DNA polymorphisms in new isolates of '*Deinococcus radiopugnans*'

C. IAN MASTERS,¹ ROBERT G. E. MURRAY,² BEVAN E. B. MOSELEY³ and
KENNETH W. MINTON^{1*}

¹Department of Pathology, F. E. Hébert School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD 20814-4799, USA

²Department of Microbiology and Immunology, The University of Western Ontario, London N6A 5C1, Canada

³Agricultural and Food Research Council, Institute of Food Research, Reading Laboratory, Shinfield, Reading, Berkshire RG2 9AT, UK

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Nineteen new *Deinococcus* isolates from soil, 18 of which were from samples immediately adjacent to a lake in Nottingham, UK, were characterized by conventional criteria, and found to be identical to each other and to conform most closely to the species *D. radiopugnans*. However, we detected three different restriction enzyme activities in three different isolates. Because of this suggestion of heterogeneity, we examined the isolates for restriction fragment length polymorphisms (RFLPs). RFLP analysis of the 19 original isolates, using three different probes, distinguished 17 divergent groups. This extraordinary diversity cannot be attributed to geographical differences, nor to the method of isolation, which employed UV-radiation selection.

Introduction

The genus *Deinococcus* (Brooks & Murray, 1981; Murray, 1986; Murray & Brooks, 1986) consists of four species of nonsporing bacteria that share an exceptional resistance to UV and ionizing radiation (Moseley, 1983) and to desiccation (Murray, 1986; Sanders & Maxcy, 1979). Since the first isolation of the genus type strain, *D. radiodurans* R1 (Anderson *et al.*, 1956), from irradiated canned meat, the other species of this genus which have been recognized are: *D. radiopugnans*, from irradiated haddock tissue in Massachusetts (Davis *et al.*, 1963) and from weathered granite in Antarctica (Counsell & Murray, 1986); *D. radiophilus*, from irradiated Bombay duck in India (Lewis, 1971); and *D. proteolyticus*, from irradiated faeces of *Llama glama* (Kobatake *et al.*, 1973) and irradiated sewage sludge cake and animal feeds in Japan (Ito *et al.*, 1983). In contrast to the latter three species, *D. radiodurans* has been isolated many times with a world-wide distribution (Murray, 1986).

The diversity of natural bacterial populations is believed to be far greater than previously detected by culture-dependent laboratory methods (Ward *et al.*, 1990, and citations therein). Recent evidence in favour of this proposition has been obtained by cloning and

sequencing of 16S rRNAs directly from natural biomass in two bacterial populations, Sargasso sea bacterioplankton (Giovannoni *et al.*, 1990) and a cyanobacterial mat from Yellowstone hot springs (Ward *et al.*, 1990). One obstacle to detecting the full range of natural bacterial diversity is that laboratory-based culture is itself a form of selection. Another obstacle is that closely related organisms may appear identical by various taxonomic criteria, and yet be quite diverse in genetic organization. In the current communication we characterized every *Deinococcus* isolate obtained from a single source, via a stringent selection method. Although the organisms were identical by conventional criteria we found an extraordinary diversity of DNA polymorphisms.

Methods

Bacterial strains and plasmids. Previously isolated strains and plasmids are listed in Table 1. *Deinococcus* spp. were grown in TGY nutrient broth (0.8% tryptone, 0.4% yeast extract, 0.1% glucose) or on TGY plates, solidified by 1.5% (w/v) agar. *Escherichia coli* was grown at 37 °C in LB broth.

Isolation of deinococci. Solid and water samples (1–2 g) were collected from various sites and mixed with 15 ml sterile 67 mM-potassium phosphate buffer, pH 7.0. Debris and soil were allowed to settle out and the supernatant placed in a sterile glass Petri dish to a depth no greater than 1 mm and exposed to 254 nm UV-radiation from a germicidal

Abbreviation: RFLP, restriction fragment length polymorphism.

