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EXPERIMENTAL ARTICLES

Isolation and Characterization of a Novel Strain of Genus *Dietzia* Capable of Multiple-Extreme Resistance[1]

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Abstract—An ultraviolet (UV) radiation resistant gram-positive bacterium, *Dietzia* sp. MG4 strain, was isolated from the Sirch Hot Spring (Kerman, Iran), then it was identified on the basis of morphological and biochemical characteristics, and 16S rRNA gene sequencing. The effects of temperature, pH, desiccation, different percentage of NaCl, hydrogen peroxide (H_2O_2), mitomycin C (MMC) and high levels of radiation on viability or growth rate of MG4 strain were investigated. Also heavy metal tolerance of MG4 strain was assayed. 16S rDNA sequence of the isolate exhibited 99.69% similarity with *Dietzia* sp. and this result was confirmed by phylogenetic analysis. Viability of this strain was obtained D_{91} according to D index after exposure to 25 J/cm^2 UV radiation dose, and D_{30} after desiccation stress (for 28 days) using flow cytometery. The D_{10} value for a microorganism is defined as the stress dose necessary to provide 10% survivors. Therefore, this strain showed high resistance to UV-C radiation and moderate resistance to desiccation. Optimal growth of MG4 strain was observed at pH 9, temperature of 30°C and 5% (w/v) NaCl. Isolated *Dietzia* was resisted up to 3 mM of nickel and 0.2 mM of mercury ions. Also this strain could tolerate 1–4% (v/v) H_2O_2 and 8 μg/mL of MMC as oxidant agents. To the best of our knowledge, this is the first study on multiple extreme resistant *Dietzia* sp. MG4 strain.

Keywords: UV-resistance, flow cytometry, oxidative stress, polyextremophilic bacteria

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Many organisms grow in normal environmental conditions, but some of them can survive and even grow under extreme conditions. Microorganisms which require extreme environments for optimum growth are called extremophiles [1]. Hot springs are ideal habitats for research on the interactions between organisms and their ability to adapt to extreme conditions. According to the studies on extreme environments, cellular processes are influenced by specific physical and chemical constraints [2]. The best example of a physical limitation for life is water. Life without water is impossible, because it is needed for all biochemical reactions [3]. Other constraints are extreme conditions such as low and high temperatures, low and high pHs, high salinity, radiation doses and various concentrations of toxic compounds [2]. Ionizing radiation and desiccation cause fragmentation of the single-stranded and double stranded DNA, mitomycin C (MMC) and UV radiation can cause DNA cross-reactivity and various forms of pyrimidine dimmers respectively. Hydrogen peroxide (H_2O_2) can damage a few nucleotides [4].

Microorganisms that are resistant to multiple extreme conditions are named poly extremophiles. Those are resistant to high doses of ionizing radiation and ultraviolet radiation termed radiophiles. Some of the radiophile bacteria including: *Deinococcus radiodurans* R1, *Deinococcus radiophilus, Kocuria rosea, Exigoubacterium acetylicum, Thermococcus marinus* and *T. radiotolerans*. *D. radiodurans* R1 is among remarkable bacteria which is highly resistant to chemicals, oxidative damages, high levels of radiation and dehydration [1]. The studied strain is named polyextremophile. *D. radiodurans* R1 is the most radiation-resistant organism known and could survive at 1 kJ/m^2 UV-light irradiation, and higher than 20 kGy of gamma radiation [4]. For each studied extreme environmental condition, it has been demonstrated that extremophilic microorganisms not only can tolerate these situations, but also they often require such conditions to survive [5].

High correlation has been typically observed between tolerance to radiation, desiccation, and DNA damaging chemicals [6]. Evolution of both drought and radiation resistance has been already presented by Shukla and colleagues [6], who showed that bacteria resisted to ionizing radiation from various habitats also represented resistance to desiccation, MMC and H_2O_2.

Extreme environmental conditions may arise naturally or by simulated forces, which the survival and development of living systems encountered with prob-

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lems. Presence of toxic chemicals, heavy metals, halogenated solvents and radionuclides in nuclear waste create problems for many species separation and disposing of individual pollutants [1]. *D. radiodurans* R1 and other microorganisms could be used in detoxification of halogenated organic compounds and toxic metals such as mercury. Theoretically, they could be used to eliminate this class of compounds selected from the waste mixture under moderate conditions [1].

The *Actinobacteria* are ubiquitous highly diverse microorganisms involved in the turnover of organic matter. The genus *Dietzia* in this family was first proposed on the basis of phylogenetic analysis to accommodate strains previously classified as *Rhodococcus maris*. At the present time, *Dietzia* species are known as gram-positive bacteria with high G+C content, which belong to the order *Actinomycetales*. *Dietzia* strains have been found to inhabit a wide variety of environments including deep sea mud, Hot Spring and oil contaminated soil [7]. In addition, secretion of high level of extracellular enzymes, high stability under multiple-extreme conditions such as: UV-radiation, desiccation, pH, salt, temperature and chemical denaturants have generated considerable attention [8]. It has been shown that at least some strains of *Dietzia cinnamea* such as P4 strain is highly resistant to ultraviolet A (UV-A), ultraviolet B (UV-B) and ultraviolet C (UV-C). The radiation-resistant bacteria could be suggested for treatment of environments where radiation is the principle factor limiting the microbial survival and function [9]. Therefore, the aims of this study were isolation and molecular identification of multiple-extreme resistant bacteria from various habitats in order to achieve the isolates for further researches on bioremediation of contaminated environments containing various toxic chemicals.

