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► **To cite this version:**

Arjan de Groot, Laurence Blanchard. DNA repair and oxidative stress defense systems in radiation-resistant *Deinococcus murrayi*. *Canadian Journal of Microbiology*, 2023, 10.1139/cjm-2023-0074 . hal-04242226

HAL Id: hal-04242226

<https://hal.science/hal-04242226>

Submitted on 14 Oct 2023

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DNA repair and oxidative stress defense systems in radiation-resistant *Deinococcus murrayi*

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Abstract

Deinococcus murrayi is a bacterium isolated from hot springs in Portugal, and named after Dr. Robert G.E. Murray in recognition of his research on the genus *Deinococcus*. Like other *Deinococcus* species, *D. murrayi* is extremely resistant to ionizing radiation. Repair of massive DNA damage and limitation of oxidative protein damage are two important factors contributing to the robustness of *Deinococcus* bacteria. Here, we identify, among others, the DNA repair and oxidative stress defense proteins in *D. murrayi*, and highlight special features of *D. murrayi*. For DNA repair, *D. murrayi* does not contain a standalone uracil-DNA glycosylase (Ung), but it encodes a protein in which Ung is fused to a DNA photolyase domain (PhrB). UvrB and UvrD contain large insertions corresponding to inteins. One of its endonuclease III enzymes lacks a [4Fe-4S] cluster. *Deinococcus murrayi* possesses a homolog of the error-prone DNA polymerase IV. Concerning oxidative stress defense, *D. murrayi* encodes a manganese catalase in addition to a heme catalase. Its organic hydroperoxide resistance protein Ohr is atypical because the redox active cysteines are present in a CXXC motif. These and other characteristics of *D. murrayi* show further diversity among *Deinococcus* bacteria with respect to resistance-associated mechanisms.

Key words: *Deinococcus murrayi*, DNA repair, oxidative stress defense, regulation, cell envelope

Introduction

Bacteria belonging to the genus *Deinococcus* are famous for their extreme resistance to UV and gamma radiation and to other DNA damage and oxidative stress-generating conditions such as desiccation. *Deinococcus* bacteria have been isolated from diverse environments and locations (e.g., soil, water, and air). Many are mesophilic, but psychrophilic and thermophilic representatives have also been described (Slade and Radman 2011). They are diderm bacteria with an outer membrane (Thompson and Murray 1981; Sexton et al. 2022). Depending on the species, cells are spherical or rod shaped. The type species of this genus is *Deinococcus radiodurans*, isolated in 1956 as a contaminant in a can of ground meat that was supposed to be sterilized by a high dose of ionizing radiation (Anderson et al. 1956). This species was initially named “*Micrococcus radiodurans*”, but taxonomic research by the Canadian microbiologist Dr. Robert G.E. Murray and collaborators revealed that this species, and some other related radiation-resistant strains and species, did not belong to *Micrococcus* but to a new genus that was called *Deinococcus* (from the Greek *deimos*, strange or unusual) (Brooks et al. 1980; Brooks and Murray 1981). The new family *Deinococcaceae* was also proposed in this work. Dr. Murray has also contributed to the characterization of the multilayered cell envelope of *D. radiodurans* (Lancy and Murray 1978; Thompson et al. 1980; Thompson and Murray 1981; Counsell and Murray 1986).

In 1997, a new species of *Deinococcus* was described for strains that were isolated from hot springs in Portugal (Ferreira et al. 1997). This species, *Deinococcus murrayi*, was named after Dr. Murray in recognition of his research on the genus *Deinococcus*. The *D. murrayi* strains are slightly thermophilic and have an optimum growth temperature of about 45–50 °C. The *D. murrayi* type strain ALT1-b (=DSM 11303), isolated from non-irradiated water samples, showed similar resistance to gamma radiation as *D. radiodurans*. Also similar to *D. radiodurans*, cells of *D. murrayi* are spherical, they divide as tetrads, and they have a multilayered cell envelope (Ferreira et al. 1997).

Extreme resistance to radiation, desiccation, and oxidative stress has been investigated most extensively for *D. radiodurans*. Several mechanisms are required for this extreme resistance, including repair of massive DNA damage and limitation of oxidative protein damage (Slade and Radman 2011; Daly 2012). The structure and composition of the cell envelope are expected to contribute to the robustness of these bacteria. Many genes and proteins, including regulators, and also non-enzymatic antioxidants such as carotenoids and Mn²⁺-containing complexes, that contribute to radiation and/or oxidative stress resistance, have been identified (Daly et al. 2010; Slade and Radman 2011; Dulermo et al. 2015; Lim et al. 2019). The conservation of proteins involved in these stress resistance mechanisms has been investigated in a set of other *Deinococcus* species isolated worldwide, which not only

revealed conservation but also remarkable diversity of these proteins among deinococci (Lim et al. 2019; de Groot et al. 2022).

A high quality draft genome sequence of the *D. murrayi* type strain has been recently obtained by the Joint Genome Institute in Berkeley, CA, USA (Mukherjee et al. 2017; García-López et al. 2019). Here, to go further in the characterization of *Deinococcus* bacteria and honor the contributions of Dr. Murray to microbiology and to the *Deinococcus* field, we identify the DNA repair and oxidative stress defense proteins in *D. murrayi*, and highlight the special features compared to *D. radiodurans* and other *Deinococcus* species. Radiation and oxidative stress resistance-associated regulatory proteins as well as important cell envelope/surface proteins were included in the analysis.

Materials and methods

The genome sequence of the *D. murrayi* type strain has a size of about 2.77 Mb, and contains about 2755 predicted genes. Specific proteins were searched in databases using protein or domain names and descriptions as keywords, and using BLAST (Altschul et al. 1997). Many protein sequences from *D. murrayi* appear to be highly similar or even identical to those from *Deinococcus* sp. RL, which was isolated from hot spring sediments in India (Mahato et al. 2014), suggesting that RL might be another *D. murrayi* strain. The online tools CD-search at the Conserved Domain Database (Lu et al. 2020), SMART (Letunic et al. 2021), ProSite (de Castro et al. 2006), and UniProt (UniProt Consortium 2023) were used for protein characterization. The 3D structures of the intein-containing UvrB and intein-containing UvrD (>1000 residues) were predicted using a local AlphaFold2 installation (version 2.3.1) (Jumper et al. 2021). The 3D structures of mature UvrB and UvrD, the PhrB-Ung fusion, standalone PhrB, Nth, and Ohr were predicted using AlphaFold2 via the software ColabFold (Jumper et al. 2021; Mirdita et al. 2022). All parameters were kept to default values. The quality of each model, presented in supplementary figures, was evaluated using the confidence measures pLDDT (predicted local distance difference test) that indicates the local accuracy, and PAE (predicted aligned error) that assesses the packing between domains or protein chains. Three-dimensional structure or model images (rank_1 model for each AlphaFold2 predicted 3D structure) were generated using PyMOL (PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC). Protein alignment was made with UniProt ClustalO and ESPript (Robert and Gouet 2014). Conserved DNA motifs in promoter regions were searched using MEME (Bailey and Elkan 1994). *Deinococcus* bacteria were grown at 37 °C in or on 10-fold diluted tryptic soy broth supplemented with trace elements (Vujicic-Zagar et al. 2009).

Results and discussion

DNA repair

Deinococcus bacteria are able to repair massive DNA damage, including hundreds of double-strand breaks, generated by high doses of radiation or prolonged desiccation (Battista

1997; de Groot et al. 2009). Therefore, efficient and essentially error-free DNA repair mechanisms are crucial for the extreme resistance. Indeed, strains lacking DNA repair gene *recA*, *recF*, *recO*, *recR*, or *polA* are among the most radiation-sensitive mutants of *D. radiodurans* (Lim et al. 2019). DNA repair proteins encoded by *D. murrayi* are listed in Table 1. Because most studies concerning deinococcal DNA repair have been performed with genes and proteins from *D. radiodurans* (Lim et al. 2019; Cheng et al. 2020; He et al. 2020; Kota et al. 2021; Ujaoney et al. 2021; Jeong et al. 2021b; Li et al. 2022; Sun et al. 2022; Seck et al. 2023; Yang et al. 2023) (and references therein), the gene locus tags of this species are included. For every protein from *D. murrayi* and/or *D. radiodurans*, Table 1 also lists the number of homologs in 10 other previously analyzed *Deinococcus* species (Lim et al. 2019).