Table 1. *Bacterial strains and plasmids*

Strain or plasmid*	Description (reference)
<i>Deinococcus</i>	
<i>D. radiodurans</i> R1 (UWO 288 ^T ; ATCC 13939)	Brooks & Murray (1981)
<i>D. radiodurans</i> SARK (UWO 298)	Brooks & Murray (1981)
<i>D. radiopugnans</i> (UWO 293 ^T ; ATCC 19172)	Brooks & Murray (1981)
<i>D. radiophilus</i> (UWO 1055 ^T ; ATCC 27603)	Brooks & Murray (1981)
<i>D. proteolyticus</i> (UWO 1056 ^T)	Brooks & Murray (1981)
<i>Escherichia coli</i> MM294 (ATCC 33625)	K12 derivative
Plasmids	
pUE58	5.6 kb <i>Eco</i> RI fragment of <i>mtcA</i> ⁺ <i>D. radiodurans</i> R1 chromosomal DNA, cloned in pAT153 (Al-Bakri <i>et al.</i> , 1985)
pUE200	8.1 kb <i>Eco</i> RI- <i>Sal</i> I fragment of <i>uvrCDE</i> ⁺ <i>D. radiodurans</i> R1 chromosomal DNA, cloned in pAT153
pS19	Full-length clone of natural <i>D. radiodurans</i> SARK plasmid pUE11 (45 kb), into the <i>Eco</i> RV site of Km ^R ColE1 derivative pS27 (Smith <i>et al.</i> , 1989)

* UWO, University of Western Ontario culture collection, London, Ontario, Canada; ATCC, American Type Culture Collection, Rockville, Maryland, USA.

lamp at a dose rate of 1.05 J m⁻² s⁻¹. Samples were taken after 15 and 20 min of irradiation (945 and 1260 J m⁻² total fluence, respectively), plated on TGY agar, and incubated at 30 °C. Red-pigmented colonies were selected and colony isolates derived from them.

Phenotypic characterization. (i) UV resistance was assessed by the agar plate streak test (Naumovski & Friedberg, 1982), with the modification that higher UV fluences were employed. (ii) UV survival curves were obtained as described by Moseley & Evans (1983). (iii) Measurement of DNA G + C content was by thermal denaturation (Marmur & Doty, 1962). Salmon sperm DNA (43 mol% G + C) was used as control. (iv) β -Galactosidase was assayed as previously described (Lennon & Minton, 1990). (v) Electron microscopy was performed as described by Brooks *et al.* (1980). (vi) Natural transformability was assessed as follows. All isolates were plated on TGY agar containing 25 μ g rifampicin ml⁻¹. Spontaneous Rif^R mutants were selected and DNA prepared from them. All original Rif^S isolates, as well as Rif^S *D. radiodurans* strains R1 and SARK, were transformed with their own (Rif^R) DNA, as described by Tirgari & Moseley (1980). Transformants were selected on TGY plates containing 25 μ g rifampicin ml⁻¹. (vii) Diamino acids in the peptidoglycan layer were determined as follows. Peptidoglycan cell walls were prepared according to Schleifer & Kandler (1972); 1 mg of peptidoglycan was then hydrolysed in 6 M-HCl at 110 °C for 18 h, and analysed in a Beckman model 120C amino acid analyser.

Screening for restriction endonucleases. Plateau-phase cultures were harvested and washed with TE buffer (10 mM-Tris, 1 mM-EDTA), resuspended in 10 ml 10 mM-Tris/HCl pH 7.5, 1 mM-EDTA, 1 mM-phenylmethylsulphonyl fluoride, and lysed by French press at 20000 p.s.i. (138 MPa) at 4 °C. Cell debris was removed by centrifugation at

27000 g for 10 min. Restriction endonucleases were detected by incubating aliquots of supernatant with 1 μ g of DNA (λ , pBR322 and ϕ X174) in a final volume of 20 μ l with 10 mM-MgCl₂, 10 mM-Tris/HCl pH 7.8, 1 mM-dithiothreitol, 0–100 mM-NaCl at 37 °C for 2 h. Cleavage products were resolved by agarose gel electrophoresis (0.6–0.8%) and the calculated DNA fragment sizes compared with restriction patterns of other known restriction endonucleases.

Assessment of polar lipids. The cultures of each isolate grown in TGY broth were harvested, extracted using the procedure of Bligh & Dyer (1959), and the extracts submitted to one-dimensional thin-layer chromatography (TLC) on 20 × 20 cm plates coated with 0.25 mm thick silica gel G-25. The two solvent systems and several detection reactions applied to the developed plates were those of Counsell & Murray (1986). Solvent 1 was chloroform/methanol/water (65:25:4, by vol.) and solvent 2 was chloroform/acetone/methanol/acetic acid/water (10:4:2:2:1, by vol.). Extracts of a set of *Deinococcus* reference strains, *D. radiodurans* SARK UWO 298, *D. radiophilus* UWO 1055^T, *D. radiopugnans* UWO 293^T and *D. proteolyticus* UWO 1056^T were run on the same TLC plates for comparison.

