electron micrograph showed rod-shaped nanoparticles with dimensions of about 20 nm × 180 nm. The energy dispersive X-ray and X-ray diffraction spectra respectively demonstrated that the extracted nanoparticles consisted of only tellurium and have a hexagonal crystal structure. This is the first study to demonstrate a biological method for synthesizing rod-shaped elemental tellurium by a *Bacillus* sp., its extraction and its antibacterial activity against different clinical isolates.

1. Introduction

The field of nanotechnology has seen impressive developments in the synthesis of different inorganic and organic nanoparticles (NPs). Different nanosized materials exhibit unique optical, chemical, biological, photoelectrochemical, and electronic properties because of their large surface/volume ratio and high surface area [1–4]. Different chemical and biological procedures for the synthesis of metal NPs have been published [5–7]. Among the synthetic procedures studied, clean, nontoxic, and eco-friendly methods have received considerable attention from biologists and chemists [5,6,8]. Different prokaryotic microorganisms and eukaryotic cells, i.e., fungi, bacteria, algae, and plants, have been tested for this purpose and many synthesis protocols have been developed for the fabrication of metal NPs [9–11]. Silver and gold NPs are frequently prepared by biological or chemical methods, and have found wide application in medicine and health sciences. In the past, researchers focused primarily on the extracellular or intracellular biosynthesis of important NPs, such as Ag and Au NPs [11,12]. Numerous studies have addressed the biological synthesis of Ag or Au NPs of various shapes and sizes [8,11–14]. In recent years, increasing attention has also been directed toward the biosynthesis of other important metal or mineral NPs, such as those of selenium [8,15,16]. The low toxicity of elemental NPs makes them excellent materials for application in medicine and veterinary sciences [7,9,10,15]. Over the past few years, tellurium (Te) and its related inorganic compounds have also been extensively used in various industries. In much the same way as sulfur and selenium, Te can be found in four oxidation states: −2 (H_2Te), +2 (TeO_2^{2-}), +4 (TeO_3^{2-}), and +6 (TeO_4^{2-}) [10]. It has applications in different fields, including use in metal alloys to improve the properties of various steels, in solar panels, glasses, new rechargeable batteries, and the metallurgy, electronics, textile, mining, and chemical industries [17–19]. In medicine, Te compounds have traditionally been used as an antimicrobial agent in the treatment of infectious diseases [20,21], such as leprosy, tuberculosis, dermatitis, cystitis, and severe eye infections [17,20,21]. Historical evidence attests to the antibacterial effect of K_2TeO_3 (potassium tellurite), which was studied with penicillin by Alexander Fleming in 1932 [20,21]. New significant biological effects, such as the inhibition of cytokine production by T cells, antisickling, and immunomodulatory activities that protect dopaminergic neurons, have been reported for this element [21,22]. As a novel application, Te is used in fluorescent detection probes as CdTe quantum dots [19,23]. However, it is rarely found in

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nontoxic and elemental form (Te°). It exists in nature as a soluble oxyanion, with forms such as tellurite (TeO_3^{2}) and tellurate (TeO_4^{2}). These Te salts have exhibited considerable toxicity to living organisms even at very low concentrations [18]. Toxic metal ions, including TeO_3^{2} or TeO_4^{2}, are usually transformed into inert metal particles using microorganisms [21,24,25]. These particles are deposited within the cytoplasm or cytoplasmic envelope of bacteria or fungi and, in some cases, generate extracellular diffusion [21,24,25].

In the present study, we screened different samples from various areas of Iran, namely, the Caspian Sea, Maharlo Salty Lake, Sarcheshmeh Copper Mine, and Ali-Sadr Cave, to isolate a Te NP-producing microorganism. After identifying the isolated strain, we used it to biosynthesize Te NPs. The prepared NPs were separated by a liquid–liquid solvent partition method and fully characterized using different instruments. The antibacterial effects of these biogenic Te NPs were evaluated against *Staphylococcus aureus, Salmonella typhi, Klebsiella pneumonia*, and *Pseudomonas aeruginosa* using the conventional serial agar dilution method.