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

Isolated MG4 strain (Accession no. JX534198) and *D. radiodurans* R1 (DSM 20539) were grown in Tryptone Glucose Yeast extract (TGY) broth medium containing: 0.5% (w/v) tryptone; 0.1% (w/v) glucose; 0.5% (w/v) yeast extract, pH 7.2, incubated at 30°C under aerobic conditions. *Escherichia coli* (from Microbiology laboratory) as a radiosensitive bacterium was grown in Luria Bertani broth (LB) at 37°C.

Sample Collection, Enrichment and Screening Procedures

Sludge and soil samples were collected from Sirch Hot Spring in Kerman, Iran. In order to enrich the bacterial strains from samples collected, one gram of each sample was added to 99 mL of TGY broth medium and incubated for 3 days at 30°C on rotary shaker (160 rpm). Then, 100 μL of each enriched sample inoculated onto TGY agar plates. After 3–4 h pre-incubation at 37°C for vegetation of spore forms, they were exposed to UV light radiation (CROSSLINKER CL-E508.G) with a 254 nm UV source at intensity of 10 J/cm^2 h for 15 J/cm^2 as total doses. The plates wrapped with aluminum foil and incubated in dark under appropriate conditions for monitoring the cells' survival after a week [6]. In order to confirm its resistance to UV radiation, colonies obtained after primary screening were sub-cultured on TGY agar, then they were grown in TGY broth for 48 h. 1 mL from broth culture was inoculated into new medium and was incubated at 30°C while agitated at 160 rpm for 24 h. The cells concentrated by centrifugation (4000 *g*) for 5 min, washed twice with 0.9% NaCl and resuspended in the same buffer. 2 mL aliquots of the cell suspension containing 10^8 cells/mL were exposed to UV radiation in an open sterile petri dish at a distance of 14 cm from a 254 nm UV source (CROSSLINKER CL-E508.G) at intensity of 10 J/cm^2 h for total dose 25 J/cm^2. The plates were wrapped with aluminum foil and incubated in the dark under appropriate condition for monitoring the survival of cells after 15 days of incubation at 30°C [10]. The sub-cultured of isolated strains on TGY agar slant from primary screening were used as radioresistant strains for subsequent studies.

Survival of the Isolated MG4 Strain under Multiple-Stress Conditions

Measurement of the UV-C resistance on the isolated MG4. Isolated MG4 strain and *D. radiodurans* grown in TGY broth and *E. coli* grown in LB broth were harvested by centrifugation (at 7000 *g* for 10 min) in the late log phase and suspended in normal saline (0.9% NaCl) to reach $A_{600} = 0.5$. Then, 2 mL of each sample was transferred to sterile petri dishes and were exposed to UV irradiation (254 nm) at a distance of 14 cm from a mercury lamp (CROSSLINKER CL-E508.G). The total dose of radiation was 25 J/cm^2 in a sterile petri dish with the depth of liquid suspension not more than 1mm and the sample was agitated after each hour. Controls were obtained with incubation of these strains without UV radiation [6].

Preparation of Bacteria for Flow Cytometry Analysis

Flow cytometry is an appealing technique for fast cell viability assessment after staining the bacterial cells with rhodamine123 (Rho-123) (Sigma, United States) which shows excitation peaks around 507–560 nm and the emission peaks around 529–580 nm [11]. Rho-123 was made up to 1 mg/mL (wt/vol) in ethanol and wrapped with aluminum foil, then maintained at –20°C as stock solution. The working concentration of Rho-123 was 10 μg/mL (w/v) which freshly prepared in phosphate buffer saline (PBS) on

the day of the experiment [12]. First of all, 400 μL of Rho-123 was added to 100 μL of cell suspension immediately after incubation in the presence of radiation at room temperature in dark for 10 min. Then, measurement of viability with the flow cytometer (BD FACSCalibur™) was performed directly. Controls were obtained by incubation of bacteria without radiation [12]. Flow cytometer was set based on using an unstained bacterial sample without any autoflorescence. A total of 50000 bacteria were recorded for each sample.

Determination of Desiccation Effect on the Isolate

Cells of the late log-phase culture of each strain were prepared as it was described in previous section. It was added 0.2 mL of each suspension aliquot in a sterile crystal polystyrene 96-wells microplate, and dried at 30°C in desiccators [13]. After 28 days, samples were rehydrated by using 0.5 mL phosphate-buffered saline (PBS). Then, samples were vortexed and analyzed by the flow cytometer (BD FACSCalibur™) for cells viability [12].