DNA purification and manipulations. DNA isolation procedures, enzymic reagents, electrophoresis, Southern blotting, hybridization, washing of blots, autoradiography, and removal of probe from blots were as described by Lennon & Minton (1990). For most Southern blot experiments 3 μ g samples of CsCl-purified chromosomal DNAs were electrophoresed on 0.8% agarose horizontal gels at 1.5 V cm⁻¹. Hybridization with ³²P-labelled probe employed 10⁶ c.p.m. ml⁻¹ in 50% (v/v) formamide for 18 h at 37 °C. Blots were washed at high stringency with a final washing at 60 °C in 15 mM-NaCl, 1.5 mM-sodium citrate, 0.1% sodium dodecyl sulphate.

Results

Isolation of *Deinococcus* strains

Fifteen samples, 1–2 g each, from various locations within the UK were heavily UV-irradiated, as described in Methods. Four locations (sites 1, 4, 5 and 6) yielded an overall total of 19 *Deinococcus*-like red-pigmented colonies. They were all from soil taken from very damp areas. Isolate 1/10 was found in Edinburgh, next to a stream. Isolates 4/1 through 4/16, 5/1 through 5/14, and 6/1 were from three locations (sites 4, 5 and 6), all within a 20 m² area next to a lake in Nottingham. By streak test all 19 isolates were UV resistant. The UV-radiation survival curves of 14 isolates are shown in Fig. 1. Also included in this figure is *D. radiodurans* R1 and the UV-sensitive *D. radiodurans* R1 derivative, strain UVS78, that lacks two UV endonuclease repair pathways (Evans & Moseley, 1983). The high UV resistance of the new isolates is similar to and characteristic of all known deinococcal species (Moseley, 1983).

Identification of new strains

The four recognized species of *Deinococcus* are quite similar in phenotype, although distinguishable by a number of characteristics (Murray, 1986). In common with all deinococci, all 19 current isolates showed the following: (i) dyads of cocci with Gram stain variability, predominantly Gram positive during the exponential phase; (ii) a very high DNA G + C content, of 67.5 ± 1.5 mol %; and (iii) production of catalase. All the current isolates differed from *D. radiodurans*, but not from the other three *Deinococcus* species, in not being naturally transformable by DNA from spontaneous Rif^R mutants of the same isolates. All the current isolates produced β -galactosidase, similar only to *D. radiopugnans*. These observations suggest assignment of all of the new isolates to *D. radiopugnans*, as only this species meets these criteria: red-pigmented radiation-resistant cocci with very high DNA G + C content, that are non-transformable and produce β -galactosidase. In addition, DNA from *D. radiopugnans* (UWO 293^T) hybridized strongly to DNA from the new isolates, but not to DNA from *D. radiodurans* R1, *D. radiodurans* SARK, *D. proteolyticus*, or *E. coli* (Fig. 2). This observation supports the assignment of the new isolates to *D. radiopugnans*.

Five representative isolates were selected for more detailed characterization: the isolate from site 1 (1/10), two isolates from site 4 (4/1 and 4/12), one isolate from site 5 (5/3), and the isolate from site 6 (6/1). It should be noted that three of these sites (4, 5, and 6) were essentially the same, as these soil samples were collected within a few metres of each other. Micrographs of thin

Fig. 1. Survival of the new isolates following UV-irradiation.

sections of the five isolates were identical, showing cocci with a complex cell wall profile with an outer membrane displaying a looped appearance widely separated from a thick peptidoglycan layer, features typical of the genus. Fig. 3 shows a representative micrograph of one of these isolates. The peptidoglycan layer is not uniformly smooth, but has irregularities every 15–18 nm, indicative of fenestrations that are found in only two species, *D. radiodurans* and *D. radiopugnans* (Brooks *et al.*, 1980; Brooks & Murray, 1981; Murray, 1986). A well developed S-layer, a feature of *D. radiodurans*, was not present. The diamino acid in the peptidoglycan of all five isolates was ornithine, an unusual feature characteristic of all deinococci. None of the five new isolates grew on 5% (w/v) NaCl, distinguishing them from *D. radiophilus*, and none produced acid from glucose, distinguishing them from *D. proteolyticus*. All five isolates reduced nitrate, similar only to *D. radiopugnans*.

