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Engineering *Deinococcus geothermalis* for Bioremediation of High-Temperature Radioactive Waste Environments

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Deinococcus geothermalis **is an extremely radiation-resistant thermophilic bacterium closely related to the mesophile *Deinococcus radiodurans*, which is being engineered for in situ bioremediation of radioactive wastes. We report that *D. geothermalis* is transformable with plasmids designed for *D. radiodurans* and have generated a Hg(II)-resistant *D. geothermalis* strain capable of reducing Hg(II) at elevated temperatures and in the presence of 50 Gy/h. Additionally, *D. geothermalis* is capable of reducing Fe(III)-nitrilotriacetic acid, U(VI), and Cr(VI). These characteristics support the prospective development of this thermophilic radiophile for bioremediation of radioactive mixed waste environments with temperatures as high as 55°C.**

The bacterium *Deinococcus geothermalis* (13) is remarkable not only for its extreme resistance to ionizing radiation but also for its ability to grow at temperatures as high as 55°C (13) and in the presence of chronic irradiation (8). The organism was isolated by Ferreira et al. (13) from hot springs together with *Deinococcus murrayi*. Both bacteria are moderately thermophilic and belong to the bacterial family *Deinococcaceae* (4, 7, 22), currently comprised of seven distinct nonpathogenic radiation-resistant species, of which *Deinococcus radiodurans* strain R1 is the best characterized (4). Advances in genetic engineering for *D. radiodurans* (9–12, 29) were a stimulus for its genome sequencing (17, 33), annotation (22), and proteomic (18) and transcriptome (19) analyses. The other deinococcal species have been reported as nontransformable or have not yet been tested for transformability by chromosomal or plasmid DNA and have been left unexplored by recombinant DNA technologies. Other genetic approaches including conjugation and protoplast fusion have not been successful in the *Deinococcaceae* (16).

A present genetic engineering goal for *D. radiodurans* is its development for bioremediation of U.S. Department of Energy (DOE) mixed radioactive environmental waste sites left over from nuclear weapons production during the Cold War (21, 25, 27, 28). These sites contain immense volumes of waste (3×10^6 m^3) that include radionuclides, heavy metals, and toxic organic compounds and have contaminated 40 million cubic meters of soil and 4 trillion liters of groundwater since 1946 (1, 21, 25, 27, 28). While there has been significant progress in engineering *D. radiodurans* for remediation of radioactive DOE waste environments (5, 8, 15), prospective treatment of contaminated sites with engineered *D. radiodurans* will be limited to temperatures below 39°C, its maximum growth temperature. However, there is a need to develop bioremediating bacteria that are resistant to both radiation and high temperatures because of the existence of thermally insulated contaminated environments where temperatures are elevated by the decay of long-lived radionuclides (e.g., ^{137}Cs and ^{90}Sr) (1). For example, soil columns beneath at least 67 radioactive leaking tanks at DOE's Hanford Site in south-central Washington State have been contaminated and have recorded temperatures as high as 70°C at depths of greater than 18 m (1). Since *D. geothermalis* and *D. murrayi* are both radiation resistant and thermophilic, they have become desirable targets for genetic development of bioremediating strains similar to those developed for *D. radiodurans* (5, 8, 15) but capable of survival and growth at higher temperatures. Given the need to develop bioremediating bacteria for treatment of radioactive high-temperature waste environments, *D. geothermalis* and *D. murrayi* were tested for their transformability with the autonomously replicating *Escherichia coli-D. radiodurans* shuttle plasmid pMD66 (9), which expresses kanamycin (KAN) and tetracycline (TET) resistance in *D. radiodurans* and additionally expresses ampicillin resistance in *E. coli*.

pMD66 and its numerous derivatives (9–12) have been used successfully to functionally express cloned genes in *D. radiodurans* growing under chronic irradiation. Examples include the *mer* operon of *E. coli* (5), which encodes Hg(II) resistance and reduction, and the *Pseudomonas* operon *todC1C2BA* (15), which encodes partial degradation of toluene. The present work shows that *D. geothermalis* is capable of expressing Hg(II)-reducing functions cloned in pMD66 at elevated temperatures and under chronic radiation and, like *D. radiodurans* (14), is naturally capable of reducing a variety of other metal contaminants present in DOE waste sites.