Effect of pH, Temperature and NaCl on the Isolated MG4 Strain

Optimized temperature for growth of isolate was determined by inoculation of prepared cells (A_{600} = 0.5) in TGY broth incubated at range of 0–65°C. The pH tolerance was determined in TGY broth buffered over a pH range from 1 to 11, which prepared by using 1 N of NaOH or HCl. Tolerance to various salt concentrations was determined in TGY broth supplemented with various ranges of NaCl between 0% (as control without salt) up to 25% (w/v). 100 μL of TGY broth with desired ranges of pH or NaCl concentration was added separately to the each well in triplicates [1]. Then, 50 μL of prepared cell suspension was added to all the wells except for negative control and was incubated at 30°C for 24–48 h. After incubation time, the optical density was determined by using a microtiter plate reader (AWARENESS, Technology INC, stat fax 2100) at 630 nm [14].

Tolerance to Hydrogen Peroxide (H_2O_2) and Mitomycin C (MMC)

Bacteria were grown overnight at 30°C in TSB medium to an absorbance of 0.2–0.5 at 600 nm (A_{600} nm). The minimal inhibitory concentration (MIC) of the H_2O_2 (i.e. minimum level of H_2O_2 exposure required to inhibit 90% of bacterial growth) for the strains, MG4 and R1, were determined using a high throughput 96-well microtiter plate assay [15]. 100 μL of TSB broth supplemented with a range of 1–5% H_2O_2 (equivalent to 0.3–1.46 M) and 1, 2, 4, 6, 8 μg from MMC were added to the 96-wells microplates in triplicates and optical density was read by microtiter plate reader after incubation at 30°C for 24–48 h.

Tolerance to Cadmium, Nickel and Mercury Ions

Heavy metal tolerance in isolated *Dietzia* was also assayed. For this, 100 μL of TSB broth supplemented with a range of 1–3 mM of cadmium and nickel ions, and 0.2–1 mM from mercury ion were added to the 96-wells microplates in triplicates. Then 50 μL from prepared cell suspension was added to all the wells except for negative control and was incubated at 30°C for 24–48 h. After incubation time, the optical density was determined by using a microtiter plate reader at 630 nm.

The 16S rDNA Sequencing

Bacterial genomic DNA was extracted from overnight culture of isolated strain using DNA extraction kit (Fermentas, Diagnostics). The 16S rDNA of MG4 strain was amplified using the universal primers RW01 (5'AACTGGAGGAAGGTGGGGAT3') corresponding to bp 1170 to 1189 in the *E. coli 16S rRNA* gene as forward, and DG74 (5'AGGAGGTGATCCAACCGCA3') corresponding to bp 1522 to 1540 in the *E. coli 16S rRNA* gene as reverse primers [16]. The PCR reaction contain 2.5 μL 10× buffer, 0.7 μL $MgCl_2$, 0.5 μL dNTP mix, 1 μL of each primer, 0.5 μL *Taq* DNA polymerase and 2 μL of extracted DNA template in a total volume of 25 μL. PCR conditions were as follows: 5 min 95°C initial DNA denaturation step, followed by 30 cycles consisting of: denaturation for 45 s at 94°C, annealing for 30 s at 55°C, and extension for 45 s at 72°C. A final extension was carried out after the amplification reaction at 72°C for 5 min [17]. The purified reaction mixtures were electrophoresed in 1.5% agarose gel. Both strands of the PCR product were sequenced by the dideoxy chain termination method (Microgen, South Korea). The identity of the 16S rRNA gene sequences (370 bp) was determined using the BLASTN network service (www.ncbi.nlm.nih.gov/Blast.cgi,NCBI).

Phylogenetic Analysis

The 16S rRNA gene sequence of MG4 strain was compared with those available in the GenBank databases using the Blastn algorithm to detect the closest bacterial sequences within the GenBank database [18]. The sequences of closely related strains were retrieved and aligned using the CLC genomic workbench (limit mode). Alignment was corrected manually. Finally, the evolutionary history was inferred using the Unweighted Pair Group with Arithmetic means (UPGMA) based on neighbor-joining method. UPGMA is a sequential clustering algorithm that builds a distance-based tree in a stepwise manner, by grouping sequences or groups of sequences that are