All species of *Deinococcus* contain large quantities of polar lipids, mostly phosphoglycolipids (Counsell & Murray, 1986; Huang & Anderson, 1989). The five isolates examined showed polar lipid profiles that were identical to each other (Fig. 4). The profile most closely resembled *D. radiopugnans*. Distinguishing features in solvent system 1 (Fig. 4a) were two prominent and closely adjacent phospholipids at R_F approx. 0.35 present in the new isolates as well as in *D. radiopugnans* and *D. radiodurans*; and a phospholipid at R_F approx. 0.56, which was present only in the new isolates and in *D. radiopugnans* and *D. proteolyticus* (Fig. 4b). Solvent system 2 showed a phospholipid at R_F 0.35 present only in the new strains and *D. radiopugnans* (Fig. 4c, d) and another at R_F 0.57 present only in the new strains and in *D. proteolyticus* and *D. radiopugnans*. The observations on these five isolates are most compatible with the assignment to *D. radiopugnans*.

Fig. 2. Southern blots probed with genomic DNA of *D. radiopugnans*. The blots shown in Figs 5 and 6 (stripped of label) were hybridized and washed under stringent conditions. The probe was 2 µg 32 P-labelled genomic DNA of *D. radiopugnans* (UWO 293^T). (a) Lanes: 1, *D. proteolyticus*; 2, *E. coli*; 3, *D. radiodurans* SARK; 4, *D. radiodurans* R1; 5, *D. radiopugnans* (UWO 293^T); 6, 1/10; 7, 6/1; 8, 4/1; 9, 4/5; 10, 4/8; 11, 4/9; 12, 4/11; 13, 4/12; 14, 4/13; 15, 4/16. (b) Lanes: 1, 5/14; 2, 5/13; 3, 5/10; 4, 5/9; 5, 5/7; 6, 5/6; 7, 5/5; 8, 5/1; 9, *D. radiodurans* R1; 10, *D. radiodurans* SARK; 11, *D. radiodurans* R1; 12, *E. coli*; 13, *D. proteolyticus*. Lanes 1–15 of (a) are lanes 3–17 of Fig. 5. Lanes 1–7 and 8–13 of (b) are lanes 1–7 and 9–14, respectively, of Fig. 6. One of the isolates, 5/3, was not studied.

Fig. 3. Thin section electron micrograph of strain 1/10 (fixed using OsO₄). Bar, 100 nm.

Restriction endonucleases in the new D. radiopugnans isolates

The 19 new isolates were evaluated for the presence of restriction endonucleases, as described in Methods. Strain 4/9 contained an isoschizomer of *Xho*II; strain 5/3 contained an isoschizomer of *Pvu*I; and strain 6/1 contained an isoschizomer of *Bst*EII. No restriction enzyme activities were detected in the other 16 isolates. The activities of all three restriction endonucleases were optimal at approximately 37 °C at 50 mM-NaCl (data not shown). The *Bst*EII isoschizomer found in isolate 6/1 could be useful since *Bst*EII reactions are performed at ≥ 50 °C.

Restriction fragment length polymorphisms

The first suggestion of heterogeneity amongst these isolates was the presence of three different restriction enzyme activities in three different isolates, as described above. To further differentiate the colony isolates, we employed analysis of restriction fragment length polymorphisms (RFLPs), a technique that has recently proved useful for distinguishing strains in a variety of bacterial species (Kaijalanin & Lindstrom, 1989, and citations therein). Southern blots of *Eco*RI-cleaved genomic DNA were hybridized with the three different probes described in Table 1: pUE58, pUE200 and pS19.

Cross-hybridization of these probes to other previously described species of *Deinococcus*, as well as the new isolates, occurred under stringent conditions (Figs 5 and 6). All the known species varied from each other as expected, but the new strains showed unexpected variety. While many isolates appeared different by hybridization to two or all three of the probes, differences between some of the strains were limited to only one of the three probes used; for example strains 4/8, 4/9 and 4/11 had identical patterns for pUE58 and pUE200 but 4/9 differed when probed with pS19.

The chromosomal probes, pUE58 and pUE200, permitted differentiation of the 19 isolates into 12 RFLP groups (Table 2). Polymorphisms detected with the SARK plasmid probe, pS19, increased the number of polymorphism groups from 12 to 17. Cross-hybridization of these three probes to genomic DNA of the other species and the new isolates was not as prominent as hybridization to the *D. radiodurans* strains; however, hybridization was distinct and highly polymorphic.

