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Isolation and identification of a new radiation-resistant bacterium *Deinococcus guangxiensis* sp.nov. and analysis of its radioresistant character

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Abstract: [Objective] To isolate and identify a new ionizing-radiation resistant strain capable of surviving under highly ionizing radiation conditions as UV and gamol/La radiation and to characterize its radioresistant properties. [Methods] The isolates were sampled from Radiation Centre of Guangxi University, Nanning, China, and the medium used for isolation and cultivation of the bacterium was General Bacterial Medium (GBM). The new ionizing-radiation resistant strain WGR700^T was identified by its morphology, biochemical and physiological characteristics, fatty acids, G + C content of DNA, UV and gamol/La radiation resistance and 16S rRNA gene sequence homology. [Results] The strain WGR700^T is of rod-shape, Gram-negative, non- spore-forming, non motile, aerobic and red-pigmented. The optimum temperature and pH for strain WGR700^T growth is 37℃ and pH7.0, respectively. The predominant respiratory quinone is MK-8 and its cell well contains ornithine. The major cellular fatty acids found in the cell wall are 16:1ω7c, 16:0, 15:1ω6c, iso-15:0 and iso-17:0. DNA of strain WGR700^T had a G + C content of 64.7mol%. WGR700^T was highly resistant to UV (> 728 J/m^2) and gamol/La radiation (D_{10} = 9.8 kGy). Phylogenetic analysis of the 16S rRNA gene sequences showed 87.1 ~ 95.6% similarities with other recognized *Deinococcus* species. [Conclusion] Based on the high 16S rRNA gene sequence divergence and phenotypic differences, it is proposed that the new isolated strain should be classified as a novel member in the genus *Deinococcus* with the name *Deinococcus guangxiensis* sp. nov. The type strain is WGR700^T (= CGMCC 1.7045^T = CICC 10360^T = JCM 15082^T).

Keywords: radioresistant bacterium; *Deinococcus*; phylogenetic analysis

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1 INTRODUCTION

The species and taxonomy of genus *Deinococcus* had been extensively studied. Different species of *Deinococcus* were recovered and identified from various environments, including rhizosphere, arid soils, foods, air dust, hot springs, feces and foul water [1-3]. *Deinococcus* species are members of non-motile, non-sporeforming, rod-shaped or coccoid bacteria. Most species of this genus exhibit a remarkable capacity to survive the lethal effects of ionization [4]. Among all Deinococcus species, *D. radiodurans* R1 is most extensively studied for the mechanisms of radiation resistance. The genome of *Deinococcus radiodurans* was the first to be sequenced [5]. To further delineate the genes underlying the resistance phenotypes, a second Deinococcus species (*D. geothermalis*) has also been sequenced [6]. The mechanism of how *D. radiodurans* manages to survive lethal dose of ionizing radiation, however, is still unclear [4,7]. Identification and characterization of new radiation resistant *Deinococcus* species would help researchers to reveal such mechanisms. In a study of the ionizing-radiation-resistant bacterial comol/Lunities from waste water that had been

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discontinuously radiated by a ^{60}Co irradiator for 10 years, we isolated an unknown gamol/La and UV-radiation resistant *Deinococcus* strain. This new species, designated as strain WGR700^T, was characterized and named *Deinococcus guangxiensis* sp. nov.

2 MATERIALS AND METHODS

2.1 Strain isolation and cultivation

Strain WGR700^T was isolated from waste water sample, Radiation Centre of Guangxi University, China. The medium used for isolation and cultivation of the bacterium was GBM medium. For isolation of strain WGR700^T, water sample was collected and 100 μl was spread on GBM solid agar plate. Plates were incubated at 28℃, 30℃, 32℃ and 37℃, respectively for 5 days before single bacterial colonies were isolated and streaked several times onto new GBM agar plates to obtain pure culture. GBM medium was used for growth, maintenance and biochemical tests of strain WGR700^T and *D. indicus* Wt/1a^T (DSM15307). Luria-Bertani (LB) medium and Tryptone-Glucose-Yeast (TGY) medium were used for cultivation of *Escherichia coli* DH5α and *D. radiodurans* R1 at 37℃ and 30℃, respectively.