2. Experimental

2.1. Isolation of Te NP-producing bacteria

Freshwater or soil samples from the Caspian Sea (located in the northern part of Iran), Maharlo Salt Lake (southern Iran), Sarcheshmeh Copper Mine (central Iran), and Ali-Sadr Cave (western Iran) were collected and transferred into sterile bottles or cans. All the collected samples were diluted with a sterile NaCl solution (0.9%). The samples were then spread on agar plates containing a nutrient agar (NA) medium (Merck, Germany), and supplemented with 200 mg l^{-1} of Te^{4+} ions (equivalent to 482.8 mg of K_2TeO_3, $3H_2O$ per liter) by a filter-sterilized stock solution of potassium tellurite (Merck). The plates were incubated aerobically at 30 °C. Several black colonies were observed on the plates after 24 h. Black is the color of precipitated metalloid Te NPs and therefore serves as a provisional marker of Te^{4+} ion reduction by the bacterial colony [17,21]. To ensure that the color was not an organic bacterial pigment, i.e., melanin, we re-streaked the colonies on plain NA plates without K_2TeO_3, after which all the plates were macroscopically examined for black organic pigment production during the incubation period (48 h) at different temperatures (25, 30, and 37 °C). The organism was constantly conserved on the NA plates supplemented with Te^{4+} ions (200 mg l^{-1}) at 4 °C. A bacterial sample was also cultivated in glycerol-supplemented nutrient broth and preserved at −70 °C.

2.2. Identification of the isolate

The phenotypic and physiological characterization of the isolate was carried out by the methods described in Bergey's *Manual of Determinative Bacteriology* and 16S rDNA (ribosomal DNA) sequence analysis [26,27]. To obtain genomic DNA, bacterial cells were harvested from an overnight culture, and the genomic DNA of the isolate was extracted with a Genelute DNA extraction kit (Sigma) following the manufacturer's recommended procedure. The bacterial cell mass was washed three times with sterile distilled water and boiled for 10 min to release total DNA. The suspension was centrifuged at 11,000 × *g* for 5 min, and then the supernatant was used as the DNA template for the PCR amplification of the 16S rDNA gene. The PCR amplification included an initial denaturation at 94 °C for 180 s, 30 cycles of denaturation at 94 °C for 60 s, annealing at 60 °C for 45 s, and a final extension at 72 °C for 90 s. A large fragment of the 16S rDNA gene was amplified using primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1525R (5′-AAGGAGGTGATCCAGCC-3′). These primers are located in the 16S rDNA of *Escherichia coli* at nucleotide positions 8–27 and 1525–1541 respectively. The amplified DNA fragment was purified from 1% agarose gel using the QIAquick Gel Extraction Kit (Qiagen) according to the supplier's instructions. The DNA fragment was subsequently sent for automated sequencing using the abovementioned primers (Takapouzist Co., Iran). Sequence similarity searches were conducted using the BLAST database (National Center for Biotechnology Information). The sequence was submitted to the GenBank® Nucleotide Sequence Database (GenBank: HQ011918.1).

2.3. Determination of Te^{4+} ion concentration

A fast, simple, and reliable chemical method recently described for the direct spectrophotometric determination of residual Te^{4+} ion concentration in bacterial culture media was used in this study [28]. With this method, the Te^{4+} ions were reduced to elemental Te using $NaBH_4$ (4.5 mM). Then the turbidities of formed colloids were measured at 500 nm with a UV–visible spectrophotometer (UVD-2950; Labomed). A standard calibration curve was constructed and used to determine the residual Te concentrations in NB media. Appropriate amounts of K_2TeO_3 (Merck) were precisely weighed and added to the NB culture medium (100 ml) in order to obtain Te ionic solutions with concentrations of 6.25, 12, 25, 50, 100, 150, and 200 mg ml^{-1}. Subsequently, all prepared standard solutions (1 ml) were reduced using freshly prepared $NaBH_4$ solution (4.5 mM). The optical densities of all the prepared colloids were measured at a wavelength of 500 nm. The standard curve was constructed by plotting the Te ion (Te^{4+}) concentrations versus the obtained optical densities at each concentration (r^2 = 0.994).