UV-irradiation was used as the selective agent to acquire these new deinococcal isolates. The great majority of all known *Deinococcus* strains have been isolated using similar protocols (UV- or X-irradiation; Murray, 1986). Thus, the chromosomal variation could be due to the selection procedure itself, as UV might induce mutations which are detected as RFLPs. To investigate this hypothesis, one of the new *D. radiopugnans*

Fig. 4. TLC of polar lipids. (a) TLC of lipid extracts developed in solvent 1 and acid charred. (b) Diagram reaction to detection sprays following TLC of lipid extracts developed in solvent 1. (c) TLC of lipid extracts developed in solvent 2 and acid charred. (d) Diagram of reaction to detection sprays following TLC of lipid extracts developed in solvent 2. Reaction to detection sprays is indicated as follows: ●, Positive for α -naphthol and Bial's reagent; (○), ninhydrin positive; ⊕, phosphate positive. Lanes: 1, *D. radiopugnans*; 2, *D. radiodurans*; 3, *D. radiophilus*; 4, *D. proteolyticus*; 5, 1/10; 6, 5/3; 7, 6/1; 8, 4/1; 9, 4/12.

nans isolates, strain 4/9, and the type strain of the genus, *D. radiodurans* R1, were heavily UV-irradiated (1500 J m^{-2}). Eight colony isolates of *D. radiopugnans* strain 4/9 survivors and 11 colony isolates of *D. radiodurans* R1 survivors, as well as unirradiated controls, were evaluated by the same RFLP procedures as used for the new

isolates above. In the case of *D. radiopugnans* 4/9, hybridization of all three probes was precisely identical amongst all eight 4/9 survivors and the unirradiated 4/9 control. The same results were found in the case of *D. radiodurans* R1, i.e. all survivors and the unirradiated control were precisely identical.

Fig. 5. Comparison of RFLP patterns from the new isolates and known *Deinococcus* species. All lanes contain DNA digested with *Eco*RI. (a) Ethidium bromide stain; (b) Southern blot probed with pUE58; (c) Southern blot probed with pUE200; (d) Southern blot probed with pS19. Lanes: 1, pUE58; 2, pUE200; 3, *D. proteolyticus*; 4, *E. coli*; 5, *D. radiodurans* SARK; 6, *D. radiodurans* R1; 7, *D. radiopugnans*; 8, 1/10; 9, 6/1; 10, 4/1; 11, 4/5; 12, 4/8; 13, 4/9; 14, 4/11; 15, 4/12; 16, 4/13; 17, 4/16.

Fig. 6. Comparison of RFLP patterns from the new isolates and known *Deinococcus* species. All lanes contain DNA digested with *Eco*RI. (a) Ethidium bromide stain; (b) Southern blot probed with pUE58; (c) Southern blot probed with pUE200; (d) Southern blot probed with pS19. The DNA in lane 8 is taken from a different gel to the rest of the lanes. Lanes: 1, 5/14; 2, 5/13; 3, 5/10; 4, 5/9; 5, 5/7; 6, 5/6; 7, 5/5; 8, 5/3; 9, 5/1; 10, *D. radiodurans* R1; 11, *D. radiodurans* SARK; 12, *D. radiodurans* R1; 13, *E. coli*; 14, *D. proteolyticus*; 15, pUE58; 16, pUE200.

Table 2. RFLP groups

The division into RFLP groups is analysed sequentially: first pUE58, then pUE200, and finally pS19. Strains are listed under a probe only if that probe differentiated a new polymorphism group not already distinguished by a prior probe. Probes pUE200 and pS19 showed multiple polymorphisms amongst isolates already differentiated by pUE58 (Figs. 4c, d, 5c, d).

Group	Probe pUE58	Probe pUE200	Probe pS19
1	1/10		
2	4/1, 4/5	4/1	
3		4/5	
4	4/8, 4/9, 4/11		4/8, 4/11
5			4/9
6	4/12, 4/13, 4/16		4/12, 4/13
7			4/16
8	5/1		
9	5/3		
10	5/5		
11	5/6, 5/9, 5/10, 5/13		5/6
12			5/9
13			5/10
14			5/13
15	5/7		
16	5/14		
17	6/1		