2.2 Morphology, biochemical and physiological characteristics

Morphology and motility of cells were examined at two different times using a fluorescence microscope (model BX51-DP70, Olympus) and a transmission electron microscope (JEM-1200 EX/S, JEOL), 24h and 48h after growth on GBM agar respectively. Motility and flagella observation was performed using semi-solid agar and Leifson [8] staining method. For observation under transmission electron microscope, cells were negatively stained as described by Zhang et al [9]. Gram staining was performed following standard Gram reaction procedures [10]. The temperature range for growth was determined on GBM agar plates incubated for 5 days at temperatures from 10℃ to 50℃. The pH range for growth was determined in buffered GBM at temperature 37℃ between pH 5.0 and pH10.0. NaCl tolerance (0.6%, 1.0%, 1.2%, 1.5% and 2.0%) was tested using GBM medium. The antibiotic sensitivity of the culture was tested using antibiotics supplied by Sangon Company (Shanghai, China) according to the manufacturer's instructions. Metabolic tests were performed as described by Smibert & Krieg [11]. Single carbon source assimilation tests were performed as described by Suresh et al [12].

2.3 Peptidoglycan, fatty acids, respiratory quinone and G+C content of DNA

Purified peptidoglycan was prepared and analyzed by the method of Schleifer and Kandler [13]. Analysis of the cellular fatty acid pattern was according to the previously described methods using the MIDI system (Microbial ID, Inc., USA) [14]. The respiratory quinone was isolated following the method of Minnikin et al [15]. And separated by HPLC following previously published protocols [16]. Isolation of DNA and determination of its G+C content were performed according to the thermal denaturation (T_m) as described previously [17,18] and E. coli K-12 DNA was used as control.

2.4 16S rRNA gene sequence analysis and phylogenetic investigation

The 16S rRNA gene was amplified by PCR according to Rainey et al [20]. The 16S rRNA gene sequence of strain WGR700^T was compared with reference sequences from GenBank. Multiple alignments with sequences of related species and calculations of levels of sequence similarity were carried out using CLUSTAL X program [21]. A phylogenetic tree (Figure 1) was constructed by the MEGA 3.1 program, using neighbour-joining method of Saitou and Nei [22] and the Knuc values [23]. Topology of the phylogenetic tree was evaluated by bootstrap resampling method of Felsenstein [24], with 1000 replicates.

2.5 UV and gamol/La radiation resistance

UV and gamol/La radiation-resistance of the bacteria were tested following protocols described by Rainey et al [2] with slight modification. The cell culture was grown in GBM medium at 37℃ for 36 h until it reached late-exponential phase. Cells were harvested by centrifugation at 8, 000 *xg* for 2 min at room temperature, followed by washing with 0.01 M phosphate buffer (PH 7.0). Cells were then diluted serially and 100 μl was spread onto GBM agar plates. For UV exposure, plates (with lids open) were exposed to a 254nm UV light at a distance of 30 cm (1.0Wm^{-2}) and a model RX003 UV detector (UVI Tech) was used to determine UV dose [19]. For the

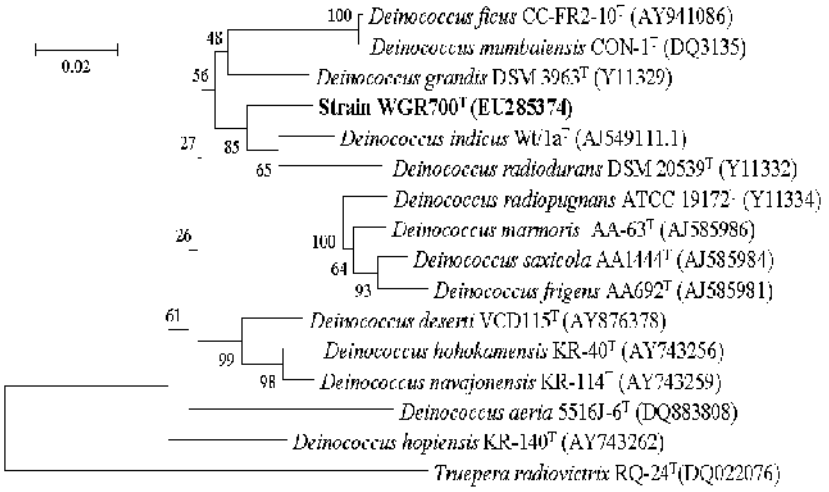

Fig.1 Phylogenetic dendrogram showing the position of strain WGR700^T among its phylogenetic neighbours. The 16S rRNA gene sequences are available from the NCBI database (accession numbers are given in parentheses). The dendrogram was constructed from distance matrices by using the neighbour-joining method. Numbers at branching points represent bootstrap percentages based on 1000 replicates. Bar, 2% sequence divergence. The sequence of *Truepera radiovictrix* RQ-24^T GenBank accession no. DQ022076) was used as the root.