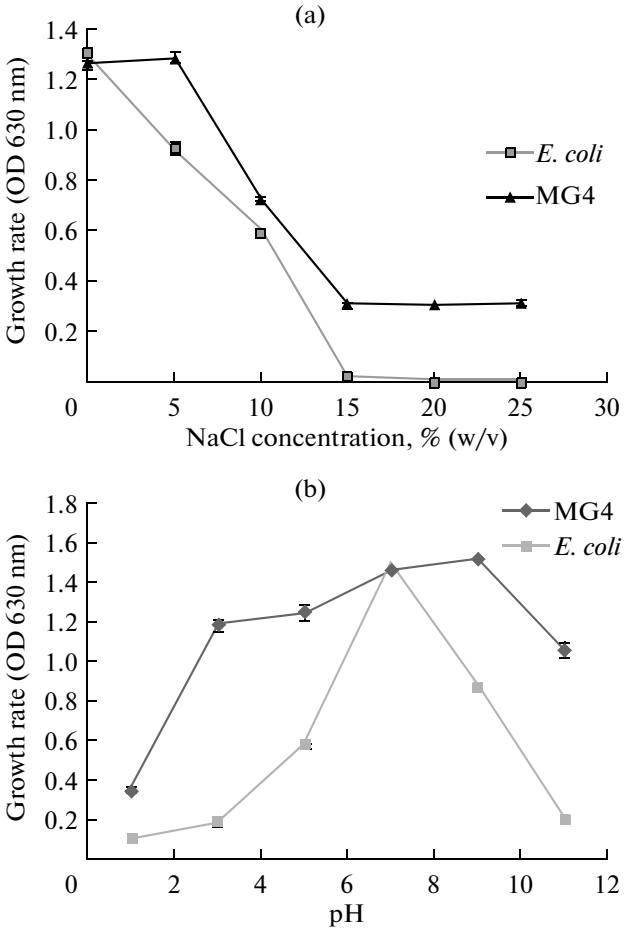

Fig. 1. Effect of two stress conditions on growth rate of isolated MG4 strain and control strain (*Escherichia coli*). (a) various concentrations of NaCl and (b) the effect of pH.

most similar to each other, i.e., for which the genetic distance is the smallest. *Rhodococcus* sp. MBEN382 was used as the out group taxon for rooting purposes. The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analyzed [19]. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree [19]. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. Evolutionary analyses were conducted in MEGA5 software package [20].

Statistical Analysis

Data were analyzed by ANOVA followed by the Duncan comparison test using the statistical program SPSS (version 17.0). All statistical tests were carried out at $\alpha = 0.05$ significance level.

RESULTS AND DISCUSSION

Identification of the Isolated MG4

In primary screening among the isolates from different habitats, three of them which were exposed to 15 J/cm^2 dose of radiation, were survived. Upon secondary screening, only one of them which was isolated from Sirch Hot Spring in Kerman, Iran could tolerate radiation dose above 25 J/cm^2. This strain which named MG4 was used for further experiments. MG4 strain was a mesophilic gram-positive non-motile, catalase and oxidase positive rod bacterium with orange pigmented colonies. The isolated MG4 grew well at temperature ranges between 25°C and 37°C with a temperature optimum at 30°C on TGY medium. It was able to grow in the presence of 0 to 10% (w/v) NaCl and optimal growth occurred at 5% (w/v) NaCl (Fig. 1a). The pH range for growth was found between 3 and 11, and the maximum growth occurred in pH 9 at 30°C (Fig. 1b). The nucleotide BLAST results of 16S rDNA sequence of the isolated MG4 strain exhibited 99.69% homology with the 16S rDNA sequence of *Dietzia* sp. WR-3 strain. Cluster analysis was performed by a dendrogram generated by the Unweighted pair-group method using arithmetic averages (UPGMA) algorithm neighbour-joining method and scale bar indicates the distance in substitutions per nucleotide, one substitution per 1000 nt. (Fig. 2). The results presented here clearly showed that the new isolated strain from the Hot Spring in Kerman (Iran) belongs to the genus *Dietzia*. The 16S rDNA sequence identified in this study has been deposited in the NCBI database under the following accession number: JX534198 as *Dietzia* sp.

Viability Assessment after Treatment with UV Radiation and Desiccation by Flow Cytometry

In nature and under stress conditions, bacterial cultures represent significant heterogeneity in terms of the percentage of viable cells according to the cellular metabolic activities [21]. Assessment of bacterial viability and identification by classical microbiological methods always had a major drawback [22]. Looks promising using of flow cytometry technique, because it allows identifying features of individual cells in the population [21]. In order to evaluate the biological responses of MG4 strain after UV-radiation or desiccation, florescence peak intensity in control sample for all the strains were determined about 1000, whereas after UV radiation and desiccation treatment florescence peak intensities were about 100, 10–100 and 10 for R1 strain, MG4 strain and *E. coli*, respectively. That is an indication of a decrease in viable cells. These results indicate that MG4 strain had higher resistance to UV radiation and desiccation than *E. coli* and lower than *D. radiodurans* R1 (Fig. 3). Viability of non-radiated cells of R1 strain, MG4 strain and *E. coli*