Discussion

This work identifies a new set of 19 isolates of *Deinococcus* from the UK. In phenotypic characteristics the isolates conformed most closely with *D. radiopugnans*. Eighteen of these colony isolates were obtained from three closely adjacent 2 g samples of damp soil next to a lake in Nottingham. Investigation of the 19 isolates for RFLPs using three probes revealed that almost every one was unique (Table 2). The plasmid pUE58 contains the *mtcA* gene, and pUE200 the *uvsC*, *uvsD* and *uvsE* genes from *D. radiodurans* R1. The gene products of these loci are two independent UV-endonucleases that are required for resistance to UV and bulky alkylating agents (Evans & Moseley, 1983; Moseley & Copland, 1978; Moseley & Evans, 1983; Tempest & Moseley, 1980), and we expected them to be highly conserved as all members of the genus are remarkably UV-resistant (Moseley, 1983). The third probe, pS19, is the full length of the naturally occurring *D. radiodurans* SARK cryptic plasmid pUE11, which has been cloned in the *E. coli* plasmid pS27 (Smith *et al.*, 1989).

Cross-hybridization of pS19 with chromosomal DNA of *D. radiodurans* strains SARK and R1, and hybridization to pS16, a cryptic plasmid of strain R1, had led to the speculation that pUE11 and pS16 may be related episomes in their respective host strains (Smith *et al.*, 1990). There are four *EcoRI* sites in pUE11; however, hybridization to *EcoRI*-cleaved genomic DNA of strains R1 and SARK results in the appearance of numerous bands under stringent conditions (Figs 5d and 6d; Smith

et al., 1989, 1990). Therefore, the results of hybridization with pS19 may reflect both plasmid and chromosomal polymorphisms.

Site 4 yielded six different strains, while site 5, a few metres away, yielded nine different strains, with no overlap of RFLP types between these two adjacent soil samples. The two samples, though very close, may represent isolated populations, allowing variation to occur; but this does not account for the differences found within each sample.

To our knowledge, the extent of diversity of laboratory-grown colony isolates from single samples (either environmental or clinical) as shown in the current work, has not been previously demonstrated for any other bacteria. A wide spectrum of studies have addressed bacterial diversity by RFLP analysis, but in all cases there has been little diversity (Clark-Curtiss & Walsh, 1989; Kakoyiannis *et al.*, 1984), or in those studies in which extensive diversity was demonstrated it was attributable to host specificity (Fukushi & Hirai, 1989; Kaijalanin & Lindstrom, 1989), isolations from widely different sites, or the importation of new strains in animals or humans from other sites (Kakoyiannis *et al.*, 1984; Caufield & Walker, 1989; Saunders *et al.*, 1990; Harrison *et al.*, 1990; van Ketel *et al.*, 1984; Steele *et al.*, 1990; LiPuma *et al.*, 1989).

The large number of *D. radiopugnans* strains from a small sample, as distinguished by RFLPs, is unlikely to be the result of point mutations adding and subtracting *EcoRI* sites in the chromosome. Instead, our observation suggests the frequent occurrence of chromosomal rearrangements. We have recently detected highly conserved long reiterated sequences widely distributed in the chromosomes of both *D. radiodurans* SARK (Lennon *et al.*, 1991) and R1 (unpublished results). These sequences could serve as recombination sites at which rearrangements occur. It can also be speculated that bacteriophages or transposons induce rearrangements; however, despite intense efforts to identify such agents over many years, none have been found (unpublished results).

This study suggests much greater heterogeneity of genomes within *D. radiopugnans* than ever previously imagined. Typically, if multiple isolates cultured from a single natural sample appear identical by classical methods, it is tacitly assumed that these isolates are monoclonal. If at any point during the course of this study we had taken one colony as representative, we would have failed to detect the diversity of chromosomal arrangement in these isolates. The frequency of rearrangements in *D. radiopugnans* may be species- or genus-specific. Alternatively, thorough characterization of natural isolates from single sources of other bacterial species may reveal similar phenomena.