Deinococcus murrayi has many genes for the various DNA repair pathways in common with *D. radiodurans* and other bacteria, but several interesting differences were also observed. The base excision repair pathway is responsible for repair of damaged or modified bases, and is initiated by DNA glycosylases. *D. murrayi* possesses less DNA glycosylases than *D. radiodurans*. For example, *D. murrayi* lacks homologs of the DR_1751 (Udg4) and DR_0022-like uracil-DNA glycosylases. A standalone homolog of DR_0689 (Ung), proposed to represent the major uracil-DNA glycosylase activity in *D. radiodurans* (Sandigursky et al. 2004; Timmins and Moe 2016), is also absent. However, *D. murrayi* encodes a protein in which Ung is fused to a PhrB deoxyribodipyrimidine photolyase domain (Table 1; Fig. 1; Fig. S1). The C-terminal Ung part of this two-domain protein is very similar to standalone Ung (more than 70% sequence identity with DR_0689). A standalone PhrB protein is also present in *D. murrayi* (but not in *D. radiodurans*) (Table 1; Fig. 1; Fig. S2). Although this protein shares only about 25% identity with the N-terminal domain of the PhrB-Ung fusion, the predicted 3D structures of these two PhrB are similar to each other (Fig. 1) and to experimentally determined structures of other PhrB proteins (Fig. S3). Conserved domain search not only showed the COG0415 (PhrB) domain for both proteins but also similarity to phr2 (TIGR00591; photolyase PhrII) for the standalone PhrB, and crypto_DASH (TIGR02765; cryptochrome, DASH family) for the PhrB part of the PhrB-Ung fusion. Cry-DASH proteins form a subfamily of the cryptochrome/photolyase family, named after members from *Drosophila*, *Arabidopsis*, *Synechocystis*, and human. Cry-DASHs may have photolyase activity repairing cyclobutane-pyrimidine dimers in single-stranded DNA, and/or may function as a photoreceptor and act in light-responsive regulatory processes (Selby and Sancar 2006; Kiontke et al. 2020). It will be interesting to elucidate the function of the PhrB (Cry-DASH) part of the PhrB-Ung fusion and, in particular, of the entire fusion protein. The PhrB-Ung fusion is not unique to *D. murrayi*. It is also present in several other *Deinococcus* species (Table 1), but we did not find it in other genera.

Deinococcus radiodurans encodes three endonuclease III enzymes (Nth or EndoIII) (DR_2438, EndoIII-1; DR_0289, EndoIII-2; DR_0928, EndoIII-3), although DNA glycosylase activity has not been detected for the latter despite conserved catalytic residues and a 3D structure very similar to other Nths (Sarre et al. 2015, 2019; Rollo et al. 2022). The function of DR_0928

Table 1. DNA repair-related proteins in *Deinococcus murrayi*.

Name	<i>D. murrayi</i> protein	<i>D. radiodurans</i> locus tag	10 other <i>Deinococcus</i> species									
			<i>ge</i>	<i>de</i>	<i>ma</i>	<i>go</i>	<i>pr</i>	<i>pe</i>	<i>rp</i>	<i>so</i>	<i>ac</i>	<i>pu</i>
Base excision repair												
AlkA	WP_027460563.1	DR_2584	1	1	1	1	1	2	1	1	1	1
Mpg	WP_027459565.1	DR_2074	1	1	2	2	1	1	2	2	2	1
Ung	—	DR_0689	1	1	1	1		1				1
Udg4	—	DR_1751	1		1	1	1	2	1			1
DR0022-like Udg	—	DR_0022										
Mug	WP_157468844.1	DR_0715	2	1	1	1	1	1	1	1	1	1
Nfi	—	DR_2162			1	1			1			1
MutY	WP_027461450.1	DR_2285	1	1	1	1	1	1	1	1	1	1
MutM (Fpg)	WP_027459436.1	DR_0493	1	1	1	1	1	1	1	1	1	1
Nth (EndoIII-2)	WP_027459803.1	DR_0289	1	1	1	1	1	1	1	1	1	1
Nth (EndoIII-3)	WP_245576191.1	DR_0928	1	1	1	1	1		1	1	1	1
Nth (EndoIII-1)	frameshift	DR_2438	1	1	1	1	1	1	1	1	1	1
XthA	WP_051363356.1	DR_0354	2	2	1	1	1	2	1	1	1	1
AlkD	—	—										1
Mismatch repair												
MutL	WP_027459243.1	DR_1696	1	1	1	1	1	1	1	1	1	1
MutS	WP_034407789.1	A2G07_08325	1	1	1	1	1	1	1	1	1	1
MutS2	WP_027461154.1	DR_1976	1	1	1	1	1	1	1	1	1	1
Vsr	—	—			1	1		1	1			
YcjD	WP_245576232.1	DR_0221					1			1	1	1
Dcm	—	—			1	1		4	2		2	
Dam	WP_027461495.1	—			1							
Dam	WP_051363638.1	—					1					
Dam	WP_211232135.1	—										
YhdJ	—	A2G07_16000										
Direct reversal												
Ada	—	—			1							
PhrB	WP_027461422.1	—				1				2	2	1
SpIB	—	—		1	1	1						
Dut	—	—					1		1			
Dcd	WP_027459983.1	—	1	1	1	1	1	1	1			1
RdgB	WP_027460282.1	DR_0179	1	1	1	1	1	1	1	1	1	1
DJ-1/PfpI family	—	DR_0491					1		1	1	1	
DJ-1/PfpI family	WP_027461411.1	DR_1199	1	1	1	1	1	1		1	1	
Two-domain fusion proteins												
PhrB-Ung	WP_051363410.1	—				1	1		1	1	1	
Nth-Dcm	—	—						1				
AdaA-AlkA	—	—				1				1	1	
Nucleotide excision repair												
UvrA	WP_027460878.1	DR_1771	1	1	1	1	1	1	1	1	1	1
UvrA2	—	DR_A0188		1	2	1	1	1	1	2	2	1
UvrB	with intein (no WP)	DR_2275	1	1	1	1	1	1	1	1	1	1
UvrC	WP_027460519.1	DR_1354	1	1	1	1	1	1	1	1	1	1
UvrD	WP_027461416.1	DR_1775	1	1	1	1	1	1	1	1	1	1
UvrD-like	WP_027460515.1	—	1	1		3	2	1		1		
SSL2 (Rad25)	—	DR_A0131		1	1	1	2	4	1			
Mfd	WP_027459305.1	DR_1532	1	1	1	1	1	1	1	1	1	1
Uve (UvsE)	WP_084542882.1	DR_1819	1	1	1	1	1	1	1	1	1	1
AtI1	WP_034408286.1	DR_0428	1	1	1	1	1	1	1	1	1	1
Recombinational repair												
RecA	WP_027459646.1	DR_2340	1	2	1	1	1	2	1	1	1	1
RecF	WP_027459469.1	DR_1089	1	1	1	1	1	1	1	1	1	1

Table 1. (continued).