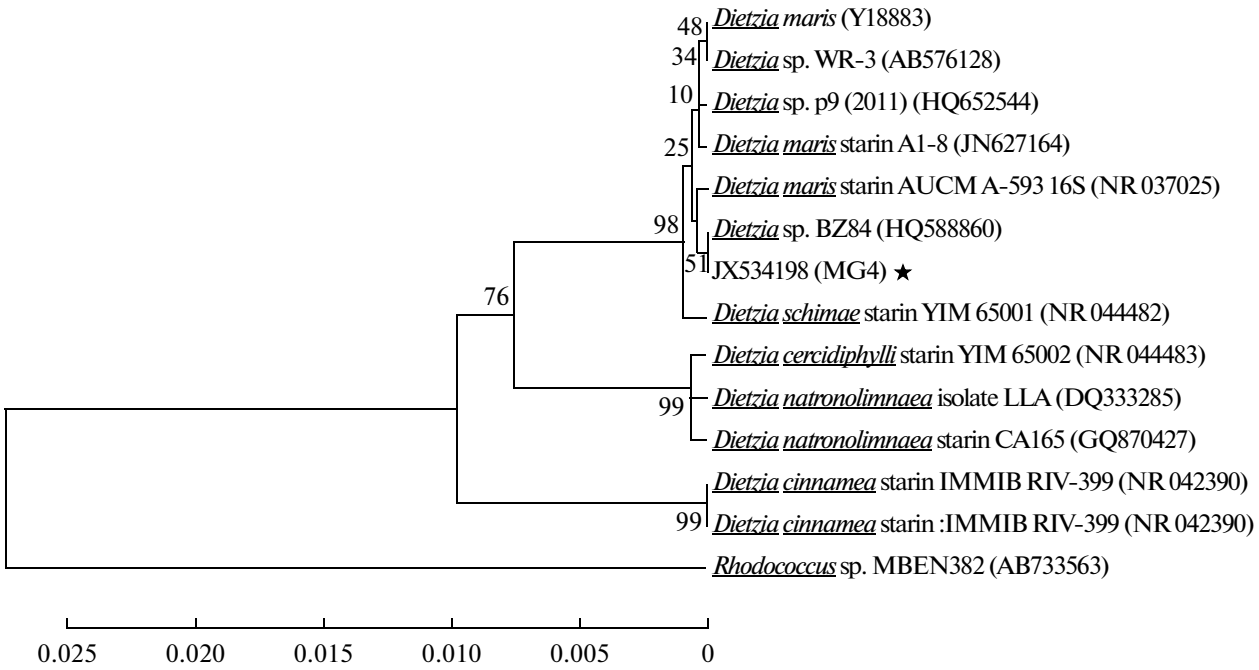

Fig. 2. Phylogenetic tree was made for bacterial isolate, MG4 strain. Cluster analysis was performed by Unweighted Pair Group with Arithmetic means (UPGMA) method. The *Rhodococcus* sp. MBEN382 was used as the out group taxon for rooting purposes. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown next to the branches.

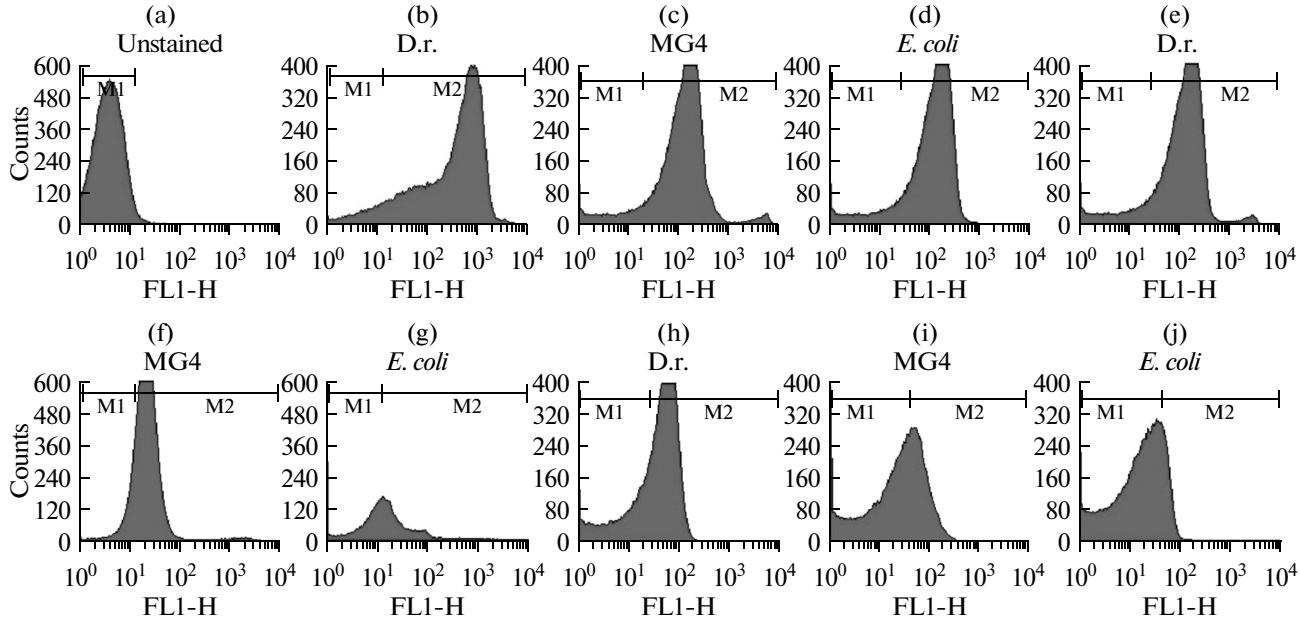

Fig. 3. Flow cytometry histograms of *Deinococcus radiodurans* R1, isolate MG4, and *Escherichia coli* cell suspensions stained with rhodamine-123 after exposure to UV radiation (25 J/cm^2) and desiccation after 28 days. Unstained bacteria (negative control) (a); non-exposure bacteria (positive control): *D. radiodurans* R1 (b), MG4 strain (c) and *Escherichia coli* (d); ultraviolet radiation exposure bacteria: *D. radiodurans* R1 (e), MG4 strain (f) and *Escherichia coli* (g); desiccation exposure bacteria: *D. radiodurans* R1 (h), MG4 strain (i) and *Escherichia coli* (j). M1: the percent of dead bacteria and M2: the percent of alive bacteria.

