EXPERIMENTAL ARTICLES

The Effect of γ-Radiation and Desiccation on the Viability of the Soil Bacteria Isolated from the Alienated Zone around the Chernobyl Nuclear Power Plant

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Abstract—*Methylobacterium extorquens, M. mesophilicum*, and *Bacillus subtilis* strains were found to be resistant to γ -radiation, irrespective of whether they were isolated from the alienated zone around the Chernobyl Nuclear Power Plant or outside this zone. The LD₉₀ of *Methylobacterium* and *B. subtilis* strains with respect to γ -radiation was 2.0–3.4 and 3.7–4.4 kGy, respectively, whereas their LD_{99.99} values were 4.5–6.9 and more than 10 kGy, respectively. The high threshold levels of γ -radiation for *Methylobacterium* and *B. subtilis* imply the efficient functioning of DNA repair systems in these bacteria. Unlike *Bacillus polymyxa* cells, the cells of *M. extorquens, M. mesophilicum*, and *B. subtilis* were also resistant to desiccation. *Pseudomonas* sp., *Nocardia* sp., and nocardioform actinomycetes were sensitive to both γ -radiation and desiccation. Similar results were obtained when the bacteria studied were exposed to hydrogen peroxide and ultraviolet radiation. The results obtained indicate that the bacteria that are resistant to γ -radiation are also resistant to desiccation, UV radiation, and hydrogen peroxide. The possibility of using common laboratory tests (such as the determination of bacterial resistance to UV light and desiccation) for the evaluation of bacterial resistance to γ -radiation is discussed.

Key words: ChNPP, γ -radiation, desiccation, bacterial resistance, Methylobacterium, Bacillus.

Earlier, we investigated the susceptibility of the soil bacteria isolated from the alienated zone around the Chernobyl Nuclear Power Plant (ChNPP) to ultraviolet radiation and hydrogen peroxide (factors inducing DNA damage) and found that γ -radiation in doses lower than 0.6 kGy virtually did not influence the viability of Methylobacterium strains [1, 2]. However, the results obtained in those studies were insufficient to derive the γ -radiation dose–survival rate curves. In the present study, we investigated the effect of γ -radiation on the survival of the soil bacteria isolated from the alienated zone around the Chnpp, as the irradiation of these bacteria with ionizing radiation in their habitat could modify their natural radioresistance. Along with this, we studied the sensitivity of these bacteria to desiccation, since there is evidence that the highly radioresistant bacterium Deinococcus radiodurans is resistant to desiccation too [3, 4]. The desiccation of D. radiodurans induced the fragmentation of DNA in this bacterium, i.e., inflicted damage on DNA similar to that inflicted by ionizing radiation. DNA strand breaks were also found in some other bacteria subjected to drying [5–8].

MATERIALS AND METHODS

Bacteria. Experiments were carried out with the strains of the genera *Methylobacterium, Bacillus, Pseudomonas*, and others, which were isolated earlier from the alienated zone around the ChNPP and from other regions of Ukraine [9, 10]. The strains used in the study are listed in Table 1.

Cultivation conditions. *Methylobacterium extorquens* and *M. mesophilicum* strains were grown on a mineral agar containing 0.5 vol % methanol as the carbon source [9]. This medium was designated MMA. The other bacteria were grown on glucose–potato agar (GPA). One-day-old cultures grown on the agar media were used to inoculate a liquid mineral medium containing 0.5 vol % methanol (*Methylobacterium* strains), or 1% glucose and 0.05% yeast autolysate (*Bacillus* strains) [10], or 1% glucose (all of the other strains studied). The bacteria were cultivated at 30°C in shaken flasks for 18 h.