2.4. General procedure for growth experiments and Te ion reduction

The agar dilution method was used to determine the minimal inhibitory concentration (MIC) of the Te ions [29]. A set of growth experiments was carried out to study the ability of the isolate to convert the Te^{4+} ions into Te NPs in NB medium (pH 7). Sub-MIC concentrations (50 and 100 mg l^{-1}) of the Te^{4+} ions (corresponding to 120.7 and 241.4 mg of K_2TeO_3 and $3H_2O$ per liter respectively) were prepared in sterile NB by adding these ions from a filter-sterilized stock solution of K_2TeO_3. Approximately 100 ml of the medium were transferred into sterile 500 ml Erlenmeyer flasks and inoculated with 1 ml fresh inoculums (OD 600 nm: 0.1). For aerobic cultivation, the flasks were plugged with cotton and incubated at 30 °C in a shaker incubator (150 rpm). In a similar trial, uninoculated media that contained Te^{4+} ions (200 mg l^{-1}) and inoculated media without Te^{4+} were used as the controls. Samples were withdrawn periodically under aseptic conditions to measure OD_{600} and perform analysis for the residual Te^{4+} ions in the culture media. Before Te analysis, the cells and NPs were removed from the culture by centrifugation at 11,000 × *g* for 30 min with a Mikro 200 centrifuge (Hettich). For the spectrophotometric detection of the remaining Te^{4+} ions, 1 ml of the supernatant from the inoculated media containing Te^{4+}ions, and 1 ml of the supernatant from the inoculated media without Te^{4+} ions were separately used as the test and blank controls respectively. These experiments were repeated three times on different days. To investigate the ability of the cell-free supernatant to reduce Te^{4+}, 100 ml of sterile NB was inoculated using fresh inoculums of bacterial isolate (D_{600} 0.1). After 24 h, the cells were separated by centrifugation at 4000 × *g* for 10 min. The supernatant was filtered aseptically in a sterile 500 ml Erlenmeyer flask using a filter (0.22 μm), after which the Te^{4+} ions were added to the supernatant (final concentration, 100 mg l^{-1}). The flasks were plugged with cotton and incubated at 30 °C in a shaker incubator (150 rpm), and checked periodically for Te NP formation.

2.5. Preparation of Te NPs

A bacterial isolate from the Caspian Sea was used for the biosynthesis of the Te NPs. Sterile NB was prepared and supplemented with a sterile potassium tellurite solution (482.8 mg l^{-1}) under aseptic conditions. Subsequently, the sterile NP was inoculated with 1 ml of the fresh inoculums of *Bacillus* sp. BZ (D_{600} 0.1) and incubated aerobically at 30 °C in a shaker incubator (150 rpm) for 48 h. Thereafter, the bacterial cells and Te NPs were removed from the culture medium by centrifugation at 10,000 × *g* for 30 min. The pellets were washed with a 0.9% NaCl solution by centrifugation and transferred into a mortar. By adding liquid nitrogen, the pellets were frozen and then disrupted by a pestle. The resultant slurry was ultrasonicated at 100 W for 5 min and washed three times by sequential centrifugation (11,000 × *g*, 5 min) with 1.5 M Tris/HCl buffer (pH 8.3) containing 0.5% SDS and deionized water. The pellets were suspended in deionized water, and the resultant suspension containing Te NPs and cell debris was collected. Further purification of the Te NPs was conducted following Shakibaie et al. [9]. Approximately 4 ml of the above-mentioned suspensions were separately transferred into test tubes, with 2 ml of n-octyl alcohol added to each tube. The mixtures were then shaken vigorously. Two mixed phases were completely separated by centrifugation at 2000 × *g* for 5 min and stored at 4 °C for 24 h. After storage, the generated Te NPs could be observed at the bottom of the tubes. The lower and upper phases were discarded, and settled NPs were washed with chloroform, ethyl alcohol, and distilled water. A stock solution of Te NPs was prepared (10 mg ml^{-1}) in deionized water and stored at 4 °C for further experiments. To ensure that all cell debris was removed, the purified Te NPs were re-suspended in the liquid–liquid phase system following the same steps.

2.6. Characterization of Te NPs

The Te NPs were characterized using different techniques. The UV–visible spectrophotometer, operated at a resolution of 1 nm, was used to determine the optical absorption property of the extracted Te NPs. Infrared spectra were recorded as KBr pellets or films by Fourier Transform Infrared (FTIR) spectroscopy (Bomen MB-154), a technique used to detect whether any functional groups appear on the surface of NPs. For transmission electron microscopy, micrographs were obtained using a TEM Zeiss 902A, operated at an accelerating voltage of 200 kV. To observe the surface features of *Bacillus* sp. BZ and determine the elemental composition of the NPs, scanning electron microscopy (SEM) coupled with energy dispersive X-ray (EDX) analysis, was conducted. The samples were analyzed using a scanning electron microscope (Philips XL30) operated at 16 kV, and EDX spectra were recorded by focusing on a cluster of the NPs. Moreover, the powder X-ray diffraction patterns of the NPs were recorded on a PW3710 MPD instrument using Cu Kα radiation in the range $2\theta = 4–90^{\circ}$.