are about 91, 95 and 95%, respectively (Figs. 3a, 3d and 3g), but after exposure to UV radiation (25 J/cm^2), viability decreased to about 2, 9 and 49% for *D. radiodurans* R1, MG4 strain and *E. coli*, respectively (Figs. 3b, 3e and 3h). Moreover, viability according to D index after 25 J/cm^{-2} radiation dose

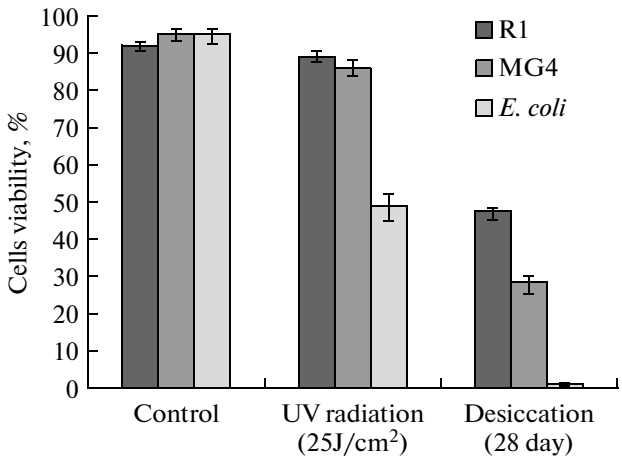

Fig. 4. Survival of *Deinococcus radiodurans* R1, MG4 strain, and *Escherichia coli*, exposed to ultraviolet radiation (25 J/cm^2) and desiccation stress (after 28 days) analysed by flow cytometry technique.

for *D. radiodurans* R1, MG4 strain and *E. coli* were D_{98}, D_{91} and D_{51}, respectively. The D_{10} value for a microorganism is defined as the radiation (or other stresses) dose necessary to provide 10% survivors, or a 90% reduction in colony-forming units. It has been indicated that at least a subset of the cellular functions are necessary to survive exposure to ionizing radiation and also necessary to survive in desiccation [23]. Also viability at 28 days after desiccation was decreased to about 47, 70 and 99% for *D. radiodurans* R1, MG4 strain and *E. coli*, respectively (Figs. 3c, 3f and 3i). According to the results, the MG4 strain showed higher resistance to desiccation than *E. coli*, whereas, its radio-resistance is moderate comparing to *D. radiodurans* R1. Evaluation of the bacterial reduction was calculated by using the following formula:

$$\frac{A-B}{A} \times 100 = \text{Bacterial reduction percent}$$

(A: percentage of viable cells before radiation and B: percentage of viable cells after radiation).

Interestingly, based on survival curves, a similar response was observed in MG4 strain and *D. radiodurans* R1 after challenging with UV-C. However, under drought conditions, microorganisms adapted to the situation by producing both outside and inside cellular compounds [24]. There is a correlation between resistance to desiccation and radiation [6], so this experiment was performed on the isolated MG4 strain in TGY broth. MG4 showed relatively moderate desiccation resistance after 28 days (Fig. 4). This was almost comparable to the desiccation tolerance of *D. radiodurans* R1, one of the most UV-resistant organisms amongst the bacteria [6]. The extent of *D. radiodurans* R1 resistance to ionizing radiation depends strongly on physiological conditions, such as the age of the culture, cell concentration, growth medium, pH, irradiation medium, irradiation temperature and plating medium [25]. Shukla et al. showed that ionizing-radiation-resistant bacteria from various habitats are also highly resistant to desiccation, MMC, and H_2O_2 [6]. In addition to DNA damage and DNA fragmentation in the cells exposed to UV radiation, protein synthesis decreases [26]. The removal of water from a cell is a severe, often lethal stress due to protein denaturation and the formation of ROS, which cause lipid peroxidation, protein oxidation, and oxidative DNA damage [27]. Drought-tolerating microorganisms use different mechanisms for protecting cellular macromolecules against harmful effects of drought. In these conditions, trehalose and sucrose could be replaced to the lost water and cytoplasm becomes a very cold liquid with severe adhesion and also secretion of free radicals decreases [27]. The most important factor in response to the environmental stresses is changing membrane lipid contents. Maintaining membrane integrity in anhydrobiotic organisms (xereophiles) is a central mechanism of resistance to drought [28]. An iron superoxide dismutase (SodF) also exists at high levels in active form in dried colonies and probably protects cells from damaging by reactive oxygen species [28]. UV-resistance is probably related to desiccation resistance, because both kinds of stress produce the same type of damage to DNA. On the other hand, UV-radiated sensitive mutant also becomes sensitive to desiccation [29]. High desiccation tolerance shown by MG4 with a very high UV resistance in comparison to type strain *D. radiodurans* R1 supports the co-evolution hypothesis of desiccation and UV-radiation resistance [6]. However, certain numbers of evidences have suggested that resistance to UV radiation and desiccation related to antioxidant defense apparatus including enzymes such as superoxide dismutase, peroxidase, catalase, and non-enzymatic systems such as vitamins A, E and carotenoids [30]. It is known that *D. radiodurans* and *Halobacterium salinarium* mutants deficient in carotenoid production are more sensitive to ionizing radiation and H_2O_2 than their wild type strains [30]. Carotenoids are efficient scavengers of ROS especially singlet oxygen ($^1O_2$) and peroxyl radicals (ROO). These pigments protect DNA from oxidative damages, proteins from carbonylation and membranes from lipid peroxidation [31]. Both radiation and desiccation resistant bacteria are marked with a high Mn/Fe ratio, and their proteins are less susceptible to oxidation than sensitive bacteria [32].