Exposure to \gamma-radiation. Radiological studies, as well as exposure to UV radiation, hydrogen peroxide, and desiccation, were carried out using the same 18-h bacterial culture containing from 10⁸ to 10⁹ cells/ml in

Spacios	C turn in	Source			
Species	Strain	region	ecosystem and its radioactivity		
Methylobacterium	19ch	10-km ChNPP zone, Novo-Shepelichi	Soil under pine forest, $R = 6.5 \times 10^{-6}$ Ci/kg soil		
extorquens	9-01	2-km ChNPP zone, Kopachi	Oak leaves in rust-colored forest $R = 5.2 \times 10^{-6}$ Ci/kg soil		
	X4	Khar'kov region	Soil under vineyard $R \le 5.0 \times 10^{-9}$ Ci/kg soil		
	B6	Environs of Kiev	Grape leaves, $R \le 5.0 \times 10^{-9}$ Ci/kg soil		
	B9	Environs of Kiev	Soil under vineyard, $R \le 5.0 \times 10^{-9}$ Ci/kg soil		
Methylobacterium mesophilicum	8-18	10-km ChNPP zone, Novo-Shepelichi	Soil under pine forest $R = 4.7 \times 10^{-6}$ Ci/kg soil		
	9-18	10-km ChNPP zone, Novo-Shepelichi	Soil under spruce forest, $R = 1.0 \times 10^{-6}$ Ci/kg soil		
	P93	Volynsk region	Soil under clover field, $R \le 5.0 \times 10^{-9}$ Ci/kg soil		
	4181	Collection strain (ATCC 29983)	-		
	Ch8	Environs of Kiev	Celandine leaves, $R \le 5.0 \times 10^{-9}$ Ci/kg soil		
Bacillus subtilis	17-16	10-km ChNPP zone, Pripyat'	Soil under grasses, $R = 1.7 \times 10^{-6}$ Ci/kg soil		
	28-95	Environs of Kiev	Soil under grasses, $R \le 5.0 \times 10^{-9}$ Ci/kg soil		
Bacilus polymyxa	8-2	10-km ChNPP zone, Novo-Shepelichi	Soil under pine forest, $R = 4.7 \times 10^{-6}$ Ci/kg soil		
Pseudomonas sp.	17-12	10-km ChNPP zone, Pripyat'	Soil under grasses, $R = 1.7 \times 10^{-6}$ Ci/kg soil		
	3-21	2-km ChNPP zone, Kopachi	Soil under grasses, $R = 2.2 \times 10^{-7}$ Ci/kg soil		
Nocardia sp.	17-3	10-km ChNPP zone, Pripyat'	Soil under grasses, $R = 1.7 \times 10^{-6}$ Ci/kg soil		
Nocardioform bacteria	3-23/1	2-km ChNPP zone, Kopachi	Soil under grasses, $R = 2.2 \times 10^{-7}$ Ci/kg soil		
	3-11	2-km ChNPP zone, Kopachi	Soil under grasses, $R = 2.2 \times 10^{-7}$ Ci/kg soil		
	3-9/1	2-km ChNPP zone, Kopachi	Soil under grasses, $R = 2.2 \times 10^{-7}$ Ci/kg soil		
	6-1	2-km ChNPP zone, Kopachi	Mountain ash leaves in rust-colored forest, $R \le 5.1 \times 10^{-6}$ Ci/kg soil		
	17-26	10-km ChNPP zone, Pripyat'	Soil under grasses, $R = 1.7 \times 10^{-6}$ Ci/kg soil		

Table 1. Bacteria used in this study a	and their sources
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Note: R is the radioactivity of soddy podzolic soil at the time of sampling (1993 through 1996).

the test and control experiments. To this end, 1-ml aliquots of each of the strains studied were placed in sterile 2-ml Eppendorf tubes, and the tubes were placed in a petri dish. The petri dish was inserted into a device equipped with a source of γ -radiation (radioactive ⁶⁰Co). In all of the experiments, the dose rate was 0.05 kGy/s. Bacteria were exposed to radiation doses varying from 0.5 to 10 kGy. The number of bacterial cells survived after their exposure to y-radiation was determined by enumerating colonies grown on the respective agar media after these media had been inoculated with the serial tenfold dilutions of the control (unexposed) and experimental (exposed) cell suspensions. Measurements were conducted in triplicate. Colonies were enumerated after 1 to 7 days of growth, depending on the growth rate of the particular bacterial strain. The results were expressed in colony-forming units (CFU).

Desiccation. Experiments of this type were carried out by the Mattimore and Battista method [4] with minor modifications. Aliquots (0.5 ml) of 18-h-old bacterial cultures were poured into 4-cm sterile petri dishes, which were then placed in a desiccator with calcined CaSO₄. The desiccator was sealed and kept in a

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dry place for 6 weeks. Then dry bacterial cells were resuspended in 0.5 ml of physiological saline solution, and the serial tenfold dilutions of this suspension were plated onto GPA and MMA to determine the number of viable cells.