MATERIALS AND METHODS

Bacterial strains, growth conditions, and transformation. *D. radiodurans* R1 (ATCC BAA-816) (33), *D. geothermalis* DSM11300, and *D. murrayi* DSM11303 were grown in TGY broth (1% Bacto Tryptone, 0.1% glucose, 0.5% Bacto Yeast Extract) (Difco) or minimal medium (MM) (see Table 2) (32). Liquid cultures were inoculated at ~5×10^6 cells/ml. For solid medium, Bacto Agar (Difco) or Noble agar (Difco) was added to TGY or MM, respectively, to 1.5% (wt/vol). *D. radiodurans* was grown at 32°C, and *D. geothermalis* and *D. murrayi* were grown at 37°C or at higher temperatures as indicated. *E. coli* was grown in Luria-Bertani

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medium at 37°C (9). pMD66 (9) (purified from *E. coli*) encodes ampicillin resistance (Ap^r), Km^r, and Tet^r in *E. coli* and Km^r and Tet^r in *D. radiodurans*. When pMD66 is prepared from *E. coli*, the plasmid transforms *D. radiodurans* with low efficiency (~50 transformants/μg) (9). However, when the same plasmid is purified from *D. radiodurans*, it transforms wild-type *D. radiodurans* with efficiencies as high as 10^6 transformants/μg (9). Plasmid transformation-restriction in *D. radiodurans* (9), therefore, distinguishes between the sources of plasmids. When purified from *E. coli*, the plasmid is called pMD66, and when purified from *D. radiodurans*, it is called pMD68 (9). The situation for a derivative (pMD300) of pMD66 encoding chloramphenicol resistance (Cm^r) is analogous (10). pMD300 purified from *E. coli* encodes Cm^r in *E. coli* and *D. radiodurans* and when purified from *D. radiodurans* is called pMD308 (10). Expression of heterologous genes and antibiotic resistance markers cloned into pMD66-type vectors is driven by two different deinococcal constitutive promoters (P1 and P2) (e.g., see Fig. 2A) that are active on autonomous plasmids in *D. radiodurans* or when integrated into *D. radiodurans* chromosomes (11, 12). pMD66-type plasmids contain a deinococcal origin of replication (dORI); an *E. coli* origin of replication (eORI); and resistance genes including *aphA* (encoding Km^r), *bla* (encoding Ap^r), and/or the *mer* operon (encoding mercury resistance) (3, 5).

The selective drug concentrations for deinococci were 8 μg of KAN per ml, 2.5 μg of TET per ml, and 3 μg of chloramphenicol per ml. For *E. coli*, antibiotics were added to Luria-Bertani medium as follows: KAN (50 μg/ml), TET (25 μg/ml), and chloramphenicol (30 μg/ml). Transformation of deinococci was by a $CaCl_2$-dependent technique described previously for *D. radiodurans* (16) but with the following modifications. Exponential cultures of deinococci were resuspended at 10^8 cells per ml in TGY broth–0.1 M $CaCl_2$–glycerol (20:8:3, vol/vol/vol). For transformation, 100 μl of the cell suspension and 5 μl of water containing various amounts of transforming DNA were added. The cell mixture was held on ice for 15 min and then incubated at 32°C for 30 min with gentle agitation. TGY (0.9 ml) was then added, and the mixture was incubated at 32°C (for *D. radiodurans*) or 37°C (for *D. geothermalis*) for 16 h with aeration before being plated on drug-selected agar.

Irradiation. Growth of cells in the presence of chronic irradiation, 50 Gy/h (^{137}Cs Gammacell 40 irradiation unit [Atomic Energy of Canada Limited]), was carried out as described previously (5, 8, 15). For high-level acute irradiation exposures, early-stationary-phase deinococcal cultures (optical density at 600 nm [OD_{600}] of 0.9 corresponds to ~10^8 CFU/ml) were irradiated without change of broth on ice at 10 kGy/h (^{60}Co Gammacell irradiation unit [J. L. Shepard and Associates; Model 109]). For the deinococcal species under investigation, three independent cell cultures and irradiation treatments of the same kind were performed. Following exposure to the indicated doses, cell suspensions were appropriately diluted and assayed for viability by plate assay on rich (TGY) medium (9). Viability data were used to construct survival curves with standard deviations according to conventional formats (9, 24). The effect of chronic exposure to gamma radiation and Hg(II) on the growth of engineered *D. geothermalis* was determined using TGY agar plates with and without 30 μM merbromin [Hg(II)] (5). Plates were spotted with ~10^5 cells and following plate inoculation were placed into the ^{137}Cs irradiator (50 Gy/h) for incubation at 50°C for 5 days.