Tolerance to Hydrogen Peroxide (H_2O_2) and Mitomycin C (MMC)

Based on obtained results in this study shown in Fig. 5a, MG4 response to H_2O_2 in the range of 2–4% (v/v) was similar to R1 strain of *Deinococcus*. However, 5% concentration of H_2O_2 showed little effect on *D. radiodurans* R1 whereas MG4 strain was highly sensitive to it (Fig. 5a). Figure 5b shows growth of MG4 strain in the presence of MMC. The type strain

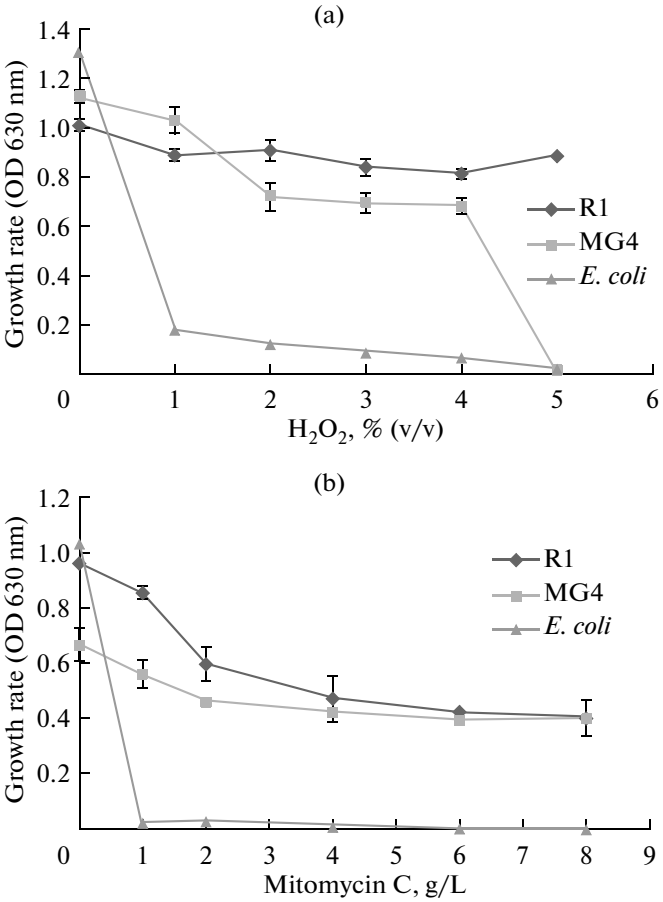

Fig. 5. Growth rates of *Deinococcus radiodurans* R1, isolated MG4 strain, and control strain (*Escherichia coli*) in the presence of different concentrations of hydrogen peroxide (a) and mitomycin C (b).

D. radiodurans R1 and isolated MG4 which showed high UV-radiated resistance also survived in the presence of MMC up to 8 μg (Fig. 5b). Since MMC is known to cause double-strand breaks in DNA, relatively high tolerance of these two strains might be due to efficient double-strand break repair. *E. coli* was used as a sensitive strain for MMC and H_2O_2 (Figs. 5a, 5b). Significant differences between treated and non treated strains with MMC and H_2O_2 were seen. Results of ANOVA test (GLM-unvariate) indicated that not only the concentrations of MMC and H_2O_2 but also the types of bacteria have significant effect on optical density ($P < 0.05$). The similarity between resistance to UV, H_2O_2 and MMC may indicate that there are regulatory systems, which control the expression of the genes involved in cellular protection from variety of stress [33]. MG4 strain probably repairs DNA damage through nucleotide excision, as it was shown by finding of *uvr*A, *uvr*BC and *uvr*D-like helicases, genes for DNA alkylation repair and DNA glycosylases in *Dietzia cinnamea* P4. Also, P4 strain exhibited systems to allow tolerance to environmental

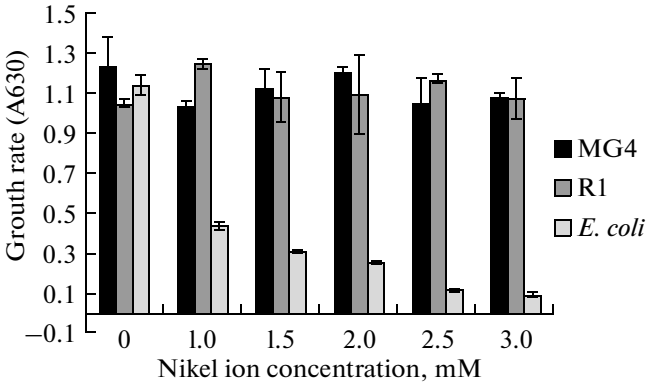

Fig. 6. Tolerances of MG4 strain, *D. radiodurans* R1, and *E. coli* to nickel ions.