Ultraviolet irradiation. To determine the resistance of bacterial cells to UV light, they were plated onto GPA and MMA and irradiated with the UV light ($\lambda = 254$ nm) from a DB-30 lamp. Illumination doses were determined using a DAU-81 dosimeter and expressed in J/m². The procedure was described in detail in our previous publications [1, 2]. The survival rate of bacteria exposed to UV light was determined in the same manner as in the case of γ -irradiation.

Survival curve processing. The survival curves of bacterial cells exposed to UV light and ionizing radiation were processed to determine the threshold dose D_{q} , the dose that kills 90% of exposed cells (LD₉₀), and the dose that kills 99.99% of exposed cells (LD_{99.99}). The threshold dose D_{q} (a measure of the DNA repair ability of cells) corresponded to the point of interception of the extrapolated straight section of the dose–response curve with the straight line corresponding to 100% bacterial survival [11, 13].



Fig. 1. Curves characterizing the tolerance of (a) *M. mesophilicum* strains 8-18 and Ch8*, *M. extorquens* strains 19ch and B9*, (b) *B. subtilis* strains 17-16 and 28-95*, *Pseudomonas* sp. 17-12, and *Nocardia* sp. 17-3 to various γ -radiation doses. The asterisks mark collection strains in the legends to all figures.

Exposure to hydrogen peroxide. The effect of H_2O_2 on bacterial survival was investigated as described earlier [10].

RESULTS AND DISCUSSION

The effect of γ -radiation and desiccation on bacterial survival was investigated using the bacterial strains that were isolated from the alienated zone around the ChNPP and the collection strains of the same bacterial species, which were isolated from areas with natural background radiation.

The effect of γ -radiation on bacterial survival. As is evident from the dose–response curves of the bacteria studied (Fig. 1a), *Methylobacterium* strains are resistant to γ -radiation, irrespective of whether they were isolated from the alienated zone around the ChNPP or outside this zone. One of these strains, *M. extorquens* B9, which was isolated from the plant phyllosphere outside the ChNPP zone, exhibited the highest radioresistance; LD₉₀ and LD_{99.99} for this strain were equal to 3.4 and 6.9 kGy, respectively (Table 2). For the other *Methylobacterium* strains studied, these parameters, as well as D_q , were lower (Table 2). Considerable intra- or interspecies differences within the genus *Methylobacterium* were not detected. The high threshold doses for *Methylobacterium* strains (up to 2.2 kGy) indicate that they possess active DNA repair systems.

B. subtilis strains were highly resistant to γ -radiation (Fig. 1b), showing LD₉₀ and LD_{99,99} values equal to

Species	Strain	γ-Radiation, kGy		UV radiation, J/m ²			Desicca-	U. 0* 0	
		Dq	LD ₉₀	LD _{99.99}	Dq	LD ₉₀	LD _{99.99}	tion*, %	$H_2 O_2, \%$
Methylobacterium extorquens	19ch	1.1	2.1	5.0	70	120	315	46.6	30.9
M. extorquens	B9	2.2	3.4	6.9	93	160	360	90.0	98.0
M. mesophilicum	8-18	1.2	2.2	5.2	95	150	330	53.3	53.3
M. mesophilicum	Ch8	1.3	2.0	4.5	87	130	290	62.8	98.6
Bacillus subtilis	17-16	1.9	4.4	>10	50	180	>360	90.0	90.0
B. subtilis	28-95	1.3	3.7	>10	10	190	>360	68.0	85.0
Nocardia sp.	17-3	<0.2	0.2	0.8	<16	16	70	0.01	6.5
Pseudomonas sp.	17-12	< 0.02	0.02	0.09	<16	16	70	0.002	0.5

Table 2. Parameters characterizing the survival of some bacteria under the action of various stress factors

* Percentage of cells that survived dry storage in a desiccator for 42 days. ** Percentage of viable cells in a cell suspension exposed to 0.1 M H_2O_2 for 5 min. D_q is the threshold dose (see the *Materials and Methods* section for explanation). LD_{90} and $LD_{99.99}$ are the doses that kill 90 and 99.99% of cells exposed to γ -radiation.