DNA isolation and manipulation. Isolation of plasmid DNA and total DNA from *E. coli*, *D. radiodurans*, and *D. geothermalis*; use of enzymatic reagents; gel electrophoresis; plasmid rescue in *E. coli*; radiolabeling of DNA; hybridization; washing of blots; and autoradiography were performed as previously described (9–12). For Fig. 1B, *D. geothermalis* (wild-type) and *D. geothermalis*/pMD66 total DNA preparations were digested with *Eco*RI. The blot was double hybridized with a 1.5-kb *Xba*I genomic *recA* (*D. radiodurans*) probe and a 1.5-kb *Eco*RI-*Bpu*10I probe of pBR322 that is specific to pMD66.

Mercury volatilization assay. Cells were pregrown to an OD_{600} of 0.5 in the presence of 20 μM merbromin [Hg(II)] as described previously (5). Cells of each strain were harvested, washed twice in fresh medium lacking Hg(II), and concentrated to an OD_{600} of 2.0 in fresh medium, followed by the inoculation of 10^7 cells of each into 200 μl of medium containing 30 μM merbromin contained in 300-μl wells of a microplate, respectively. The plates were covered with a sheet of X-ray film, held together with a weight, and incubated in the dark at 32 or 40°C. Following exposure for 14 h, the films were developed.

Metal reduction by *D. geothermalis*. The native metal reduction capabilities of *D. radiodurans* have been examined previously (14). The protocols for examining metal reduction by *D. geothermalis* are essentially identical to those used for *D. radiodurans* (6, 14, 30, 31) but at higher temperatures. The ability of *D. geothermalis* to reduce Fe(III) (as Fe-nitrilotriacetic acid [NTA]) was examined in cultures containing 10 mM lactate in basal medium at 45°C. For the experiment with Cr(VI) and U(VI), cultures were incubated in TGY at 40°C.

RESULTS

Transformation of *D. geothermalis* and *D. murrayi*. Plasmid transformation of *D. geothermalis* by using pMD66/68 was successful (Table 1), with stable introduction proven by hybridization with a pMD66 probe (Fig. 1A). *D. geothermalis* was also transformable with the Cm^r-encoding *D. radiodurans* plasmid pMD300/308 (data not shown). While the transformation efficiencies were much lower for *D. geothermalis* than for *D. radiodurans*, irrespective of the source of plasmid (Table 1), this was not a problem because the transforming DNA could be prepared and used in bulk. Electroporation as an alternative method of transformation was previously shown not to be effective for *D. radiodurans* (M. J. Daly, unpublished data), where the low electroporation efficiencies were attributed to the unusually thick cell wall structures of deinococci. Figure 1B shows the assessment of the copy number of pMD66 in *D. geothermalis* relative to the chromosomal content by comparing the hybridization of two similarly sized nonhomologous probes to total DNA prepared from *D. geothermalis*/pMD66. The *recA* probe (1.5 kb, derived from *D. radiodurans*) is a reporter of chromosome copy number, and the pMD66 probe (1.5 kb, derived from pBR322) is specific to the plasmid. The hybridization signal for the *recA* probe was determined by densitometry to be about one-third of the intensity arising from the pMD66 probe, suggesting that pMD66 exists in multiple copies in *D. geothermalis*.

Stability of *D. geothermalis*/pMD66. Deinococcal sequences in pMD66/68 are derived exclusively from *D. radiodurans* strain SARK (9, 16), which has no detectable homology to *D. radiodurans* strain R1 or *D. geothermalis* (Fig. 1A). To test whether pMD66 is uniformly retained and repaired in *D. geothermalis* following acute irradiation, *D. geothermalis*/pMD66 was assessed for survival following various exposures to ionizing radiation and recovery on TGY, or on TGY supplemented with KAN as a marker for the presence of pMD66 (Fig. 1C). The survival of *D. geothermalis*/pMD66 plated on TGY-KAN was indistinguishable from that found for wild-type *D. geothermalis* on TGY. Plasmid rescue in *E. coli* from total DNA purified from a culture of *D. geothermalis*/pMD66 following recovery from 12 kGy showed that, of 1,000 Km^r *E. coli* colonies rescued, 100% were also Tet^r and Ap^r, supporting the idea