gamol/La radiation-resistance test, cells were prepared as above. After washing with 0.01 mol/L phosphate buffer, the suspensions were exposed to radiation levels between 0 and 15 kGy at room temperature, using a Shephard model 484 ^{60}Co irradiator at a dose rate of 1 kGyh^{-1}. After 1 kGy, 3 kGy, 5 kGy, 8 kGy, 10 kGy, and 15 kGy gamol/La radiation, suspensions were diluted serially and 100 μl was spread on GBM agar plates. All plates were then incubated at 37℃ for 5 days and colony formation frequencies were recorded. *D. radiodurans* R1 and *E. coli* DH5α were used as controls for both radiation exposures. Relative survival was determined by comparing with unirradiated cultures.

3 RESULTS AND DISCUSSION

Characterization of strain WGR700^T identified it as an aerobic, Gram negative, non-motile and non-sporeforming rods. Five species (*D. deserti*, *D. grandis*, *D. indicus*, *D. yunweiensis* and *D. mumbaiensis*.) of the genus *Deinococcus* are Gram negative and rod shaped. *D. ficus*, *D. maricopensis*, *D. yavapaiensis* and *D. papagonensis* are also rod shaped but Gram positive. The average length and diameter of the cell of strain WGR700^T were 6.0 ~ 7.0 μm and 2.0 μm, respectively (Figure 2), which are much bigger than other *Deinococcus* species that are also in rod shape [1, 9, 12, 25 – 27].

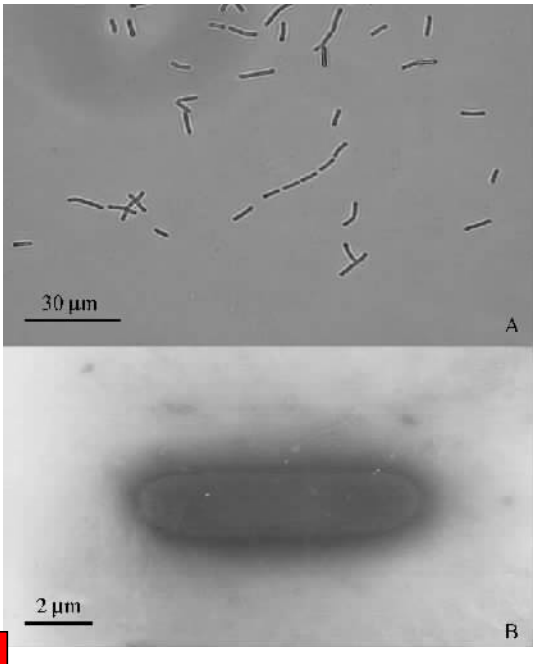

Fig.2 Fluorescence microscope and transmission electron microscope images of strain WGR700^T cells. (A) Fluorescence microscope micrograph of WGR700^T cells grown in liquid GBM medium at 37℃ for 24 h (Bar, 30.0 μm); (B) Transmission electron micrograph of a strain WGR700^T cell grown on GBM agar at 37℃ for 48 h (Bar, 2.0 μm).

Colonies of strain WGR700^T were pink to red, circular and opaque on GBM and Luria-Bertani agar plates. Size of the colonies was about 2 ~ 3 mm in diameter after incubated on GBM agar at 37℃ for 36 h . The temperature and pH range for growth was 28 ~ 45℃ and pH 6.5 ~ 9.0, respectively, with an optimum growth

condition of 37℃ and pH 7.0. Other physiological and biochemical properties of strain WGR700^T are listed in Table 1 or in the species description.

Like all other *Deinococcus* species, MK-8 is the major respiratory quinone of strain WGR700^T. The peptidoglycan of strain WGR700^T cell wall contained L-ornithine. The DNA G + C content was 64.7%. Major fatty acids of the strain are 16:1ω7c, 16:0, iso-15:0 and iso-17:0, which are also predominant species in most other *Deinococcus* strains. Different from other *Deinococcus* species, strain WGR700^T does not contain $C_{15:0}$ in its cell membrance, while possesses a unique $C_{18:1\omega5c}$. Interestingly, $C_{15:1\ iso\ F}$ is only detected in the cellular fatty acids of strain WGR700^T and *Deinococcus navajonensis* KR-33^T [2].