Stanin	Radiotolerance parameters, kGy					
Stram	Dq	LD ₉₀	LD _{99.99}			
M. extorquens B9	2.2	3.3	6.9			
E. coli B	0.04	0.15	0.45			
D. radiodurans WT	6.4	8.3	12.7			
M. radiotolerans 0-1	1.8	3.0	6.5			
The ratios of radiotolerance parameters						
Compared strains	D_q/D_q	LD ₉₀ /LD ₉₀	LD _{99.99} /LD _{99.99}			
M. extorquens B9/E. coli B	55	22	15			

Table 3. Parameters characterizing the tolerance of *M. extorquens* B9 to γ -radiation in comparison with the radiosensitive bacterium *E. coli* B and the radioresistant bacteria *D. radiodurans* WT and *M. radiotolerans* 0-1

Note: Radiotolerance parameters for *E. coli* B, *D. radiodurans* WT, and *M. radiotolerans* 0-1 were calculated from the relevant data published by Nazim and James [12], Moseley [13], and Ito and Iizuca [14].

2.9

1.2

3.7–4.4 and more than 10 kGy, respectively (Table 2). The radioresistances of *B. subtilis* strain 17-16, which was isolated from the ChNPP zone, and *B. subtilis* strain 28-95, which was isolated from a habitat outside this zone, were almost the same. The high threshold doses for these strains indicate that they possess active DNA repair systems. *Pseudomonas* sp. 17-12 and *Nocardia* sp. 17-3 were found to be sensitive to γ -radiation (Table 2).

M. extorquens B9/D. *radiodurans* WT *M. extorquens* B9/M. *radiotolerans* 0-1

Although the *Methylobacterium* and *Bacillus* strains studied were characterized by approximately equal threshold doses, the latter strains were more radioresistant than the former strains, as is evident from the almost twofold higher $LD_{99,99}$ values of bacilli (Fig. 1 and Table 2). These data suggest that bacilli possess not only active DNA repair systems, but also some other mechanisms of cell protection against detrimental factors.

The radioresistance parameters of one of the strains studied in this work. M. extorauens B9, were compared with those calculated from the relevant data reported for the radiosensitive bacterium Escherichia coli B [12], the radioresistant bacterium D. radiodurans WT [13], and the radiotolerant bacterium *M. radiotolerans* strain 0-1 [14]. As can be seen from Table 3, these bacteria greatly differ in their threshold doses, M. extorquens B9 being 55 times more radioresistant than E. coli B and almost 3 times more radiosensitive than D. radiodurans WT. This fact is not surprising, since the latter bacterium is known to possess a very active DNA repair system. At the same time, the radioresistance of M. extorquens B9 was close to that of M. radiotolerans ("Pseudomonas radiora") strain 0-1 (NCIB 10815). For clarity, these data, as well as those concerning two other bacteria studied in this work (M. extorquens B9, B. subtilis 17-16, and Pseudomonas sp. 17-12) are summarized pictorially in Fig. 2. As is evident from this fig-

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ure, the radioresistance of *Methylobacterium* and *Bacillus* strains is intermediate between those of *D. radiodurans* and *E. coli*, whereas the radioresistance of *Pseudomonas* sp. 17-12 is close to that of *E. coli*. It can be seen that the radioresistance of the *Methylobacterium* strains studied is relatively high.

2.5

1.1

The effect of desiccation on bacterial survival. Many organisms develop the ability to withstand desiccation and dry storage. Desiccation tolerance is typical of seeds of higher and lower plants, yeasts, bacteria, fungi, spores, and so on. Nevertheless, the desiccation



Fig. 2. The dose–survival curves of (1) *M. extorquens* B9, (2) *B. subtilis* 17-16, (3) *Pseudomonas* sp. 17-12, (4) *E. coli* B [12], (5) *D. radiodurans* WT [13], and (6) *M. radiotoler-ans* 0-1 [14] exposed to γ-radiation.

1.8

1.1



Fig. 3. The survival rates of various bacteria subjected to desiccation: (open bars) *M. mesophilicum* strains 8-18, 9-18, 4181*, P93*, and Ch8* and *M. extorquens* strains 19ch, 9-01, B9*, X4*, and B6*; (shaded bars) *B. subtilis* strains 17-16 and 28-95* and *B. polymyxa* 8-2; (black bars) *Pseudomonas* sp. strains 3-21 and 17-12 and nocardioform strains 3-23/1, 17-3, 3-9/1, 6-1, 3-11, and 17-26.