2.7. Evaluation of the antibacterial effect of Te NPs

Susceptibility tests were carried out by the conventional serial broth dilution method with modifications. Müller–Hinton Broth (MHB) was supplemented with different concentrations of Te NPs ranging from 0.062 to 2 mg ml^{-1}, and inoculated with 5×10^5 colony forming units (CFU ml^{-1}) of different clinical isolates (*S. aureus, S. typhi, K. pneumonia, and P. aeruginosa*). All the culture media were incubated at 37 °C for 24 h. After incubation, time aliquots (20 μl) of TTC solution (2,3,5-triphenyl-2H-tetrazolium chloride) (0.5 mg ml^{-1}) were added into the culture media and shaken. All the test tubes were incubated for an additional 2 h. The data was reported as MICs, and the lowest concentration of the Te NPs demonstrated no visible red color after the additional incubation period (2 h) at 37 °C. Because the Te NPs were black, the color may have caused difficulty in growth observation; to solve this problem, we used TTC reagent in the MIC determination assay [30]. The minimum bactericidal activity (MBC) of the Te NPs was also measured by inoculating the broths used for the MIC determination onto Te-free Müller–Hinton Agar plates. After incubation at 37 °C for 24 h, the lowest Te NP concentrations required to kill the germs of the test strains were considered the MBCs.

3. Results and discussion

3.1. Identification of the microorganism

Although different water and soil samples were collected from different areas of Iran, we chose the Ramsar Beach sample, which contains the bacterium with the highest ability to convert Te^{4+} ions into elemental state ions (Te^0). The left-hand image in Fig. 1 shows the cultures of this isolate on the plain NA (Plate A) and Te-supplemented NA (Plate B) media. The culture flasks containing *Bacillus* sp. BZ were cultivated in NB media (A) with and without (B) Te^{4+}ions. The flasks were then incubated at 30 °C for 24 h (right-hand image, Fig. 1). In the morphological studies, the isolated strain appeared as a nonmotile, rod-shaped Gram-positive and endospore-forming bacterium with yellowish white colonies on

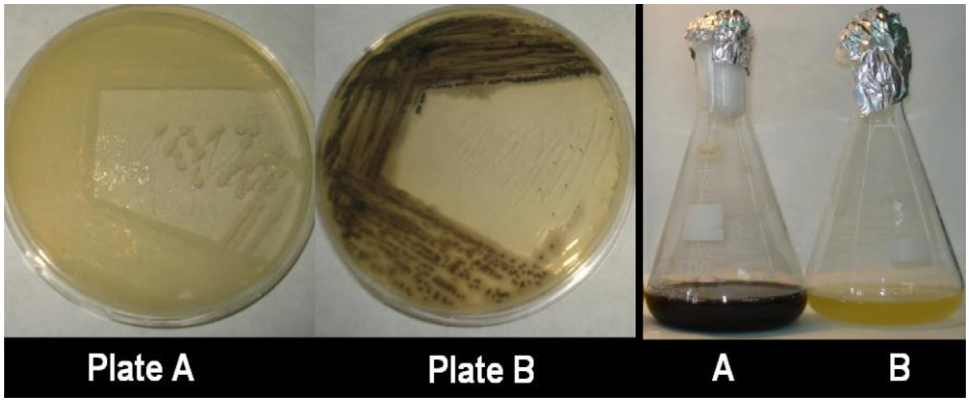

Fig. 1. Image of the nutrient agar plates with (Plate B) and without (Plate A) K_2TeO_3 salt streaked with isolated *Bacillus* sp. BZ (left-hand image). This isolate transformed Te ions into elemental Te NP black colonies (Plate B). Culture flasks containing *Bacillus* sp. BZ cultivated with (A) and without (B) Te^{4+} ions, and incubated at 30 °C for 24 h (right-hand image).

Table 1
Biochemical characteristics of *Bacillus* sp. BZ.

Characteristics	Result
Catalase production	+
Oxidase activity	–
Voges–Proskauer test	–
pH in the Voges–Proskauer broth	>6
Methyl red test	+
Acid formation from	
D-glucose	+
L-arabinose	–
D-xylose	–
D-mannitol	+
Hydrolysis of casein	–
Hydrolysis of gelatin	–
Hydrolysis of starch	+
Utilization of citrate	+
Nitrate reduced to nitrite	+
Formation of indolen	–
Formation of dihydroxyacetone	–
Growth in NaCl solution	
2%	+
5%	+
7%	+
10%	–
Growth at	
5 °C	–
10 °C	–
30 °C	+
40 °C	+
50 °C	–

the NA media. On the basis of these results and other biochemical tests (Table 1), we inferred that the isolated bacterium is a strain of the *Bacillaceae* family. A BLAST search of the 16S rDNA sequence against the NCBI GenBank® Nucleotide Sequence Database confirmed the morphological and biochemical results. Alignment results containing 102 characters revealed 98% identity with several members of the genus *Bacillus*. The *Bacillus* sp. BZ 16S rDNA sequence (1447 bp) was submitted to the NCBI GenBank® Nucleotide Sequence Database (accession number HQ011918).