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References

- AL-BAKRI, G. H., MACKAY, M. W., WHITTAKER, P. A. & MOSELEY, B. E. B. (1985). Cloning of the DNA repair genes *mtcA*, *mtcB*, *uvsB*, *uvsD*, *uvsE*, and the *leuB* gene from *Deinococcus radiodurans*. *Gene* **33**, 305–311.
- ANDERSON, A. W., NORDEN, H. C., CAIN, R. F., PARRISH, G. & DUGGAN, D. (1956). Studies on a radio-resistant micrococcus. I. Isolation, morphology, cultural characteristics, and resistance to gamma radiation. *Food Technology* **10**, 575–578.
- BLIGH, E. G. & DYER, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* **31**, 911–917.
- BROOKS, B. W. & MURRAY, R. G. E. (1981). Nomenclature for *Micrococcus radiodurans* and other radiation-resistant cocci: *Deinococcaceae* fam. nov. and *Deinococcus* gen. nov., including five species. *International Journal of Systematic Bacteriology* **31**, 353–360.
- BROOKS, B. W., MURRAY, R. G. E., JOHNSON, J. L., STACKEBRANDT, E., WOESE, C. R. & FOX, G. E. (1980). Red-pigmented micrococci: a basis for taxonomy. *International Journal of Systematic Bacteriology* **30**, 627–646.
- CAUFIELD, P. W. & WALKER, T. M. (1989). Genetic diversity within *Streptococcus mutans* evident from chromosomal DNA restriction fragment polymorphisms. *Journal of Clinical Microbiology* **27**, 274–278.
- CLARK-CURTISS, J. E. & WALSH, G. P. (1989). Conservation of genomic sequences among isolates of *Mycobacterium leprae*. *Journal of Bacteriology* **171**, 4844–4851.
- COUNSELL, T. C. & MURRAY, R. G. E. (1986). Polar lipid profiles of the genus *Deinococcus*. *International Journal of Systematic Bacteriology* **36**, 202–206.
- DAVIS, N. S., SILVERMAN, G. J. & MASUROVSKY, E. B. (1963). Radiation resistant, pigmented coccus isolated from Haddock tissue. *Journal of Bacteriology* **86**, 294–298.
- EVANS, D. M. & MOSELEY, B. E. B. (1983). Roles of the *uvsC*, *uvsD*, *uvsE*, and *mtcA* genes in the two pyrimidine dimer excision repair pathways of *Deinococcus radiodurans*. *Journal of Bacteriology* **156**, 576–583.
- FUKUSHI, H. & HIRAI, K. (1989). Genetic diversity of avian and mammalian *Chlamydia psittaci* strains and relation to host origin. *Journal of Bacteriology* **171**, 2850–2855.
- GIOVANNONI, S. J., BRITSCHGI, T. B., MOYER, C. L. & FIELD, K. G. (1990). Genetic diversity in Sargasso sea bacterioplankton. *Nature, London* **345**, 60–63.
- HARRISON, T. G., SAUNDERS, N. A., HATHTHOTUWA, A., HALLAS, G., BIRTLES, R. J. & TAYLOR, A. G. (1990). Phenotypic variation amongst genotypically homogeneous *Legionella pneumophila* serogroup I isolates: implications for the investigations of outbreaks of Legionnaires' disease. *Epidemiology and Infection* **104**, 171–180.
- HUANG, Y. & ANDERSON, R. (1989). Structure of a novel glucosamine-containing phosphoglycolipid from *Deinococcus radiodurans*. *Journal of Biological Chemistry* **31**, 18667–18672.
- ITO, H., WATANABE, H., TAKEHISA, M. & IIZUKA, H. (1983). Isolation and identification of radiation-resistant cocci belonging to the genus *Deinococcus* from sewage sludges and animal feeds. *Agricultural and Biological Chemistry* **47**, 1239–1247.
- KAIJALANIN, S. & LINDSTROM, K. (1989). Restriction fragment length polymorphism analysis of *Rhizobium galegae* strains. *Journal of Bacteriology* **171**, 5561–5566.
- KAKOYIANNIS, C., WINTER, P. J. & MARSHALL, R. B. (1984). Identification of *Campylobacter coli* isolates from animals and humans by bacterial restriction endonuclease DNA analysis. *Applied and Environmental Microbiology* **46**, 545–549.
- VAN KETEL, R. J., TER SCHEGGETE, J. & ZANEN, H. C. (1984). Molecular epidemiology of *Legionella pneumophila* serogroup I. *Journal of Clinical Microbiology* **26**, 362–364.
- KOBATAKE, M., TANABE, S. & HASEGAWA, S. (1973). Nouveau *Micrococcus* radio-résistant à pigment rouge, isolé de fèces de *Llama glama*, et son utilisation comme indicateur microbiologique de la radiosterilisation. *Comptes Rendus des Séances de la Société de Biologie* **167**, 1506–1510.