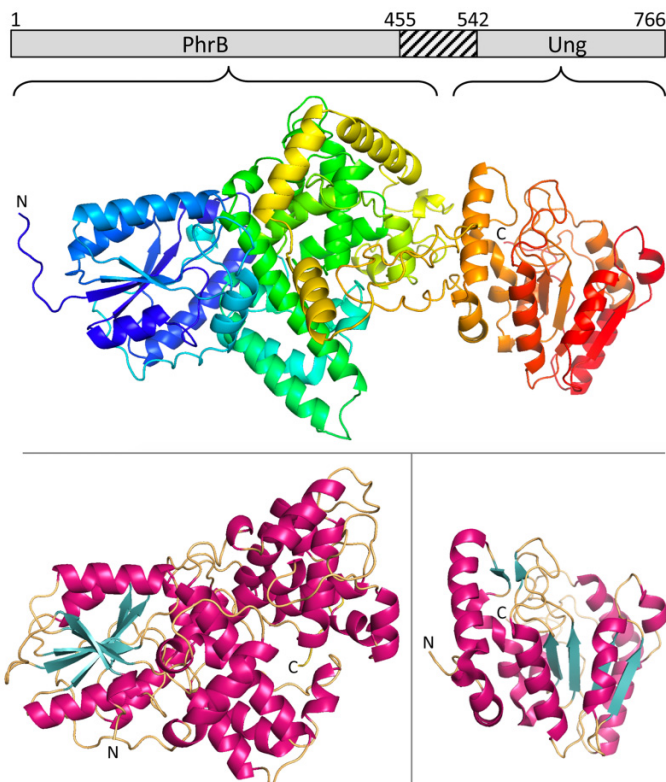
Name	<i>D. murrayi</i> protein	<i>D. radiodurans</i> locus tag	10 other <i>Deinococcus</i> species									
			<i>ge</i>	<i>de</i>	<i>ma</i>	<i>go</i>	<i>pr</i>	<i>pe</i>	<i>rp</i>	<i>so</i>	<i>ac</i>	<i>pu</i>
RecO	WP_027460968.1	DR_0819	1	1	1	1	1	1	1	1	1	1
RecR	WP_027459782.1	DR_0198	1	1	1	1	1	1	1	1	1	1
RecJ	WP_084542967.1	DR_1126	1	1	1	1	1	1	1	1	1	1
RecN	WP_027461354.1	DR_1477	1	1	1	1	1	1	1	1	1	1
RecQ	WP_027461329.1	DR_1289	fr	1	1	2	1	1	1	1	1	1
RecQ-like	WP_084542944.1	—		1	1			1	1	1		
RecQ-like multidomain	WP_084542979.1	—		1								
HRDC domain	WP_027461447.1	DR_2444	1	1	1	1	1	1	1	1	1	1
RecD	WP_027460935.1	DR_1902	1	1	1	2	2	2	1	1	1	1
SbcC	WP_027461308.1	DR_1922	1	1	1	1	1	1	1	1	1	1
SbcD	WP_027461309.1	DR_1921	1	1	1	1	1	1	1	1	1	1
RuvA	WP_027461138.1	DR_1274	1	1	1	1	1	1	1	1	1	1
RuvB	WP_027459876.1	DR_0596	1	1	1	1	1	1	1	1	1	1
RuvC	WP_027460729.1	DR_0440	1	1	1	1	1	1	1	1	1	1
RecG	WP_027460759.1	DR_1916	1	1	1	1	1	1	1	1	1	1
RecB	—	—							1			
RecX	WP_245576219.1	DR_1310	1	1	1	1	1	1	1	1	1	1
RadA (Sms)	WP_027461338.1	DR_1105	1	1	1	1	1	1	1	1	1	1
RusA	—	—								1	2	
Ligases with adjacent genes												
LigA	WP_027460876.1	DR_2069	1	2	1	1	1	1	1	1	1	1
LigB (DdrP)	—	DR_B0100			1	1				1	1	
PARG	—	DR_B0099			1							
PNKP	—	DR_B0098			1	1				1	1	
Rnl	—	DR_B0094				1						1
3'-5' exonucl	—	DR_B0095			1			fr				
Others												
PolA	WP_027459561.1	DR_1707	1	1	1	1	1	1	1	1	1	1
PolX	WP_027461057.1	DR_0467	1	1	1	1	1	1	1	1	1	1
DnaE	WP_027459902.1	DR_0507	1	1	1	1	1	1	1	1	1	1
DnaQ	WP_027459834.1	DR_0856	1	2	1	1	1	2	1	1	1	1
DnaQ	WP_051363395.1	—	1		1			1	1			1
DnaN	WP_027460151.1	DR_0001	2	1	1	2	1	1	1	1	1	1
DnaX	WP_027459650.1	DR_2410	1	1	1	1	1	1	1	1	1	1
PriA	WP_027459918.1	DR_2606	1	1	1	1	1	1	1	1	1	1
DnaA	WP_027460150.1	DR_0002	1	1	1	1	1	1	1	1	1	1
PolB	—	—		1		1		1	1			
DinP	WP_027460020.1	—			1	1	1	1	1	1	1	
ImuY	—	—		1				1				
DnaE2	—	—		1				1				
Ssb	WP_027459862.1	A2G07_13010	4	1	1	3	4	1	1	1	1	1
GyrA	WP_027460811.1	DR_1913	1	1	1	1	1	1	1	1	1	1
GyrB	WP_027459303.1	DR_0906	1	1	1	1	1	1	1	1	1	1
Top1	WP_027459941.1	DR_0690	1	1	1	1	1	1	1	1	1	1
TopA	WP_027460080.1	DR_1374	1	1	1	1	1	1	1	1	1	1
TopA-like	—	—			1	2	1	1				
RarA	WP_027461035.1	DR_1898	1	1	1	1	1	1	1	1	1	1
XseA	WP_051363408.1	DR_0186	1	1	1	1	1	1	1	1	1	1
XseB	WP_034409634.1	DR_2586	1	1	1	1	1	1	1	1	1	1
YqgF (RuvX)	WP_027460104.1	DR_2509	1	1	1	1	1	1	1	1	1	1
Exo	—	—		1				1				
NucS (EndoMS)	—	—			1	2	1	fr	1	1	1	

Table 1. (concluded).

Name	<i>D. murrayi</i> protein	<i>D. radiodurans</i> locus tag	10 other <i>Deinococcus</i> species									
			<i>ge</i>	<i>de</i>	<i>ma</i>	<i>go</i>	<i>pr</i>	<i>pe</i>	<i>rp</i>	<i>so</i>	<i>ac</i>	<i>pu</i>
HelD	—	DR_1572		1	1	2	1	1	1	1	1	1
HepA	—	A2G07_07235	1	1	1	1					1	1
HepA	WP_027459771.1	DR_B0136	1	1				1	2			
NurA	WP_027461016.1	DR_0836	1	1	1	1	1	1	1	1	1	1
HerA	WP_051363553.1	DR_0837	1	1	1	1	1	1	1	1	1	1
DdrA	WP_027461074.1	DR_0423	1	1	1	1		1	2	1	fr	1
DdrB	WP_027461534.1	DR_0070	1	1	1	1	1	1	1	1	1	1
DdrC	WP_027460160.1	A2G07_00380	1	1	1	1	1	1	1	1	1	1
DdrD	WP_027460474.1	DR_0326	1	1	1	1	1		1	1	1	1
PprA	WP_027460655.1	DR_A0346	1	1	1	1		1				

Note: The last 10 columns indicate the number of homologs, if any, in 10 *Deinococcus* species, which are: *ge* = *D. geothermalis*; *de* = *D. deserti*; *ma* = *D. maricopensis*; *go* = *D. gobiensis*; *pr* = *D. proteolyticus*; *pe* = *D. peraridilitoris*; *rp* = *D. radiopugnans* strain DY59 (previously *D. swuensis*); *so* = *D. soli*; *ac* = *D. actinoslerus* strain BM2; *pu* = *D. puniceus*. fr, frameshift or pseudogene.

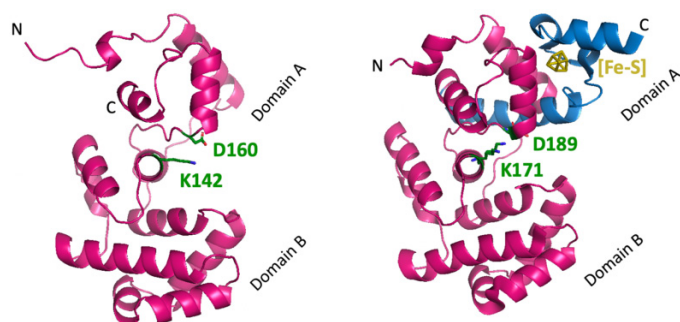
Fig. 1. The PhrB-Ung fusion protein from *Deinococcus murrayi*. The AlphaFold model for the PhrB (deoxyribodipyrimidine photolyase)-Ung (uracil-DNA glycosylase) fusion protein (WP_051363410.1; 766 amino acids (aa)) from *D. murrayi* is shown in rainbow colors, from blue (N terminus) to red (C-terminus). For comparison, the predicted structure from another, standalone PhrB protein from *D. murrayi* (WP_027461422.1; 454 aa; *bottom left*) and the crystal structure of the standalone Ung from *D. radiodurans* (DR_0689; 247 aa; *bottom right*; PDB accession 2BOO) (Leiros et al. 2005) are also shown.