3.2. Reduction of Te^{4+} ions into Te NPs

Fig. 2 shows the optical densities of the broth cultures supplemented with sub-inhibitory concentrations of Te^{4+} (50 and 100 mg l^{-1}) and inoculated by *Bacillus* sp. BZ during incubation in a shaker incubator (150 rpm) for 48 h. The spectrophotometric measurement of the Te^{4+} ions showed acceptable linearity between the absorbance obtained at 500 nm and serial Te^{4+} concentrations (1–200 mg $.l^{-1}$) (inset, Fig. 3). The time course study of the Te^{4+} reduction by the abovementioned isolate revealed that the reduction process took place with different profiles under different concentrations of Te^{4+} ions (0, 50, and 100 mg ml^{-1}) (Fig. 3). The Te ions were reduced by *Bacillus* sp. BZ in the NB culture supplemented with Te^{4+} ions. No reduction of Te^{4+} was detected in the uninoculated NB (Fig. 3).

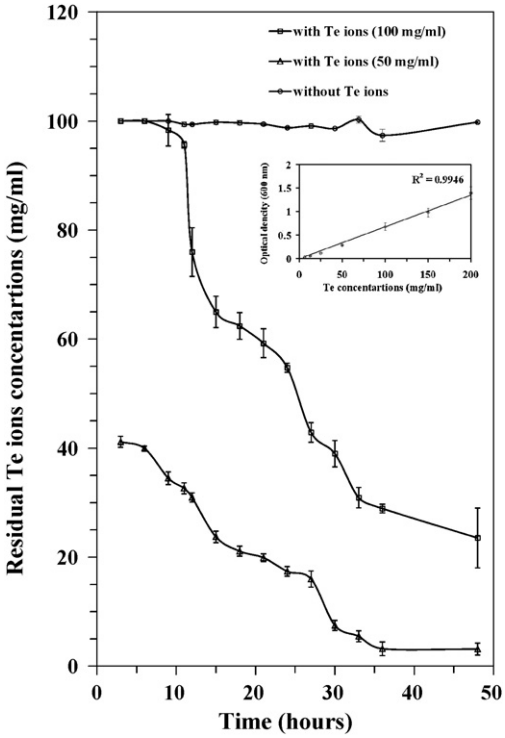

Fig. 3. Reduction pattern of Te ions at different concentrations. The symbols represent the mean values of three experimental growth cultures and the bars indicate standard deviations. The inset shows the standard curve for the determination of Te ion concentration in the nutrient broth.

3.3. Te NP preparation and characterization

The Te NPs were separated from the cell debris during extraction by a two-phase separation system following a recently described method [9]. The UV–visible spectrum of the extracted NPs is shown in Fig. 4. A band observed in the spectrum (Fig. 4),

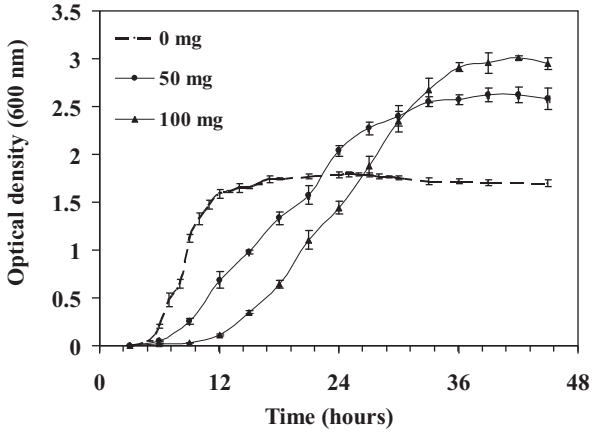

Fig. 2. Optical densities of *Bacillus* sp. BZ culture under different concentrations of Te ions. The symbols represent the mean values of three experimental growth cultures, and the bars indicate standard deviations.