Fig. 4. The dose–survival curves of (a) *M. mesophilicum* strains 8-18 and Ch8*, *M. extorquens* strains 19ch and B9*, (b) *B. subtilis* strains 17-16 and 28-95*, *Pseudomonas* sp. strain 17-12, and *Nocardia* sp. strain 17-3 exposed to UV light.

tolerance of bacteria is insufficiently studied [15]. For this reason, the range of bacteria tested for desiccation tolerance (Fig. 3) was wider than the range of bacteria tested for resistance to γ -radiation (Fig. 1). All of the Methylobacterium strains were found to be relatively tolerant to desiccation, showing survival rates from 30 to 90% (Fig. 3). The Methylobacterium strains isolated from the ChNPP zone and outside did not differ in their desiccation tolerance. B. subtilis strains were also resistant to desiccation (Fig. 3), although the other bacillar species studied, B. polymyxa 8-2, was very sensitive to desiccation. Similar results were obtained earlier for the resistance of *B. subtilis* and *B. polymyxa* species to UV irradiation. The survival rates of Pseudomonas strains and nocardioform actinomycetes with respect to desiccation were as low as 0-5%.

An analysis showed that the *Methylobacterium* and *Bacillus* strains that are resistant to γ -radiation are also resistant to desiccation. Unlike the *Methylobacterium* strains, all of which were resistant to γ -radiation and desiccation, irrespective of the level of natural background radiation at the sites from which they were isolated, some *Bacillus* strains turned out to be sensitive to γ -radiation and desiccation. Similar regularities were earlier observed for the representatives of these two bacterial genera which were investigated for their resistance to UV radiation [1].

The sensitivity of bacteria to UV light and H_2O_2 . Since the bacterial strains used in this study were not earlier investigated for their sensitivity to UV light and hydrogen peroxide, we performed such studies and found that *M. extorquens*, *M. mesophilicum*, and *B. subtilis* strains were resistant to UV radiation (Fig. 4) and

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 H_2O_2 (Table 2). The LD_{90} of the methylobacteria and *B. subtilis* with respect to UV radiation was 120–160 and 180–190 J/m², respectively. The $LD_{99,99}$ values of these bacteria were 290–360 and more than 360 J/m², respectively. At the same time, *Pseudomonas* sp. 17-12 and *Nocardia* sp. 17-3 were sensitive to H_2O_2 and UV radiation (Table 2).

A comparative analysis of bacterial resistance to DNA-damaging factors. As is evident from the data presented in Table 2, the bacterial strains that are resistant to γ -radiation are also resistant to desiccation. This fact is not surprising, since γ -radiation and desiccation are known to induce similar damage (double-strand breaks) to DNA. This was shown for D. radiodurans [4, 16], E. coli [5], B. subtilis [7, 8], and Kineococcus auranticaus [17]. There is evidence that resistance to radiation parallels resistance to desiccation. For instance, Mattimore and Battista reported that all radiosensitive D. radiodurans strains were also sensitive to desiccation [4]. The *E. coli* mutant defective in the DNA repair system *uvrA recA* was more sensitive to desiccation at the water activity $a_w = 0.53$ or lower than the parent strains [5]. Therefore, the enhanced resistance of some bacteria to γ -radiation may be accompanied by their enhanced resistance to other DNA-damaging factors (desiccation, UV radiation, and hydrogen peroxide). This suggestion, which was first made in our earlier publications [1, 10], received a strong experimental underpinning in the present work.

Thus, the *M. extorquens, M. mesophilicum*, and *B. subtilis* strains studied are resistant to γ -radiation, desiccation, UV radiation, and hydrogen peroxide, irrespective of whether they were isolated from the alienated zone around the ChNPP or outside this zone. The high resistance of these bacteria to the DNA-damaging factors implies the efficient functioning of DNA repair systems in these bacteria. The results obtained confirm the suggestion that radioresistant bacteria can be selected through the selection of desiccation-resistant strains and that the resistance of bacteria to ionizing radiation, desiccation, and other DNA-damaging factors is determined by the activity of DNA repair systems.

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