may be to protect damaged DNA rather than to repair it, or to recruit other DNA repair enzymes. DR_0289 is a highly efficient Nth enzyme with broad substrate specificity. DR_2438 has much weaker activity but was also found to process single-stranded DNA substrates. Homologous sequences for these three Nths are present in *D. murrayi*. However, there is a frameshift in the DR_2438 homolog in *D. murrayi* (but not in the homolog, RDMS_09165, in the closely related *Deinococcus* sp. RL). The DR_0289-type Nth is well conserved (84% identity between the proteins of *D. murrayi* and *D. radiodurans*). Nth enzymes, including the three Nths of *D. radiodurans*, possess a [4Fe-4S] cluster for which the role is unclear. Remarkably, in both *D. murrayi* and *Deinococcus* sp. RL, the homolog of DR_0928 (i.e., the Nth with unknown function in *D. radiodurans*) is shorter than DR_0928, and lacks the C-terminal region of about 50–60 amino acid residues that includes the [4Fe-4S] cluster. This 186-residue Nth from *D. murrayi* has 56% identity with the corresponding region of DR_0928. The absence of the C-terminal [4Fe-4S] cluster may have resulted from mutation(s) because sequences similar to the C-terminal region of DR_0928, including cysteine residues of the [4Fe-4S] cluster, are present in another reading frame downstream of the stop codon of the DR_0928 homolog in both *D. murrayi* and *Deinococcus* sp. RL. Except for the absence of the C-terminal region, the predicted structure of this shorter *D. murrayi* Nth is very similar to the DR_0928 crystal structure (Fig. 2; Fig. S4). Several regions of Nth, including the iron sulfur cluster loop (FCL), interact with DNA (Sarre et al. 2015). The absence of the FCL in the shorter *D. murrayi* Nth may affect its DNA binding properties. Blastp analyses did not indicate Nth homologs lacking the [4Fe-4S] cluster in other species. For DR_0289 (i.e., the Nth with highly efficient DNA glycosylase activity), point mutation of an iron-coordinating cysteine of the [4Fe-4S] cluster resulted in a protein with reduced stability but with similar DNA glycosylase activity compared to wild-type DR_0289 (Rollo et al. 2022). Experimental work is necessary to find a possible function of the [4Fe-4S] cluster-lacking Nth of *D. murrayi*.

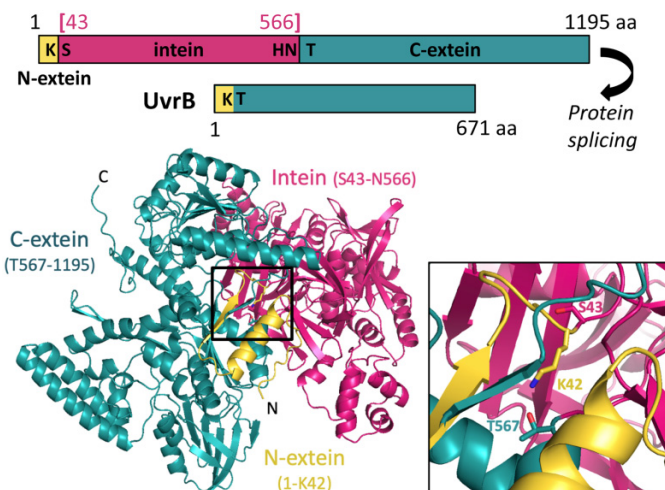
The nucleotide excision repair (NER) pathway is capable of removing a wide range of DNA lesions, including bulky

Fig. 2. An endonuclease III homolog from *Deinococcus murrayi* lacking an iron–sulfur cluster. The AlphaFold model of the (Fe–S) cluster-lacking endonuclease III (Nth) protein (WP_245576191.1) from *D. murrayi* is shown on the left-hand side. The crystal structure of the homolog from *D. radiodurans* is shown on the right-hand side (DR_0928; PDB 4UOB) (Sarre et al. 2015). The latter, like other Nth enzymes, contains a C-terminal region with an (Fe–S) cluster (shown in blue and yellow, respectively). Nth enzymes consist of two α -helical domains (A and B) with the DNA binding cleft in between (Sarre et al. 2015). Suggested active site residues Lys and Asp are indicated in both proteins. For residue numbering in DR_0928, the protein of 263 residues is considered, assuming that the start codon position of DR_0928 was misannotated initially (Sarre et al. 2019).



adducts and UV-induced pyrimidine dimers. In bacteria, this pathway involves the sequential action of the UvrA, UvrB, UvrC, and UvrD proteins to release a short single-stranded DNA fragment containing the lesion (Seck et al. 2022). UvrB is a central component in this repair pathway as it interacts with UvrA, UvrC, and the damaged DNA (Seck et al. 2023). UvrA and UvrC from *D. murrayi* were readily found. A second UvrA, called UvrA2, which is present in *D. radiodurans* and many others but which does not seem to be involved in NER, is absent in *D. murrayi*. Initially, UvrB from *D. murrayi* was not found because it is not present in the protein database. It appeared that an *uvrB* gene (locus tag H565_RS16785) in *D. murrayi* is annotated as a pseudogene (i.e., a DNA segment that resembles a gene but is unable to produce a functional protein product; for this *uvrB* locus, the description includes “incomplete; missing N terminus; derived by automated computational analysis./pseudo”). However, further analysis of this region revealed the presence of a coding sequence for a protein of 1195 amino acid residues (Fig. S5), which is much more than the 671-residue-long UvrB of *D. deserti*, for example. Sequence analysis revealed that *D. murrayi* UvrB contains an intein of 524 residues (Fig. 3). An intein is a protein segment that can excise itself and rejoin the remaining N- and C-terminal parts (the exteins) with a peptide bond in a process called protein splicing (Gogarten et al. 2002; Pavankumar 2018). The amino acid residues that will be ligated to obtain mature UvrB (i.e., the last residue of the N-terminal extein and the first residue of the C-terminal extein) are in proximity in the predicted structure of precursor UvrB (Fig. 3), although model confidence is low for these residues

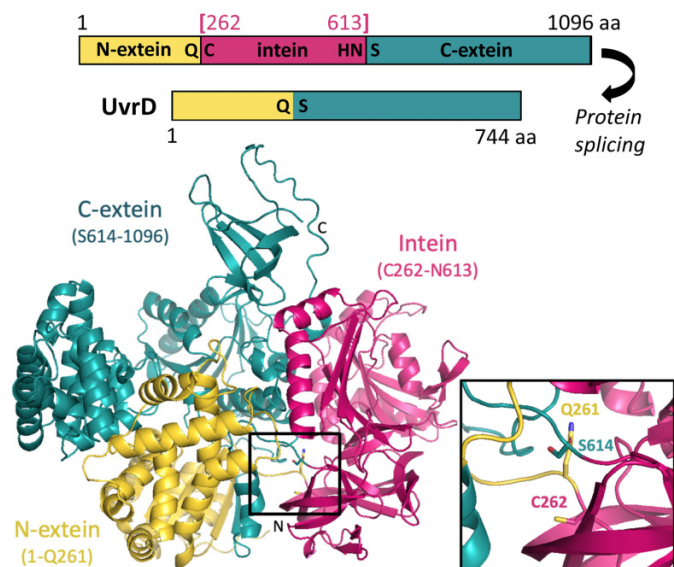
Fig. 3. Inteин-containing UvrB from *Deinococcus murrayi*. The 1195-residue precursor contains an intein as shown schematically and in the AlphaFold model. The indicated residues S43, H565, N566, and T567 are conserved and important for the splicing process. Residues K42 and T567 that will be ligated during protein splicing are in proximity in the predicted structure (bottom right).



that are present in predicted loops (Fig. S6). In the AlphaFold model of mature UvrB, and in the crystal structure of *Bacillus caldotenax* UvrB, the corresponding residues are present in an α -helix (Figs. S7 and S8). Such an intein-containing precursor UvrB was not observed in the *Deinococcus* species that we analyzed previously, but blastp analysis showed the presence of similar intein-containing UvrB in several other deinococci such as *Deinococcus* sp. RL, *D. grandis*, *D. phoenicis*, and also in species from other genera (e.g., *Rhodocaloribacter litoris*). Remarkably, UvrD from *D. murrayi* is also produced as an intein-containing precursor (Fig. 4; Figs. S9 and S10). Following blastp, similar intein-containing UvrD precursors were found in many other bacteria (e.g., *Deinococcus* sp. RL, *D. radiopugnans* strain ATCC 19172 but not in strain DY59, *D. indicus*, *Truepera radiovictrix*, *Calidithermus terrae*, and several *Clostridium* species). The inteins in *D. murrayi* UvrB and UvrD are inserted in the ATP-binding/ATPase domains, likely affecting the function of these domains (Figs. S8 and S11). Experimental work will be needed to determine to which extent the intein has an impact on the activities and molecular interactions of these proteins. At least one more *D. murrayi* protein contains an intein: the replicative DNA helicase DnaB (WP_027460698.1). In *D. radiodurans*, inteins have been identified in the Snf2 family helicase HepA (locus tag A2G07_07235) and ribonucleoside-diphosphate reductase (DR_2374). DNA repair- or replication-related proteins with an intein are also present in several other *Deinococcus* species (e.g., DnaE in *D. radiopugnans* strain DY59 but not strain ATCC 19172).