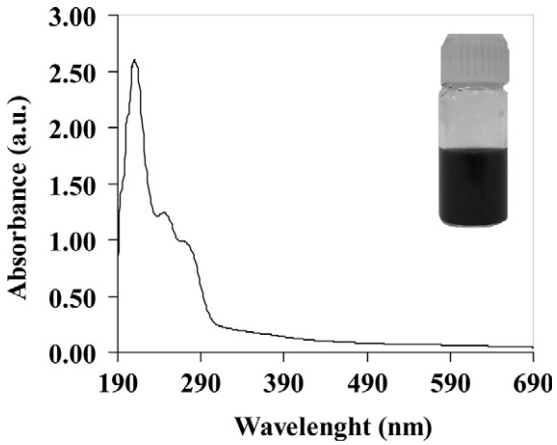

Fig. 4. UV–visible spectrum of the biogenic Te NPs synthesized by *Bacillus* sp. BZ isolated from the Caspian Sea (a). A vessel contains extracted Te NP suspension. An image of the extracted Te colloids is also shown in this figure.

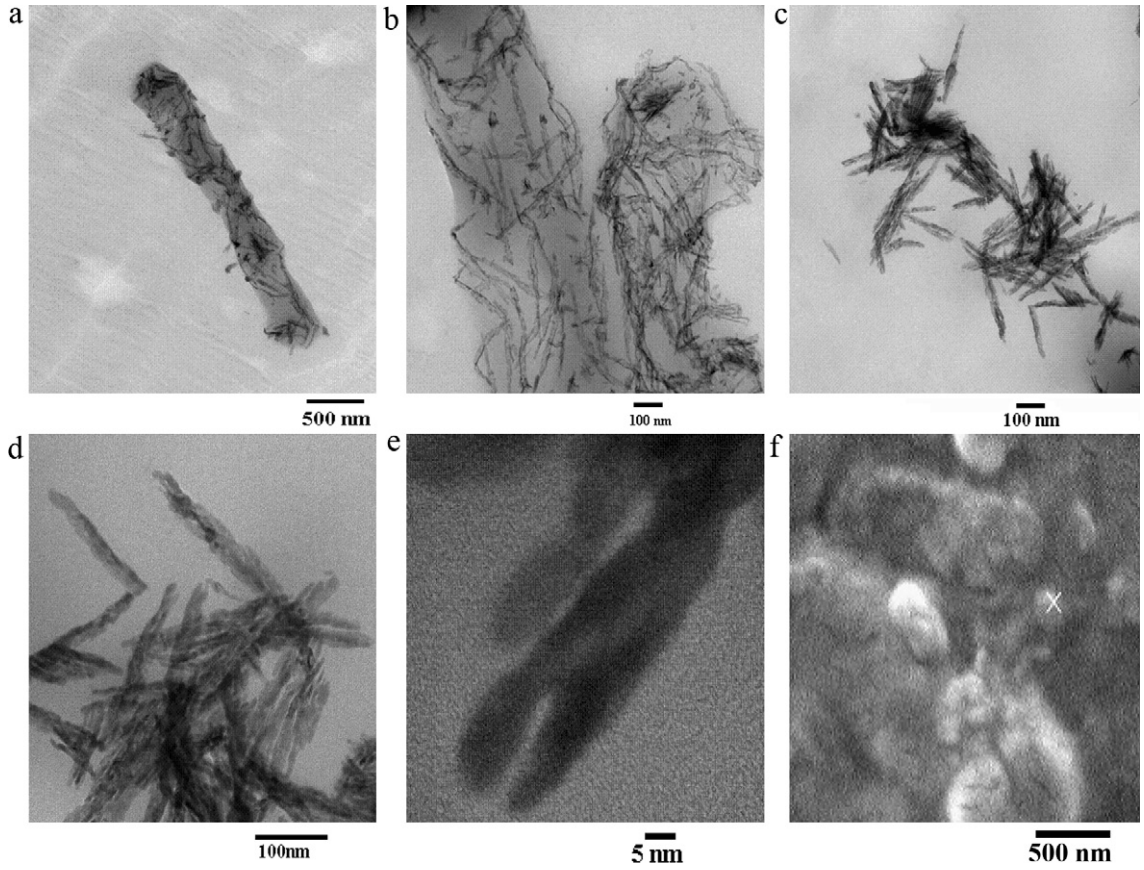

Fig. 5. TEM and SEM images of the biogenic Te NPs and *Bacillus* sp. BZ. (a and b) TEM thin section showing internal cellular accumulations of elemental Te NPs in *Bacillus* sp. BZ. (c and d) TEM micrograph of extracted Te NPs. (e) High-resolution TEM image showing the mono lattice plane of the Te NPs. (f) SEM image of *Bacillus* sp. BZ.