FIG. 1. Transformation of *D. geothermalis* with pMD66 and resistance of pMD66-transformed *D. geothermalis* to acute gamma radiation. (A) *D. geothermalis*/pMD66. Total DNA from the indicated strains was uncut or digested with *Pst*I before electrophoresis, blotting, and probing of the blot with a whole-plasmid radiolabeled pMD66 probe. Abbreviations: DEIRA, *D. radiodurans*; DEIGEO, *D. geothermalis*. (B) The copy number of pMD66 in *D. geothermalis*/pMD66 is about threefold higher than its chromosomal copy number. (C) Survival of *D. geothermalis*/pMD66 following acute gamma radiation. Symbols: open squares, *D. geothermalis* plated on TGY at 37°C; solid triangles, *D. geothermalis*/pMD66 plated on TGY-KAN at 37°C; solid diamonds, *D. radiodurans* plated on TGY at 32°C.

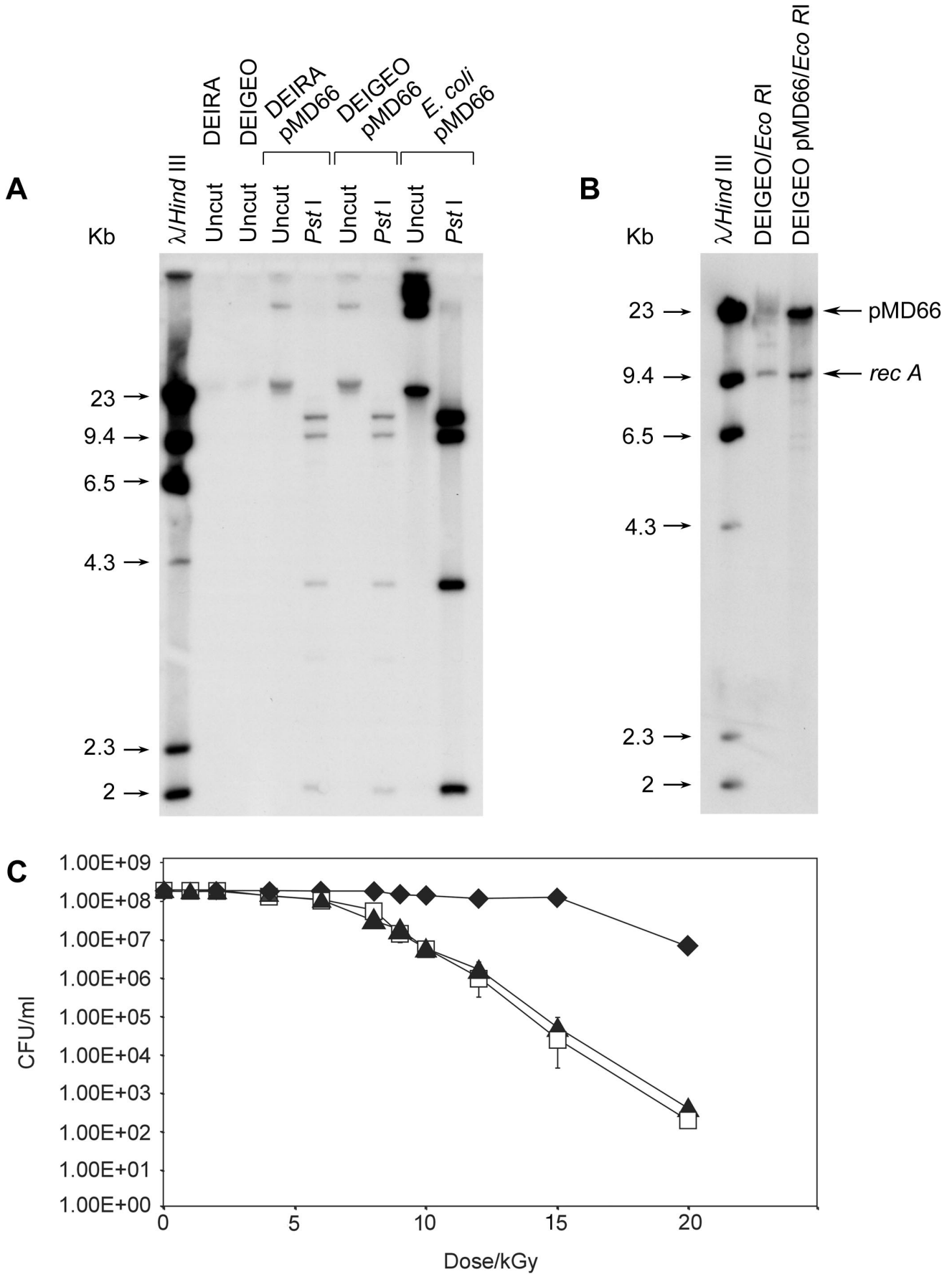

A
Kb
λ/Hind III
DEIRA
DEIGEO
DEIRA pMD66
DEIGEO pMD66
E. coli pMD66
Uncut
Pst I
23
9.4
6.5
4.3
2.3
2
B
Kb
λ/Hind III
DEIGEO/Eco RI
DEIGEO pMD66/Eco RI
pMD66
rec A
C
CFU/ml
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
0
5
10
15
20
Dose/kGy

TABLE 1. Transformation of pMD66/68 into *D. geothermalis*, *D. radiodurans* and *E. coli*

	No. of transformants/μg of DNA for recipient[a]:		
	D. geothermalis	*D. radiodurans*	*E. coli*
Plasmid source[b]			
pMD66 (purified from *E. coli*)	$1 \times 10^1 \pm 2 \times 10^1$	$1 \times 10^2 \pm 8 \times 10^1$	$9 \times 10^4 \pm 3 \times 10^3$
pMD68 (purified from *D. radiodurans*)	$5 \times 10^1 \pm 2 \times 10^1$	$8 \times 10^5 \pm 1.5 \times 10^4$	$4 \times 10^5 \pm 2.1 \times 10^4$
pMD66 (purified from *D. geothermalis*)	$5 \times 10^2 \pm 29 \times 10^1$	$4 \times 10^5 \pm 7 \times 10^3$	$4 \times 10^2 \pm 25 \times 10^1$

[a] Km^r transformants per microgram of plasmid purified from the indicated strains.
[b] pMD66, pMD68, and pMD66-*D. geothermalis* have identical restriction maps (Fig. 1A).