Deinococcus radiodurans and *D. geothermalis*, the first two *Deinococcus* species for which the genome was sequenced, do not possess error-prone DNA translesion polymerases. However, several more recently sequenced *Deinococcus* species encode homologs of one or several types of such polymerases

Fig. 4. Intein-containing UvrD from *Deinococcus murrayi*. The 1096-residue precursor contains an intein as shown schematically and in the AlphaFold model. Residues C262, H612, N613 and S614 correspond to conserved residues important for the splicing process. Residues Q261 and S614 that will be ligated during protein splicing are in proximity in the predicted structure (bottom right).



(PolB, DinP, and ImuY/DnaE2) (Table 1) (Lim et al. 2019; Blanchard and de Groot 2021). Radiation-induced mutagenesis, dependent on the operon encoding ImuY and DnaE2, has been demonstrated for two *Deinococcus* species, *D. deserti* (Dulermo et al. 2009), and *D. ficus* (Zeng et al. 2011), showing that stress survival of *Deinococcus* may be accompanied by limited error-prone repair. This may also be true for *D. murrayi*, as it contains a gene encoding DNA polymerase IV (DinP) (Table 1).

After exposure to radiation or desiccation, the expression of many genes and proteins is upregulated in *Deinococcus*, including several DNA repair genes and genes of unknown function (Tanaka et al. 2004; Basu and Apte 2012; Yuan et al. 2012; de Groot et al. 2014). Five novel genes were found to be the most highly upregulated after radiation or desiccation in *D. radiodurans* (Tanaka et al. 2004). These genes were called *ddrA*, *ddrB*, *ddrC*, *ddrD*, and *prrA*. In recent years, these five genes and the encoded proteins have been characterized in several studies (Devigne et al. 2019; Lim et al. 2019; Bouthier de la Tour et al. 2021; Rajpurohit et al. 2021; Banneville et al. 2022) (and references therein). All five proteins are able to bind to DNA (single-stranded and/or double-stranded), and contribute in DNA protection and repair processes. They are conserved in most, but not all, *Deinococcus* species. Also *D. murrayi* possesses genes encoding *DdrA*, *DdrB*, *DdrC*, *DdrD*, and *PprA* (Table 1).

Oxidative stress defense

Exposure of cells to radiation or desiccation causes redox imbalance (oxidative stress) through formation of reactive

oxygen species (ROS), which can react with and damage any molecule in the cell, including DNA and proteins. While DNA is not protected from damage, it has been shown that proteins in radiation-resistant organisms are much better protected against oxidative damage than in radiation-sensitive organisms (Daly et al. 2007). *Deinococcus* bacteria are well equipped with enzymatic and non-enzymatic antioxidant systems (Slade and Radman 2011; Daly 2012; Lim et al. 2019; de Groot et al. 2022). Identified non-enzymatic antioxidant systems and compounds are a high intracellular Mn^{2+}/Fe^{2+} ratio (limiting Fe^{2+} -catalyzed oxidative damage to proteins), small-molecule Mn^{2+} -antioxidant complexes, small peptides, carotenoids, and the low-molecular-weight thiol bacillithiol (Daly et al. 2004, 2010; Tian and Hua 2010; Berlett and Levine 2014; Jeong et al. 2021a). Antioxidant enzymes are also important for survival of *Deinococcus* when exposed to radiation (acute or chronic), desiccation, or oxidants (Lim et al. 2019; de Groot et al. 2022; Gaidamakova et al. 2022; Han et al. 2022) (and references therein). These enzymes are for example involved in ROS removal (e.g., superoxide dismutase, catalase, and peroxiredoxin), and protection and repair of oxidation-sensitive cysteine and methionine residues in proteins (thiol reductases). Most *Deinococcus* species, including *D. murrayi*, possess several superoxide dismutases, thioredoxins (Trxs) and Trx-like proteins, and catalases and other peroxidases (Table 2).

Deinococcus murrayi has a homolog of the heme catalase DR_1998 that is highly expressed and required for resistance to hydrogen peroxide and chronic ionizing radiation in *D. radiodurans*. Unlike *D. radiodurans*, *D. murrayi* also possesses a (non-heme) manganese catalase. Concerning thiol-dependent peroxidases of the OsmC family, *D. murrayi* contains homologs of OsmC, Ohr, and YhfA. However, *D. murrayi* Ohr is atypical with respect to the positions of the two redox-active cysteine residues. Interestingly, these residues are present in a CXXC motif in Ohr from *D. murrayi* (and also from *Deinococcus* sp. RL), whereas in essentially all homologs these two cysteines are distant in the protein sequence (e.g., in *D. radiodurans* Ohr) (Fig. 5). Nevertheless, in the 3D structure the two cysteine residues are close to each other in both the atypical and canonical Ohr, thus forming a similar active site (Fig. 5; Fig. S12). Ohr proteins are active as homodimers, with the two Cys from one monomer and conserved Arg and Glu residues from the other monomer contributing to the active site (Lesniak et al. 2002; Meireles et al. 2017). These Arg and Glu residues are also present in *D. murrayi* Ohr (Fig. 5).

Disulfide bond (Dsb) family proteins such as DsbA–DsbE are involved in formation or reduction of disulfide bonds in extracytoplasmic proteins, and may also repair oxidized cysteine residues and have a role in defense against oxidative stress (Ezraty et al. 2017; de Groot et al. 2022). Proteins similar to DsbA, DsbB, CcdA (analog of DsbD), and DsbE (also called CcmG) are present in *D. murrayi*. DsbE proteins contain an N-terminal transmembrane segment or a signal peptide, and a WXXWCXXC motif in a thioredoxin domain in the periplasmic part of the protein. A second homolog of DsbE was found in *D. murrayi* (WP_051363583.1), containing the motif but lacking an N-terminal transmembrane segment or a signal peptide. Such protein was also found in *Deinococcus*

Table 2. Oxidative stress defense-related proteins in *Deinococcus murrayi*.

Name	<i>D. murrayi</i> protein	<i>D. radiodurans</i> locus tag	10 other <i>Deinococcus</i> species									
			<i>ge</i>	<i>de</i>	<i>ma</i>	<i>go</i>	<i>pr</i>	<i>pe</i>	<i>rp</i>	<i>so</i>	<i>ac</i>	<i>pu</i>
Superoxide dismutases (SODs)												
SodA	WP_027460929.1	DR_1279	1	1	1	1	1	1	1	1	1	1
SodC	—	DR_1546		1		1	1					
SodC	WP_027459576.1	DR_A0202		1	1	1	1			1		1
SodC-like	WP_027461525.1	DR_0644	1	1	1	1			2	1	1	2
Catalases												
KatE1	WP_027459600.1	DR_1998	1	1	1	1	1	1	1	1		
KatE2	—	DR_A0259						1				
KatE3	—	—				1					1	1
MnCat	WP_027461456.1	—				1	1	1	1			
Peroxiredoxins (Prxs) and Prx-related proteins												
Bcp	WP_027461381.1	DR_0846	1	1	1	1	1	1	1	1	1	1
Bcp	—	DR_1208										
Bcp	WP_027461313.1	DR_1209	2	1	2	1	1	2	1	2	2	2
Bcp-like	—	—		fr		1				1	1	1
AhpE	WP_034409648.1	DR_2242	1	1	1	1	1	2	1	1	1	1
AhpD	WP_027460390.1	DR_1765	1	2	1	1	1	5	1	1	1	1
Other peroxidases												
CCP	—	DR_A0301						1				
OsmC	WP_027459320.1	DR_1538	1	1	1	1			1	1	1	1
Ohr	WP_027459819.1	DR_1857	1		2	2	2	2	1	1		1
YhfA	WP_027460307.1	DR_1177	1	2	1	1	1	2	1	1	1	1
Thioredoxin reductase, thioredoxins (Trxs) and Trx-like proteins												
TrxR	WP_027459354.1	DR_1982	2	1	1	1	1	2	1	1	1	1
TrxA	WP_027460625.1	DR_0944	1	1	1	1	1	3	1	1	1	1
TrxC	WP_027460985.1	DR_A0164	1	1								
Trx-like	WP_027459776.1	DR_2085	1	1	1	1	1	1	1	1	1	1
Trx-like	WP_027460945.1	DR_0057	1	1	1	1	1	1	1	1	1	1
Trx-like	WP_025567292.1	DR_A0072	1			2	2		1			
Trx-like	WP_027460088.1	DR_B0110	1			1	1		1	1	1	
Trx-like	WP_027460208.1	DR_0948	1	1	1	1	1		1	1	1	1
Methionine sulfoxide reductases												
MsrA	WP_027460922.1	DR_1849	1	1	1	1	1	1	1	1	1	1
MsrB	WP_027459852.1	DR_1378	1	1	1	1	1	1	1	1	1	1
MsrP	—	DR_2536	1	1	1	1	1	1	1	1	1	1
MsrQ	—	DR_2537	1	1	1	1	1	1	1	1	1	1
Dsb family proteins, periplasmic or cytoplasmic membrane												
DsbA (SP)	—	DR_2019 DR_0560	1	1	1	1	1			2	1	1
DsbA (TM)	WP_027460879.1	DR_0753	1	1	1	1	2		1	1	1	1
DsbB	WP_027460880.1	DR_0754	1	1	1	1	1		1	1	1	1
CcdA (DsbD)	WP_027460794.1	DR_1300	1	2	1	1	1	2	1	1	1	2
TlpA-like family; DsbE (CcmG)	WP_027460801.1	DR_0345 DR_0189	1	2	2	2	1	3		1	1	1
TlpA-like family (cytoplasmic?)	WP_051363583.1	—										
Other proteins involved in reactive oxygen species protection												
HSP33	WP_027459371.1	DR_0985	1	1	1	1	1	1	1	1	1	1
FrnE	—	DR_0659	1	1	1	1	1	1	1	1	1	1
FrnE-like	—	—		1			1					
DsbA-like protein	WP_027461433.1	DR_2335		1	1	1			1	1	1	1
Dps1	WP_027459930.1	DR_2263	1	1	1	1	1	1	1	1	1	1
Dps2	—	DR_B0092				1						
PNPase	WP_027459873.1	DR_2063	1	1	1	1	1	1	1	1	1	1
RhlB	WP_084542977.1	DR_0335		1	1	1				1	1	1