corresponding to surface plasmon resonance, occurred at 210 nm, indicating the formation of Te NPs [31]. The inset in the figure shows that a vessel contained extracted Te NP colloids. The TEM images of different magnifications (c–e) shown in Fig. 5 demonstrate that the extracted Te NPs were rod-shaped. The figure also shows deposited *Bacillus* sp. BZ in intercellular spaces (a and b). The lengths of these rods were nearly 180 nm and their widths were less than 20 nm. However, they appeared larger than their actual size and could accumulate. The SEM image of *Bacillus* sp. BZ shows rod-shaped microorganisms (Fig. 5f). Fig. 6 shows that the EDX microanalysis of the purified NPs exhibited main Te absorption peaks at 3.72 keV [31]. The elemental composition analysis showed the presence of strong signals from 100 wt % Te atoms without other atom signals. The phase and purity of the NPs were further examined by X-ray diffraction (XRD, Fig. 7). All of the reflections in Fig. 7 can be indexed on a hexagonal phase of Te crystal (hexagonal space group: *P*3121 (152) JCPDS 36-1452) [32].

FTIR spectroscopy was performed to analyze the functional organic groups attached to the surface of the Te NPs. Fig. 8 shows a FTIR spectrum of biogenic nanotellurium with some typical absorption bands; such as the strong absorption at 1741 cm^{-1} and 1065 cm^{-1} that were corresponded to carbonyl (C=O) and ester (C–O) stretching vibrations respectively [34,35]. The absorption band at 2967 cm^{-1} was related to hydrocarbon (C–H) stretching vibrations. Three peaks at 1458.5, 1377.8 and 769 cm^{-1} were also related to ethyl group bending vibrations. The medium peaks at

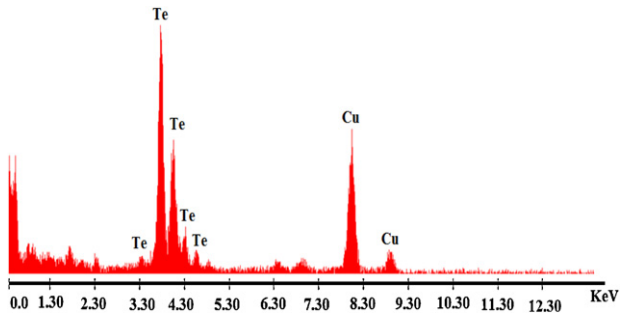

Fig. 6. Energy dispersive X-ray spectrum of the Te NPs that were extracted from *Bacillus* sp. BZ.

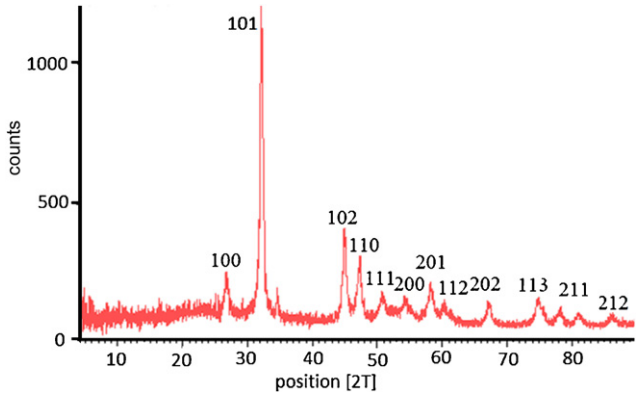

Fig. 7. XRD analysis of the Te nanocrystal synthesized by *Bacillus* sp. BZ.