stresses such as: SodA and a single Cu–Zn SodC and multiple DNA repair mechanisms [9]. Sod is the most indispensable enzyme for protecting the cells from the toxicity of the reactive oxygen species that are generated during aerobic respiration for energy production [4]. Furthermore, the ability of isolated MG4 strain to protect itself from oxidative stress may be due to these mechanisms.

Heavy Metal Resistance of MG4 Strain

Results showed high resistance of MG4 strain to nickel (up to 3 mM) (Fig. 6) and low tolerance to cadmium (Fig. 7). The growth rate of isolated *Dietzia* was not changed in presence of nickel up to 3 mM concentration. R1 strain also grown in the presence of nickel ions up to 3 mM and *E. coli* was grown, but not the same as DG4 or R1 strains. Also as it shown in Fig. 8 the isolated *Actinobacteium* could tolerated 0.2 mM of mercury ion. Máthé et al. [34] showed that *Dietzia psychralcaliphila* BGN5 tolerated lead ion up to 2 mM, cupper ion up to 1.5 and 0.6 mM of zinc ion, but there

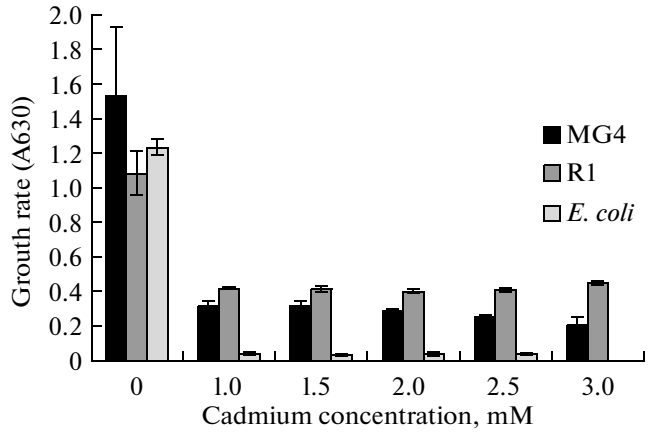

Fig. 7. Tolerances of MG4 strain, *D. radiodurans* R1, and *E. coli* to cadmium ions.

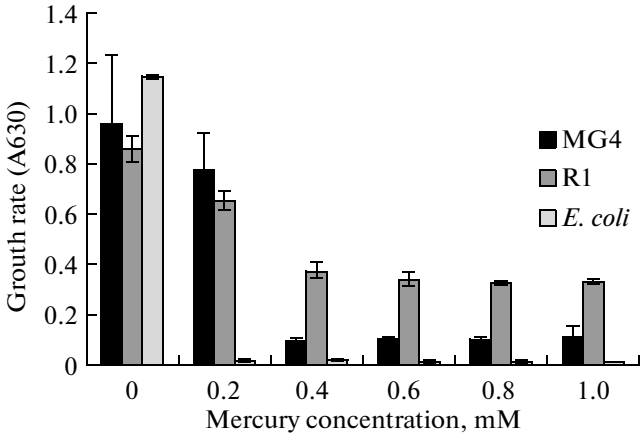

Fig. 8. Tolerances of MG4 strain, *D. radiodurans* R1, and *E. coli* to mercury ions.

is not any data for mercury, cadmium and nickel ions for this bacterium. *E. coli* as a non-resistant representative bacterium did not grow in media contain cadmium and mercury, while R1 strain as a resistant bacterium was grown in these media.

In conclusion a new mesophilic strain of *Dietzia* sp. from *Actinobacteria* was isolated from Sirch Hot Spring in Kerman, Iran. This isolate had multiple resistances to UV-C radiation, desiccation, salt concentrations, and ranges of pH, H_2O_2, MMC, and the ions of nickel, cadmium and mercury ions. Evidences show that there is a correlation between UV radiation resistance and other stresses in MG4 strain. These multiple resistances could be due to strong repair systems, enzymatic antioxidants defense system such as catalase or peroxidase, and non-enzymatic antioxidants such as carotenoid pigments and Mn accumulation. A bioremediation strategy based on genetic engineering in radiation-resistant bacterium, such as *Dietzia* sp. (MG4 strain) could be used for treatment of environments where radiation is the principle factor in limiting microbial survival and function. Therefore, with respect to multiple resistances of the isolated strain, MG4 strain could be suggested as a suitable candidate for further investigations such as bioremediation of radioactive waste sites and other contaminated environments containing various toxic oxidant chemicals. To the best of our knowledge, this is the first report about isolation of *Dietzia* sp. from Sirch Hot Spring that is able to survive in multiple extreme conditions and could be a candidate for heavy metal removal from wastes under multiple stresses.

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