UV and gamol/La-radiation-resistance of WGR700^T was compared with that of *D. radiodurans* R1 and *E. coli* DH5α. At dose of 3.0 kGy gamol/La radiation, there was no growth for *E. coli* DH5α, whereas almost no decrease in survival was observed for strains *D. radiodurans* R1 and WGR700^T. Even at 15.0 kGy gamol/La radiation, there was still growth for *D. radiodurans* R1 and WGR700^T. As for UV radiation tolerance, the lethal dose of UV radiation for *E. coli* DH5α was 40 J/ m^2, whereas strains WGR700^T and *D. radiodurans* R1 could grow at doses as high as 728 and 624 J/m^2, respectively. The γ-radiation survival curve showed that D_{10} for *D. radiodurans* and WGR700^T were 9.8 kGy and 10.2 kGy (Figure 3), respectively. These data suggested that strain WGR700^T is highly ionizing-radiation resistant.

Phylogenetic tree analysis of the WGR700^T 16S rRNA suggested 87.1 ~ 95.6% sequence similarities to recognized *Deinococcus* species. The maximum similarity (95.6%) was found between WGR700^T and *Deinococcus indicus* (Wt/1a^T), a strain isolated from an arsenic contaminated aquifer located in the Chakdah district of West Bengal, India [12]. *D. indicus* (Wt/1a^T) is resistant to arsenic, but no growth was observed for strain WGR700^T in GBM medium containing As (V) (Na_2HAsO_4, 5 mmol/L) or As (III) (As_2O_3, 0.2 mmol/L). Fatty acid composition of WGR700^T is also different from Wt/1a^T. Cells of Wt/1a^T contained $C_{15:0}$ (9.3%), but it was not detected in WGR700^T. Conversely, $C_{18:0}$ (3.08%), $C_{18:1\omega5c}$ (1.34%) and $C_{15:1\ iso\ F}$ (1.24%) are found in WGR700^T, but not in Wt/1a^T. Several phenotypic and biochemical characteristic differences were also found between the two strains (Table 1).

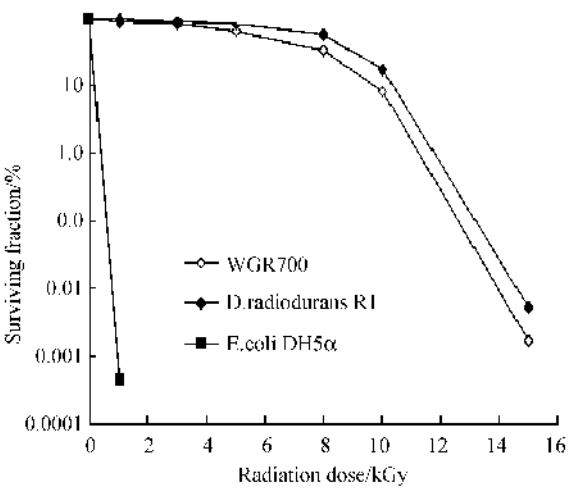

Fig.3　γ-radiation survival curve of *Escherichia coli* DH5α (■), *Deinococcus guangxiensis* (◇) and *Deinococcus radiodurans* R1 (◆).

Table 1　Comparison of the characteristics of strain WGR700^T with *D. indicus* Wt/1a^T and *D. radiodurans* R1^T

Characteristic	1	2	3
Morphology	Rods	Rods	Spherical
Colony Pigment	Pink-red	Red	Pink-red
Optimum growth: Temperature (℃)	37	30	30
pH	7.0	6.0 ~ 7.0	7.0
NaCl tolerance (%)	0 ~ 1.5	0 ~ 1.0	0 ~ 1.0
Biochemical characteristics: Gelatinase	−	+	Nd
Arginine dihydrolase	−	+	−
Utilization as carbon source: L-Histidine	−	+	−
L-Arginine	−	+	−
L-ornithine	−	+	−
α-lactose	−	+	−
Tryptophan	W	+	
L-rhamnose	W	+	−
D-melibiose	W	+	Nd
L-Arabinose	W	+	−
D-glucose	+	−	+
Sensitivity to antibiotics: Ampicillin	S	R	S
Nalidixic acid	R	S	S
Kanamycin	S	R	S
DNA G + C content (mol%)	64.7	65.8	67.0

Note: a). Strains: 1, WGR700^T; 2, *D. indicus* Wt/1a^T; 3, *D. radiodurans* R1^T. b). +, Positive; −, negative; w, weakly positive; R, resistant; S, sensitive; nd, not determined.