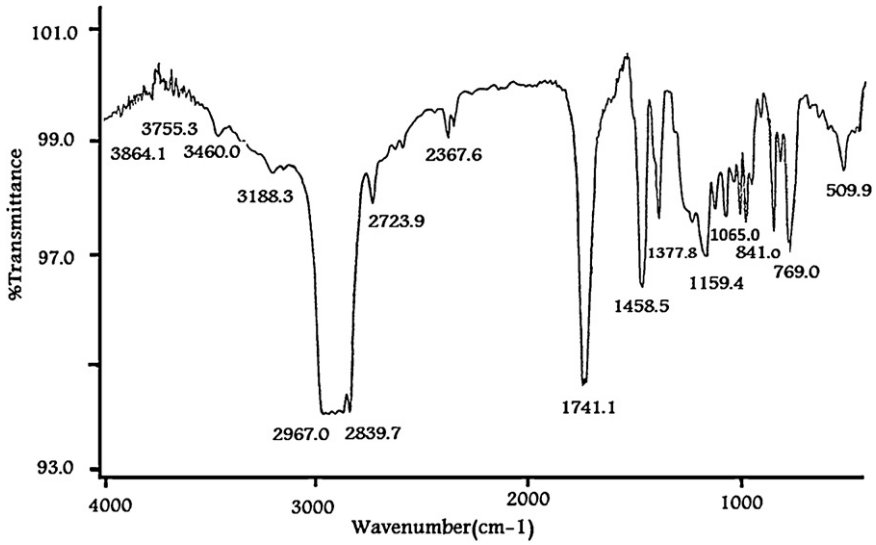

Fig. 8. FTIR spectra of the extracted biogenic Te NPs synthesized using the *Bacillus* sp. BZ isolated from the Caspian Sea.

Table 2
Minimum inhibitory concentration and minimum bactericidal concentration of the biogenic Te NPs prepared by *Bacillus* sp. BZ and evaluated against different bacteria.

Species	MIC ($\mu g\,ml^{-1}$)	MBC ($\mu g\,ml^{-1}$)	MBC/MIC ratio
Staphylococcus aureus	250	500	2
Pseudomonas aeruginosa	125	125	1
Salmonella typhi	125	125	1
Klebsiella pneumonia	125	250	2

1065.0 and 1159.4 cm^{-1} were of bending vibrations of C–C bands [34]. Overall, it was concluded that an ester ketone residue (–CO–O) exists on the surface of Te NPs.

3.4. Determination of MIC and MBC of Te NPs against pathogenic bacteria

Table 2 lists the results of the serial dilution method in which TTC reagent was used to evaluate the MICs of the Te NPs against different test strains. The table shows that the Te NPs showed good antibacterial activity against all the test strains. The obtained MICs for the Te NPs were 250, 125, 125, and 125 $\mu g\,ml^{-1}$ for *S. aureus, P. aeruginosa*, *S. typhi*, and *K. pneumonia* respectively. The MBCs were also evaluated for these NPs (Table 2). The Te NPs killed all the tested living microorganisms at similar concentrations (125–500 $\mu g\,ml^{-1}$). The calculated MBC:MIC ratios of all the tested bacteria were between 1:1 and 2:1 (Table 2). These ratios confirm that the Te NPs are bactericidal against the bacterial test strains.

4. Conclusion

Bacillus sp., designated as BZ, was isolated from the Caspian Sea. On the basis of a partial 16S rDNA sequence, we determined that the strain BZ is phylogenetically related to the *Bacillaceae* family. The ability of this isolate to form Te NPs was investigated. This bacterium was isolated from seawater, and showed good tolerance against high concentrations of Te^{+4} (MIC, 300 $\mu g\,ml^{-1}$). Few studies have reported extremophile bacteria (isolated from extreme environments) that can tolerate Te ions at high concentrations (1250 $\mu g\,ml^{-1}$) [17,24,33]. In the current work, *Bacillus* sp. BZ could remove more than 80% of the Te ions after a 48 h incubation (Fig. 3) in NB supplemented with 100 $mg\,ml^{-1}$ of Te^{+4}. We previously reported a simple method for extracting and purifying selenium NPs from culture media [9]; this method was used for the recovery of Te NPs in the present study [9]. UV–visible spectroscopy, EDX, and XRD results (Figs. 4, 6 and 7 respectively) confirm that the extracted particles were elemental Te with a nanoscale hexagonal crystal structure [31]. The TEM images of the Te NPs show that intercellular single or cluster nanorods with dimensions of about 180 nm × 20 nm were produced by *Bacillus* sp. BZ. Other types of Te NPs with different shapes and sizes have also been synthesized via chemical methods [31]. In this research, we used a bacterium isolated from the Caspian Sea to prepare the Te NPs. For the first time, Te NPs were purified, characterized, and used for antibacterial evaluation against different pathogenic test strains. The biogenic Te NPs were prepared using *Bacillus* sp. BZ and evaluated against clinical isolates of *S. aureus, P. aeruginosa*, *S. typhi*, and *K. pneumonia*. The MBC:MIC ratios of the Te NPs, determined by the serial liquid dilution method, revealed that these NPs exhibited bactericidal effects against the test strains and killed living microorganisms during the initial incubation time (24 h).

Acknowledgments

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