- LENNON, E. & MINTON, K. W. (1990). Gene fusions with *lacZ* by duplication insertion in the radioresistant bacterium *Deinococcus radiodurans*. *Journal of Bacteriology* **172**, 2955–2961.
- LENNON, E., GUTMAN, P. D., YAO, H. & MINTON, K. W. (1991). A highly conserved repeated chromosomal sequence in the radioresistant bacterium *Deinococcus radiodurans* SARK. *Journal of Bacteriology* **173**, 2137–2140.
- LEWIS, N. F. (1971). Studies on a radio-resistant coccus isolated from Bombay duck (*Harponodon nehereus*). *Journal of General Microbiology* **66**, 29–35.
- LIPUMA, J. J., STULL, T. L., DASEN, S. E., PIDCOCK, K. A., KAYE, D. & KORZENIOWSKI, O. M. (1989). DNA polymorphisms among *Escherichia coli* isolated from bacteriuric women. *Journal of Infectious Diseases* **159**, 526–532.
- MARMUR, J. & DOTY, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *Journal of Molecular Biology* **5**, 109–118.
- MOSELEY, B. E. B. (1983). Photobiology and radiobiology of *Micrococcus (Deinococcus) radiodurans*. *Photochemistry and Photobiology Reviews* **7**, 223–274.
- MOSELEY, B. E. B. & COPLAND, H. J. R. (1978). Four mutants of *Micrococcus radiodurans* defective in the ability to repair DNA damaged by mitomycin-C, two of which have wild-type resistance to ultraviolet radiation. *Molecular and General Genetics* **160**, 331–337.
- MOSELEY, B. E. B. & EVANS, D. M. (1983). Isolation and properties of strains of *Micrococcus (Deinococcus) radiodurans* unable to excise ultraviolet light-induced pyrimidine dimers from DNA: evidence for two excision pathways. *Journal of General Microbiology* **129**, 2437–2445.
- MURRAY, R. G. E. (1986). The biology and ecology of the *Deinococcaceae*. In *Perspectives in Microbial Ecology (Proceedings of the IV International Symposium on Microbial Ecology)*, pp. 153–158. Edited by F. Megusar & M. Gantar. Ljubljana: Slovene Society for Microbiology.
- MURRAY, R. G. E. & BROOKS, B. W. (1986). *Deinococcus*. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1035–1043. Edited by P. H. A. Sneath & J. G. Holt. Baltimore: William & Wilkins.
- NAUMOVSKI, L. & FRIEDBERG, E. C. (1982). Molecular cloning of eukaryotic genes required for excision repair of UV-irradiated DNA: isolation and partial characterization of the *RAD3* gene of *Saccharomyces cerevisiae*. *Journal of Bacteriology* **152**, 323–331.
- SANDERS, S. W. & MAXCY, R. B. (1979). Isolation of radiation-resistant bacteria without exposure to radiation. *Applied and Environmental Microbiology* **38**, 436–439.
- SAUNDERS, N. A., HARRISON, T. G., HATHTHOTUWA, A., KACHWALLA, N. & TAYLOR, A. G. (1990). A method for typing strains of *Legionella pneumophila* serogroup I by analysis of restriction fragment length polymorphisms. *Journal of Medical Microbiology* **31**, 45–55.
- SCHLEIFER, K. H. & KANDLER, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriological Reviews* **36**, 407–477.
- SMITH, M. D., ABRAHAMSON, R. & MINTON, K. W. (1989). Shuttle plasmids constructed by the insertion of an *Escherichia coli* cloning vector into two *Deinococcus radiodurans* plasmids. *Plasmid* **22**, 132–142.
- SMITH, M. D., MCNEIL, L. B. & MINTON, K. W. (1990). Plasmid genetics and gene expression in *Deinococcus radiodurans*. In *Genetic*

- Transformation and Expression*, pp. 125–131. Edited by L. O. Butler, C. Harwood & B. E. B. Moseley. Andover, Hants, UK: Intercept.
- STEELE, T. W., LANSER, J. & SANGSTER, N. (1990). Isolation of *Legionella longbeachae* serogroup I from potting mixes. *Applied and Environmental Microbiology* **56**, 49–53.
- TEMPEST, P. R. & MOSELEY, B. E. B. (1980). Defective excision repair in a mutant of *Micrococcus radiodurans* hypermutable by some monofunctional alkylating agents. *Molecular and General Genetics* **179**, 191–199.
- TIRGARI, S. & MOSELEY, B. E. B. (1980). Transformation in *Micrococcus radiodurans*: measurement of various parameters and evidence for multiple independently segregating genomes per cell. *Journal of General Microbiology* **119**, 287–296.
- WARD, D. M., WELLER, R. & BATESON, M. M. (1990). 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. *Nature, London* **345**, 63–65.