Journal of Environmental Radioactivity 144 (2015) 113–119

Contents lists available at ScienceDirect

Journal of Environmental Radioactivity

journal homepage: www.elsevier.com/locate/jenvrad

Isolation a new strain of *Kocuria rosea* capable of tolerating extreme conditions

M. Gholami, Z. Etemadifar*, M. Bouzari

Department of Biology, Faculty of Sciences, University of Isfahan, Hezarjarib St., Azadi Sq., 81746-73441, Isfahan, Iran

ARTICLE INFO

Article history:
Received 1 December 2012
Received in revised form
1 January 2015
Accepted 10 March 2015
Available online 31 March 2015

Keywords:
UV-radiation resistant bacteria
Kocuria rosea
Flow cytometry
Multiple-stress conditions

ABSTRACT

A new actinobacterial strain was isolated from Ab-e-Siah spring (dark water) taken from the Ramsar city in Iran, and subjected to several stress conditions investigation. The isolate, named MG2 strain, was Gram-positive, aerobic, diplococci or tetrad shaped, non-spore forming and non-motile. Phylogenetic analysis of the isolate using 16S rDNA sequence indicated that the organism matched best with the genus *Kocuria* and the highest sequence similarities (98.55%) being found with *Kocuria rosea*. The 16S rDNA sequence determined in this study has been deposited in the NCBI database with the accession no. JX534199, *K. rosea* strain MG2. The isolated strain was an alkaliphilic-mesophilic bacterium because the optimal growth was observed at pH 9.2 and temperature of 28 °C under aerobic condition. MG2 was a halotolerant strain and tolerated maximally to 15% NaCl concentraion. Viability analysis by flow cytometry indicated that this strain had highly resistance to UV-C radiation and moderately resistance to desiccation after 28 days. The viability of *K. rosea* strains MG2 and *Deinococcus radiodurans* R1 were determined D_{87} and D_{98} according to D index, respectively, by a dose radiation 25 J/cm (Appukuttan et al., 2006). Thus the UV resistance of strain MG2 was comparable with representative radiation resistant *Deinococcus*. Also MG2 was grown at 1–4% of H_2O_2 as an oxidant agent. This research is the first study on multiple extreme resistance of *Kocuria rosea* new strain (MG2) isolated in Iran.

1. Introduction

Applications of ionizing radiation for sterilizing foods and other materials have led to a number of studies on the radio-resistance of microorganisms. Radiation resistance is dispersed among the three kingdoms of life and is not in correlation with the prevalence of these organisms and the characteristics of their habitats (Cox and Battista, 2005). Environmental background radiation varies considerably due to local geological features and atmospheric conditions. In most places on the earth, the natural radioactivity varies only within narrow limits, but in some places there are wide deviations from normal levels because of abnormally high levels of radioactive minerals (Charles, 2001). The most well-known, high-level natural radiation areas are Kerala in India, the coastal region of Espirito Santo, Mineas Gerais in Brazil and many other areas, such as Yangjiang in China, the Nile Delta in Egypt and Ramsar in Iran (Charles, 2001).

Of the genera containing ionizing-radiation-resistant microorganisms, mesophilic bacterium *Deinococcus radiodurans* as a polyextremophile bacterium has been first isolated from irradiated meat cans and has been studied since 1956. It survives in a variety of environment of extreme γ-radiation and ultraviolet radiation, genotoxic chemicals such as mitomycin C (MMC) and H_2O_2, heat, desiccation. *Deinococcus* serves acceleration and deceleration force, which are lethal to almost all other microorganisms. After the discovery of *D. radiodurans*, other radiation-resistant bacteria strains, including *Deinococcus geothermalis*, *Deinococcus murrayi*, *Rubrobacter radiotolerans* and *R. xylanophilus*who exhibit variable radiation resistance were studied (Yoshinaka et al., 1973). There are also some other ionizing radiation-resistant bacteria that have been isolated and described including species of the genera *Acinetobacter*, *Chroococcidiopsis*, *Hymenobacter*, *Kineococcus*, *Kocuria*, and *Methylobacterium* (Brooks and Murray, 1981).

Ionizing-radiation-resistant bacteria have been isolated from a variety of environments which were previously exposed to X-ray, gamma irradiation and non-irradiated samples or from naturally radioactive environments (Rainey et al., 2005). Some microorganisms are growing in natural radioactive environments also under

* Corresponding author.
E-mail address: z_etemadifar@yahoo.com (Z. Etemadifar).

http://dx.doi.org/10.1016/j.jenvrad.2015.03.010
0265-931X/

other stress conditions. Thus, these microorganisms are able to develop different mechanisms to survival at these stress conditions (Shukla et al., 2007).

Over the last sixty years, the use of fissionable nuclear materials has made the development of weapons, production of energy, and medically relevant radionuclides possible. However, comprehensive strategies for eliminating nuclear waste materials and remediation of contaminated lands and facilities are still lacking. Contaminated sites contain highly energetic radionuclides, including ^{235}U, ^{238}U, ^{239}Pu, ^{137}Cs and ^{90}Sr (Atlas and Philp, 2005). Additional pollutants at these sites may include other heavy metals (e.g., mercury), organic pollutants (e.g. toluene), and fuel hydrocarbons (Brim et al., 2006). Currently available chemical methods are costly and often lack the specificity required to properly decontaminate these sites. Microbes, in contrast, offer a high degree of specificity with regards to processing their targeted materials, are potentially more cost-effective and less likely to cause dispersal of radioactive waste over a wider area (Cox and Battista, 2005). Most of used bacteria in metal biosorption are sensitive to radiation and other stress conditions, so cannot be use for bioremediation of these contaminated sites (Appukuttan et al., 2006). Thus, the isolation and characterization of radiation-resistant bacteria could be beneficial for this goal. Moreover, these organisms usually tolerate other stress conditions in these sites.