Table 2. (concluded).

Name	<i>D. murrayi</i> protein	<i>D. radiodurans</i> locus tag	10 other <i>Deinococcus</i> species									
			<i>ge</i>	<i>de</i>	<i>ma</i>	<i>go</i>	<i>pr</i>	<i>pe</i>	<i>rp</i>	<i>so</i>	<i>ac</i>	<i>pu</i>
Carotenoid												
CrtE	WP_027459470.1	DR_1395	1	1	1	1	1	1	1	1	1	1
CrtB	WP_034407839.1	DR_0862	1	1	1	1	1	1	1	1	1	1
CrtI	WP_051363338.1	DR_0861	1	1	1	1	1	1	1	1	1	1
CrtLm	WP_027460970.1	DR_0801	1		1	1				1	1	fr 1
CrtY-CruF	—	—		1				1	1			
CruF	WP_027460236.1	DR_0091	1		1	1				1	1	1 1
CrtD	WP_027460233.1	DR_2250	1	1	1	1	1	1	1	1	1	1 1
CrtO	WP_027460237.1	DR_0093	1	1	1	1	1	1	1	1	1	1 1
CYP287A1	WP_034408570.1	DR_2473			1	1	1	1	1	1	1	1 1
NOS	WP_051363406.1	DR_2597	1	1	1	1	1	1	1		1	1
Bacillithiol (BSH)												
BshA	WP_027460299.1	DR_1555	1	1	1	1	1	1	1	1	1	1 1
BshB1	WP_027460148.1	A2G07_01750	1	1	1	1	1	1	1	1	1	1 1
BshC	WP_027460828.1	DR_1647	1	1	1	1	1	1	1	1	1	1 1
Bdr	WP_034408478.1	DR_2623	1	1	1	1	1	1	1	1	1	1 1
AbxC	WP_027460378.1	DR_1832	1	1	1	1	1	1	1	1	1	1 1

Note: For DsbA: SP = signal peptide; TM = N-terminal transmembrane segment. See further the note in Table 1.

sp. RL (96% identity), but not in others. If this protein is functionally expressed, it might be located in the cytoplasm and perhaps represent an additional Trx-like protein.

Surprisingly, compared to the other analyzed *Deinococcus* genome sequences, *D. murrayi* and *Deinococcus* sp. RL do not encode homologs of FrnE, MsrP, and MsrQ. FrnE is a cytoplasmic DsbA-like thiol oxidoreductase. In *D. radiodurans*, FrnE contributes to resistance to cadmium, diamide, gamma rays, and hydrogen peroxide (Khairnar et al. 2013; Bihani et al. 2018). MsrPQ is a methionine sulfoxide reductase system that repairs oxidized methionine in periplasmic proteins (Gennaris et al. 2015). Therefore, FrnE- and MsrPQ-like activities may be absent in *D. murrayi*, or the lack of these proteins may be compensated by other enzymes.

Deinoxanthin is a major carotenoid in *D. radiodurans*. Genes required for the biosynthesis of this pigment have been identified in *D. radiodurans* (Tian and Hua 2010; Zhou et al. 2015). These genes are conserved in *D. murrayi* (Table 2). Nevertheless, colonies of *D. murrayi* are darker red than those of *D. radiodurans* when grown under the same conditions (Fig. 6), possibly because of different composition and/or amount of the produced carotenoids. For *D. radiodurans*, it was reported that nitric oxide produced by nitric oxide synthase (NOS, DR_2597) modulates carotenoid biosynthesis (Hansler et al. 2016). Deletion of *nos* caused a decrease in total carotenoid abundance and perturbations in carotenoid composition compared to the wild-type strain. An NOS homolog was found in *D. murrayi* and most, but not all, other *Deinococcus* species (Table 2). The bacillithiol biosynthesis proteins BshA, BshB, and BshC are also conserved in *D. murrayi*, as well as bacillithiol disulfide reductase (Bdr) and bacilliredoxin (AbxC). The latter shows similarity to bacilliredoxin BrxC from *Bacillus subtilis*, but because the proteins from *Deinococcus* have a different monothiol active site motif and a C-terminal extension, they were called AbxC (atypical BrxC). AbxC may be

involved in protein de-bacillithiolation and in scavenging of hydrogen peroxide (Jeong et al. 2021a).

Radiation and oxidative stress resistance-associated regulatory proteins

In *D. radiodurans*, several regulatory proteins have been described for contributing to DNA repair and radiation and oxidative stress resistance, and also to Mn/Fe homeostasis (Lim et al. 2019). These include proteins of two-component signal transduction systems (DrRRA, RadSR, and DrtSR), regulators of metal ion homeostasis (FUR family proteins, DtxR), LysR family proteins proposed to function as OxyR, a serine/threonine-protein kinase (RqkA), a cyclic AMP receptor protein (DdrI), a GntR family protein (DR_0265), and a zinc peptidase (IrrE, also called PprI) that functions together with an XRE family transcriptional repressor (DdrO). The network of regulatory pathways is complex, and expression or activity of DNA repair and oxidative stress response proteins may involve more than one regulatory protein. Adding to this complexity is the presence of small RNAs (Tsai et al. 2015; Villa et al. 2021). Transcriptomics experiments have been performed for several mutant strains lacking one of these regulators, which revealed differential expression of dozens to hundreds of genes under standard growth condition and/or after stress exposure (Lim et al. 2019). More work is needed for most of these regulatory proteins to unravel their molecular regulation mechanism and to determine the genes regulated directly by these regulators. Not all of these regulators are conserved in every other *Deinococcus* species, and some species contain homologs of stress response-associated regulators that are absent in *D. radiodurans* (Table 3).

The *D. murrayi* homologs of the radiation and oxidative stress resistance-associated regulatory proteins are shown in Table 3. The proteins that are conserved in most *Deinococcus*

Fig. 5. The CXXC motif-containing atypical Ohr from *D. murrayi*. *Top:* Alignment of Ohr (organic hydroperoxide resistance protein) from *Deinococcus murrayi* (Dmur_Ohr) and *D. radiodurans* (DR_1857). For both proteins, the Cys (two in each protein), Arg, and Glu residues contributing to the active site are indicated with triangles below the alignment. The secondary structure of DR_1857 is indicated above the sequences. *Bottom:* AlphaFold model of Ohr from *D. murrayi* (left) compared to the crystal structure of Ohr from *D. radiodurans* (right) (monomer of PDB accession 1USP) (Meunier-Jamin et al. 2004). The Ohr monomer is composed of two distinct subdomains: a small N-terminal domain (orange) and a larger C-terminal domain. The two Cys residues in both Ohr structures are shown in green.