The distance matrix dendrogram (Figure 1) showed that strain WGR700^T is encompassed by the major branch of the genus *Deinococcus*. Results of 16S rRNA gene sequence comparison and chemotaxonomic data clearly demonstrated that strain WGR700^T is a member of the genus *Deinococcus* (Figure 1). Additionally, strain WGR700^T differs from other *Deinococcus* species with validly published names in some phenotypic characteristics (Table 1). Therefore, based on the above phenotypic and genotypic data, strain WGR700^T is proposed to represent a novel species of the genus *Deinococcus* and the name *Deinococcus guangxiensis* sp. nov. is recommended.

4 DESCRIPTION OF *Deinococcus guangxiensis* sp. nov.

Deinococcus guangxiensis (guang. xi. en′sis. N. L. masc. adj. *guangxiensis*, pertaining to Guangxi, an autonomous region in south-west China).

It is aerobic, Gram-negative, non-spore-forming, non-motile rods. The colony color on GBM and TGY tested media is pink to red. Colonies are circular, opaque and approximately 2.0 ~ 3.0 mm. WGR700^T can grow at 28℃ ~ 45 ℃ and pH 6.5 ~ 9.0 and optimum growth occurred at 37℃ and pH 7.0 ~ 8.0. It can tolerate NaCl up to 1.5%. Resistant to UV (> 728 J/m^2) or gamol/La (> 15 kGy) irradiations. Positive for catalase, aesculin, casein hydrolysis and reduction of nitrate to nitrite, but negative for oxidase, lipase, gelatinase and urease. A number of compounds can be utilized as sole carbon sources, including D-cellobiose, D-mannose, L-rhamnose, D-melibiose, glycerin, D-raffinose, L-tryptophan, sucrose, D-glucose, L-arabinose, D-maltose and amylum, but not α-lactose, D-sorbitol, fructose, L-Arginine. Resistant to nalidixic acid (50 μg/mL), ceftazidime (50 μg/mL) and spectinomycin (50 μg/mL). Sensitive to chloramphenicol (50 μg/mL), kanamycin (25 μg/mL), neomycin (25 μg/mL), Ampicillin (50 μg/mL), penicillin (25 μg/mL), rifampicin (50 μg/mL), streptomycin (50 μg/mL) and tetracycline (15 μg/mL). The major fatty acids of strain WGR700^T were 16:1ω7c (35.59%), 15:1ω6c (11.08%), 16:0 (9.93%), iso-15:0 (5.73%), iso-17:0 (5.61%), 17:1ω6c (4.69%), 17:1ω8c (3.28%), 17:0 (3.17%), 18:0 (3.08%), 16:1ω5c (1.89%) and iso-16:0 (1.89%). Major respiratory quinone was MK-8 and cell wall peptidoglycan contains ornithine as the diamino acid. The DNA G + C content is 64.7 mol%. The GenBank accession number for the 16S rRNA gene sequence of strain WGR700^T is EU285374.

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一株新的耐辐射菌 *Deinococcus guangxiensis* sp.nov.的分离鉴定及耐辐射特性分析

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摘要:【目的】从广西大学辐射中心辐射源附近被常年辐射的水样中分离并鉴定出新的耐辐射菌株,并对其耐辐射特性进行研究。【方法】通过 GBM 培养基分离培养得到一株新的耐辐射菌株,命名为 WGR700^T。应用生理生化试验,脂肪酸含量,(G+C) mol%含量测定以及 16S rRNA 序列同源性分析等方法对菌株进行鉴定,同时对 WGR700^T 的耐辐射特性进行分析。【结果】菌株 WGR700^T 为革兰氏阴性,杆状,没有鞭毛,不能运动,厌氧并能产生红色素。最佳生长温度和 PH 分别为 37℃和 pH7.0,主要的呼吸醌是 MK-8,细胞壁内还有鸟氨酸,主要脂肪酸为 16:1ω7c,16:0,15:1ω6c,iso-15:0 和 iso-17:0。G+C 含量为 64.7mol%。菌株 WGR700^T 具有很强的 UV (>728 J/m^2)和电离辐射抗性 (D_{10} = 9.8 kGy)。菌株 WGR700^T 和奇异球菌属 (*Deinococcus*)内其它菌种 16S rRNA 有很高的相似性 (87.1~95.6%)。【结论】根据 16S rRNA 及生理生化特征区别,菌株 WGR700T 应是奇异球菌属的一个新种,命名为 *Deinococcus guangxiensis* sp. nov. 模式菌株为 WGR700^T (= CGMCC1.7045^T = CICC 10360^T = JCM 15082^T)。

关键词:耐辐射菌株;耐辐射奇异球菌属;系统进化树

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