Kocuria is one of the radiation-resistant bacteria and has been employed as a biotechnology tool for several goals, which are explained briefly as follow. Metal cations biosorption by psychrophilic *K. carniphila* and *K. polaris* (El-Sharouny et al., 2013); biosurfactant production by halophilic *Kocuria marina* BS-15 isolated from solar salt works (Sarafin et al., 2014); sponge-derived *Kocuria* was studied as sources of the new thiazolyl peptide antibiotic kocurin (Palomo et al., 2013); and exocellular keratinase activity is well known in *Kocuria rosea* (Gupta et al., 2012).

This study aimed for the isolation and characterization of UV-C resistance bacteria from Ab-e-siah radioactive spring, investigation of the tolerance to other environmental stresses such as: desiccation, hydrogen peroxide, pH range, and NaCl, and correlation between UV-C radiation resistance and other stress tolerances in selected isolate for future biotechnological application.

2. Experimental procedures

2.1. Site description

Ramsar city, one of the areas with high natural radioactivity in the world, is located in the West of Mazandaran province, Iran (E50 39 59.1 N36 53 54.9). Most of the radiation in this area is due to dissolved radium-226 in water of hot springs along with smaller amounts of uranium and thorium due to travertine deposits. The Ab-e-Siah radioactive spring in this city is well-known due to presence of ^{226}Ra and ^{232}Th. Maximum levels of activity in hot springs in Iran are attributed to springs located in Ramsar, particularly Ab-e-Siah with 146.5 Bq L^{-1} activity. The equivalent dose around the spring was measured previously about 13.48 μSv h^{-1} (Dabbagh et al., 2006). The temperature of hypothermal springs ranges from 28 to 35 °C so that it is classified as a hypothermal spring (Dabbagh et al., 2006).

2.2. Enrichment and isolation of bacteria resistant to ultraviolet radiation

Samples of water and sludge during the summer season were collected from the Ab-e-Siah (dark water) mineral spring. The temperature and pH of the water of Ab-e-Siah at the time of sampling was 28 °C and 6.8 respectively. More details were shown in Table 1.

Table 1
Physico-chemical composition of Ab-e-Siah radioactive spring (Dabbagh et al., 2006).

Parametrs	Value
pH	6.8–7.2
Temperature °C	28–42
Total hardness, mg/L	1128
dry residue at 180 °C, g/L	104.351
Appearance color	dark
Maximum levels of radioactivity, Bq/L	146.5
Water activity, Radium^{226}, Bq/L	112.71
Uranium, μg/L	2
Manganese, μg/L	464.26
Mercury, μg/L	10
Cadmium, μg/L	5
Chloride, μg/L	62,903
Bromine, μg/L	103,643
Antimony, μg/L	5
Beryllium, μg/L	5

2.2.1. Primary screening

In order to enrich the bacterial strains present in samples, one gram of each soil sample was added to 99 ml of TGY broth media [0.5% (w/v) tryptone; 0.1% (w/v) glucose; 0.5% (w/v) yeast extract] and incubated for 3 days at 30 °C. Then 100 μl of each enriched sample was inoculated into TGY agar plates and exposed in a UV light (CROSSLINKER CL-E508.G) with a 254 nm UV source at intensity of 10 J/cm^2 h for 15 J/cm (Appukuttan et al., 2006). In order to prevent of light-dependent repair after radiation, plates were covered with an aluminum foil and incubated at 30 °C for a week. Gram stain was used for analysis the gram reaction and cell morphology of the isolates after 24 h incubation in TGY medium. The motility of cells was assessed by using the hanging-drop method. Catalase activity was determined by assessing bubble production with 3% (v/v) H_2O_2 and oxidase activity was determined using 1% (w/v) tetra-methyl-p-phenylenediamine. The isolated colonies in this stage sub-cultured on TGY agar slants media and stored in refrigerator for further analysis.

2.2.2. Secondary screening

The stock cultures from primary screening were examined by several experiments. In order to confirm ultraviolet radiation resistance, the isolated cultures were grown in TGY broth for 48 h, then one ml of each culture was inoculated into new medium and were incubated at 30 °C while agitated at 160 rpm for 24 h. The cells concentrated by centrifugation (at 12000 g for 10 min), then washed twice with normal saline (NS) (0.9% NaCl) and resuspended in the same buffer. The cell suspensions (2 ml aliquots) were exposed in an open sterile petri dish at a distance of 14 cm from a UV light (CROSSLINKER CL-E508.G) with a 254 nm UV source at intensity of 10 J/cm^2 h from zero to 25 J/cm^2. The number of CFU (colony-forming unit) was determined after 15 days of incubation at 30 °C. Also the cell viability was measured using flow cytometry to determine viable and dead cells. The confirmed radioresistant sub-cultures (from primary screening) were kept at 4 °C.

2.3. Survival of isolated MG2 under multiple-stress condition

2.3.1. Culture conditions

For experiments, strain MG2 and *D. radiodurans* R1 were grown under aerobic conditions in TGY broth media at 30 °C and *Escherichia coli* was grown in Luria Bertani medium (LB) at 37 °C.