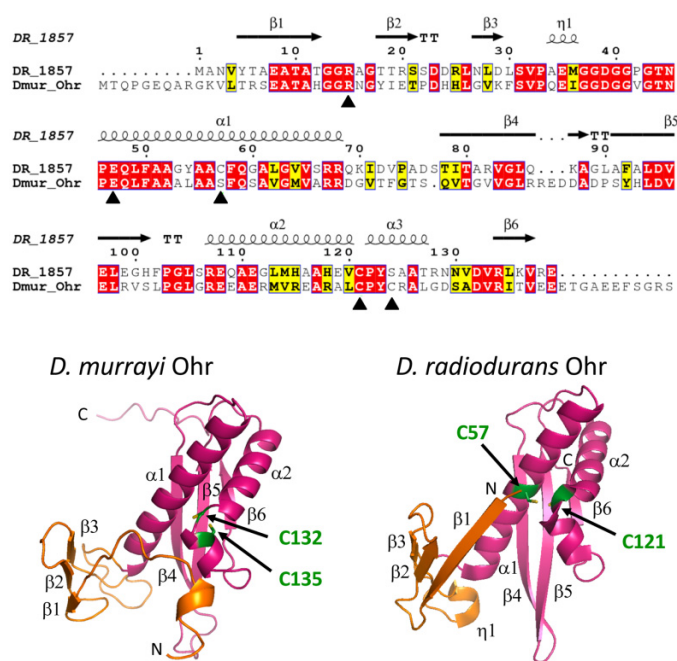
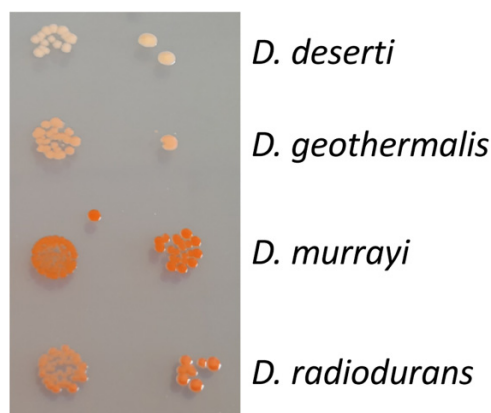


Fig. 6. Pigmented colonies of the type strains of four *Deinococcus* species.



species are also present in *D. murrayi*. The two LysR family proteins from *D. radiodurans*, DR_0615 and DR_A0336, were proposed to be 1-Cys-OxyR proteins (*Escherichia coli* OxyR has two cysteines required for hydrogen peroxide sensing) (Chen

et al. 2008; Yin et al. 2010). DR_A0336 is not well conserved in *Deinococcus* species, while homologs of DR_0615 are highly conserved. However, the *D. murrayi* homolog of DR_0615 does not contain any cysteine, as observed for the counterparts in *Deinococcus* sp. RL, *D. deserti* and *D. peraridillitoris*, and, therefore, this protein cannot function as a cysteine-dependent hydrogen peroxide sensor.

The zinc peptidase IrrE and the transcriptional repressor DdrO are highly conserved in *Deinococcus*, including *D. murrayi*. The IrrE/DdrO system is crucial for radiation resistance in *D. radiodurans* and *D. deserti*, and most likely in other *Deinococcus* bacteria as well. It is also the best studied radiation response pathway in these bacteria (de Groot et al. 2019; Lim et al. 2019; Lu et al. 2019; Lu and Hua 2020; Blanchard and de Groot 2021; Eugénie et al. 2021; Magerand et al. 2021; Narasimha and Basu 2021). This system controls expression of genes that are characterized by a palindromic motif called radiation-desiccation response motif (RDRM) in the promoter region, which is the binding site for the transcriptional repressor DdrO (Makarova et al. 2007; Wang et al. 2015; Blanchard et al. 2017; Eugénie et al. 2021). The genes regulated by IrrE/DdrO (i.e., the RDR regulon) include mainly DNA repair-related genes (e.g., *recA*, *ssb*, *gyrA*, *uvrB*, *ddrA* to *ddrD*, and *pprA*), and also *ddrO* itself and several genes of unknown function (e.g., *ddrQ* and *ddrS*). A recent study combining ChIP-seq and RNA-seq showed that the RDR regulon contains at least 35 genes in *D. radiodurans* (Eugénie et al. 2021). After exposure to radiation or other DNA damage and oxidative stress-generating conditions, IrrE peptidase cleaves and inactivates repressor DdrO, resulting in induced expression of RDR genes. This specific cleavage is essential for radiation resistance, because strains expressing point mutants of IrrE or DdrO that abolish DdrO cleavage are extremely sensitive to radiation, like *irrE* deletion mutants. The annotated IrrE of *D. murrayi* contains 256 amino acid residues. However, it appears that the start codon position is wrongly predicted, as found for many *irrE* homologs (Ludanyi et al. 2014; Magerand et al. 2021). Based on analysis of the sequence upstream of the annotated start codon, we propose that the correct *D. murrayi* IrrE is 28 residues longer at the N terminus. A similar, but not identical, set of genes is (predicted to be) regulated by IrrE/DdrO in different *Deinococcus* species (Blanchard et al. 2017), which can be expected for *D. murrayi* as well. Indeed, analysis of promoter region sequences of *D. murrayi* homologs of *D. radiodurans* and *D. deserti* RDR genes and of other DNA repair genes showed the presence of a common palindromic motif, with the same consensus sequence as for the previously reported RDRM (Makarova et al. 2007), upstream of several DNA repair genes, such as *ssb*, *gyrA*, *gyrB*, *recA* operon, *ruvB*, *uvrA*, intein-containing *uvrB*, *ddrA* to *ddrD*, and *pprA*, and also upstream of the unknown function genes *ddrQ* (WP_027460366.1, adjacent and divergently oriented to *ddrO*) and *ddrS* (WP_084542933.1; a small protein with two CXXC motifs). This bioinformatic analysis did not indicate the RDRM upstream of *dinP* (encoding DNA polymerase IV, which is absent in *D. radiodurans* and *D. deserti*), suggesting that expression of *dinP* may not be controlled (directly) by IrrE/DdrO in *D. murrayi*. However, experimental data may reveal RDR regulon

Table 3. Radiation and oxidative stress resistance-associated regulatory proteins in *Deinococcus murrayi*.

Name	<i>D. murrayi</i> protein	<i>D. radiodurans</i> locus tag	10 other <i>Deinococcus</i> species										
			<i>ge</i>	<i>de</i>	<i>ma</i>	<i>go</i>	<i>pr</i>	<i>pe</i>	<i>rp</i>	<i>so</i>	<i>ac</i>	<i>pu</i>	
IrrE (PprI)	WP_034408305.1	DR_0167	1	1	1	1	1	1	1	1	1	1	1
DdrO	WP_027460365.1	DR_2574	1	2	1	3	1	2	1	1	1	1	1
LexA1	—	DR_A0344		1		1	1	1	1				
LexA2	WP_027459292.1	DR_A0074	1	1	1				1	1	1		
RqkA	WP_027460955.1	DR_2518	1	1	1	fr			1	1	1	1	1
LysR family	WP_027459977.1	DR_0615	1	1	1	1	1	1	1	1	1	1	1
LysR family	—	DR_A0336									1	1	
OhrR	—	—		1	1				1				
SoxR	—	—		1				1				1	fr
FUR family	WP_034403483.1	A2G07_01865	1	1	1	1	1	1	1	1	1	1	1
FUR family	WP_027459325.1	DR_0865	1	1	1	1	1	1	1	1	1	1	1
FUR family	—	—	1	1		1				1			
DtxR	WP_027460118.1	DR_2539	1	2	1	1	1	1	1	1	1	1	1
MntR	—	—		1					1				
DrRRA	WP_027459634.1	DR_2418	1	1	1	1	1			1	1	1	1
RadS	—	DR_B0090					1						
RadR	—	DR_B0091					1						
DrtR	WP_027460127.1	DR_2415	1	1	1	1	1	1	1	1	1	1	1
DrtS	WP_027460126.1	DR_2416	1	1	1	1	1	1	1	1	1	1	1
DdrI	WP_027460812.1	DR_0997	1	1	1	1	1	1	1	1	1	1	1
GntR family	WP_027460048.1	DR_0265	2	2	1	1			2	1	1	1	1

Note: See note Table 1.

members not predicted bioinformatically (Eugénie et al. 2021).

Cell envelope

Although *D. radiodurans* stains Gram positive, work by Dr. Murray and others have shown that its cell envelope is multilayered, including an outer membrane and a proteinaceous surface layer, reminiscent of Gram-negative bacteria (Thornley et al. 1965; Work and Griffiths 1968; Lancy and Murray 1978; Thompson and Murray 1981). Recent studies using cryo-electron microscopy have revealed more details of the cell envelope of *D. radiodurans* and some of its proteins (Sexton et al. 2022; von Kügelgen et al. 2022; Farci et al. 2023). The envelope is composed of a cytoplasmic (inner) membrane, a thick layer of peptidoglycan and an additional periplasmic layer, an outer membrane, and a surface layer (S-layer). Two abundant surface proteins of *D. radiodurans* are hexagonally packed intermediate (HPI, DR_2508) and surface layer protein A (SlpA, DR_2577) (Rothfuss et al. 2006). HPI is probably the only protein in the S-layer (von Kügelgen et al. 2023). SlpA was also thought to be an S-layer protein, but it appears to be an outer membrane protein with an N-terminal segment in the periplasmic space. The C-terminal region of SlpA contains a 30-stranded beta-barrel in the outer membrane, connected by a long coiled-coil segment to the N-terminal S-layer homology (SLH) domain that interacts with the peptidoglycan (Misra et al. 2015; von Kügelgen et al. 2022). It was also found that SlpA is bound to the carotenoid deinoxanthin and, at the periplasmic side, to

a protein that was identified as DR_0644 (Farci et al. 2016, 2023). DR_0644 is annotated as a hypothetical protein or CHR domain-containing protein, and has limited similarity to superoxide dismutases (Table 2). Further work has indicated that DR_0644 is a Cu-only superoxide dismutase, and that SlpA in complex with deinoxanthin and DR_0644 has photoprotective and chemoprotective functions (Farci et al. 2023). Characterization of *slpA* deletion mutants showed that SlpA is also important for integrity of the outer membrane, but it is not essential for the assembly of the S-layer (Rothfuss et al. 2006; von Kügelgen et al. 2022). Biogenesis of outer membrane beta-barrel proteins in Gram-negative bacteria involves the beta-barrel assembly machinery (BAM complex) and the translocation and assembly module (TAM) (Ranava et al. 2018). In *D. radiodurans*, homologs of the outer membrane protein BamA and the single-pass inner membrane protein TamB have been identified and characterized, with mutant strains showing decreased growth rate and severely damaged outer layers of the cell envelope (Yu et al. 2017; Yu and Lu 2019).

Blastp analysis revealed an HPI homolog, WP_027460105.1, in *D. murrayi* (and in *Deinococcus* sp. RL), but not in 10 other *Deinococcus* species considered here. Homologs of *D. radiodurans* SlpA (1167 residues) are found in the other *Deinococcus* bacteria, including *D. murrayi* (WP_027460369.1, 858 residues). The SlpA proteins in six of these analyzed *Deinococcus* species contain between 857 and 946 amino acid residues, while SlpAs in the six others have between 1068 and 1167 residues. The smaller SlpA proteins have a shorter coiled-coil or less beta-strands compared to SlpA from *D. radiodurans*

(von Kügelgen et al. 2022). BamA (840 to 900 residues) and TamB (3100 to 4000 residues) are well conserved in *Deinococcus*, including *D. murrayi* (BamA: WP_027461009.1; TamB: WP_157468832.1). Homologs of the Cu-only superoxide dismutase DR_0644 were found in *D. murrayi* (WP_027461525.1) and the other *Deinococcus* species analyzed here (except for *D. proteolyticus*), but with rather low identity percentages (31%–40%). Furthermore, these homologs (145–151 residues) do not contain the unstructured threonine-rich region that is present in DR_0644 (206 residues). However, they all contain a lipoprotein signal peptide and the three Cu-binding histidine residues of the active site (Farci et al. 2023).

Concluding remarks

Characterization of several radiation-resistant red-pigmented “*Micrococcus*” bacteria isolated more than 50 years ago revealed that they formed a distinct group separate from *Micrococcus*, and Dr. Murray proposed the names *Deinococcaceae* (fam. nov.) and *Deinococcus* (gen. nov.; type genus) for these bacteria (Brooks et al. 1980; Brooks and Murray 1981). The first described *Deinococcus* species were *D. radiodurans* (type species), *D. radiophilus*, *D. proteolyticus*, and *D. radiopugnans* (Brooks and Murray 1981), and several years later the rod-shaped *D. grandis* was included in the genus *Deinococcus* (Rainey et al. 1997). Also in 1997, two more *Deinococcus* species were described, one was named *D. geothermalis* and the other *D. murrayi* in recognition of Dr. Murray’s work on the genus *Deinococcus* (Ferreira et al. 1997). At present, more than 80 *Deinococcus* species have been validly published.

The development of DNA sequencing techniques has allowed the determination and analysis of genome sequences of many organisms, including many *Deinococcus* species. Previously, we compared the radiation- and oxidative stress resistance-associated proteins of eleven *Deinococcus* species for which a complete and assembled genome sequence was available (Lim et al. 2019). Obviously, many DNA repair and oxidative stress defense proteins were found to be conserved among these *Deinococcus* species and other bacteria. However, a large diversity was also observed, with several proteins absent in the well-studied *D. radiodurans* but present in other *Deinococcus* species, and proteins contributing to radiation resistance in *D. radiodurans* that are absent in other *Deinococcus* species. Here, we analyzed a set of proteins encoded by *D. murrayi*, and discovered some interesting features compared to *D. radiodurans* and other species. For example, the sole uracil-DNA glycosylase Ung of *D. murrayi* is fused to a PhrB protein that might have single-stranded DNA-specific photolyase activity or might function as a photoreceptor and have a role in signaling. The ATPase domains of NER proteins UvrB and UvrD contain inteins, indicating that protein splicing occurs to obtain mature UvrB and UvrD. In bacteria and archaea, many identified inteins are present in DNA replication and repair proteins (e.g., helicases, recombinases, polymerases, and gyrase subunits), and often in ATPase domains of such proteins (Novikova et al. 2014, 2016). The reason for this is not fully understood and still a matter of debate, but it might be caused by selective pressure that

retains the intein in these proteins and domains, or protein splicing might be a post-translational regulatory mechanism under stress conditions (Gogarten et al. 2002; Pavankumar 2018). For example, splicing of the intein in the RecA homolog RadA of the archaeon *Pyrococcus horikoshii* is stimulated by single-stranded DNA and DNA damage (Lennon et al. 2016). For the ATPase domains containing an intein insertion, it has been suggested that this may spare ATP under conditions when splicing is inhibited (Novikova et al. 2016). To the best of our knowledge, an intein inserted in UvrB has not been reported previously. The expression of *uvrB* in *D. murrayi* is predicted to be induced upon exposure to radiation because its promoter region contains a motif (RDRM) that is found upstream of radiation-induced, IrrE/DdrO-regulated genes in *Deinococcus* bacteria. It will be interesting to study the intein-containing UvrB and UvrD in *D. murrayi* and to discover if splicing of these proteins is stimulated or inhibited under certain conditions (e.g., low or high temperature, oxidative stress, and DNA damaging conditions). Another special feature of *D. murrayi* is the atypical Ohr protein in which the redox active cysteines are present in a CXXC motif, in contrast to essentially all other identified Ohr proteins. The absence of cytoplasmic disulfide oxidoreductase FrnE and periplasmic methionine sulfoxide reductase MsrPQ in *D. murrayi* is also remarkable.

In conclusion, analysis of new genome sequences is always informative. Here, with respect to the extraordinary radiation-resistant *Deinococcus* bacteria, the characterization of *D. murrayi* showed further diversity for proteins involved in DNA repair and oxidative stress defense, opening the way to new studies.

Article information

History dates

Received: 24 April 2023

Revised: 11 July 2023

Accepted: 2 August 2023

Accepted manuscript online: 8 August 2023

Version of record online: 5 October 2023

Notes

This paper is a part of the tribute collection entitled “Dr. Robert Murray: Strong roots in Canadian microbiology - Strong global leadership and vision.” <https://cdsciencepub.com/topic/cjm-murray-tribute>

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Data availability statement

Accession numbers of genes and proteins found in this study are available in the text and in the tables, and can be accessed at public databases.

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Competing interests

The authors declare that they do not have any competing interests.

Funding information

Our work is funded by the Transverse Division No. 4 (Radiobiology) of the French Alternative Energies and Atomic Energy Commission (Programme No. 4 Radiobiologie) and by a grant from the Agence Nationale de la Recherche (ANR-19-CE12-0010).

Supplementary material

Supplementary data are available with the article at <https://doi.org/10.1139/cjm-2023-0074>.

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