that irradiation-induced mutations and deletions are rare in *D. geothermalis*, as is the case in *D. radiodurans* (9, 11). These results show that pMD66 is retained in *D. geothermalis* without alteration following high-dose irradiation and recovery and is repaired with similar efficiency to its chromosomes.

Construction and characterization of Hg(II)-resistant *D. geothermalis*. The complete *E. coli* Hg(II) resistance (*mer*) operon (4.2 kb, encoding six proteins) (3, 5) has previously been functionally expressed in *D. radiodurans* by using a pMD66 derivative, pMD727 (5) (Fig. 2A). In *D. radiodurans*, all six *mer* genes are necessary for reduction of Hg(II) to Hg(0). pMD727 was successfully transformed into *D. geothermalis* (Fig. 2B), yielding strain MD865. This construction placed the *mer* genes under the control of a constitutive *D. radiodurans* promoter (P2, Fig. 2A), and Southern analysis with a radiolabeled probe containing a 1.5-kb *Eco*RI-*Bgl*II fragment from the *mer* operon showed no significant homology with the *D. geothermalis* genome (Fig. 2B). Reduction of Hg(II) to volatile elemental Hg(0) by *D. geothermalis* strain MD865 was examined by testing for mercury volatilization, which causes film darkening (5, 26). Following 14 h of incubation with Hg(II) in a microplate at 32 or 40°C, covered by X-ray film, wild-type *D. geothermalis* showed modest Hg(0) volatilization. However, strain MD865 (*D. geothermalis*/*mer*$^+$) showed substantial Hg(0) volatilization based on film darkening compared to wild-type *D. geothermalis* at 32 or 40°C (Fig. 2C). MD865 also was resistant to 50 μM Hg(II) during growth at 50°C (Fig. 2D) and displayed luxuriant growth at 50°C in the presence of 50 Gy/h on solid medium containing 30 μM merbromin (data not shown). Wild-type *D. geothermalis* did not grow in medium containing 30 μM merbromin in the presence or absence of chronic radiation.

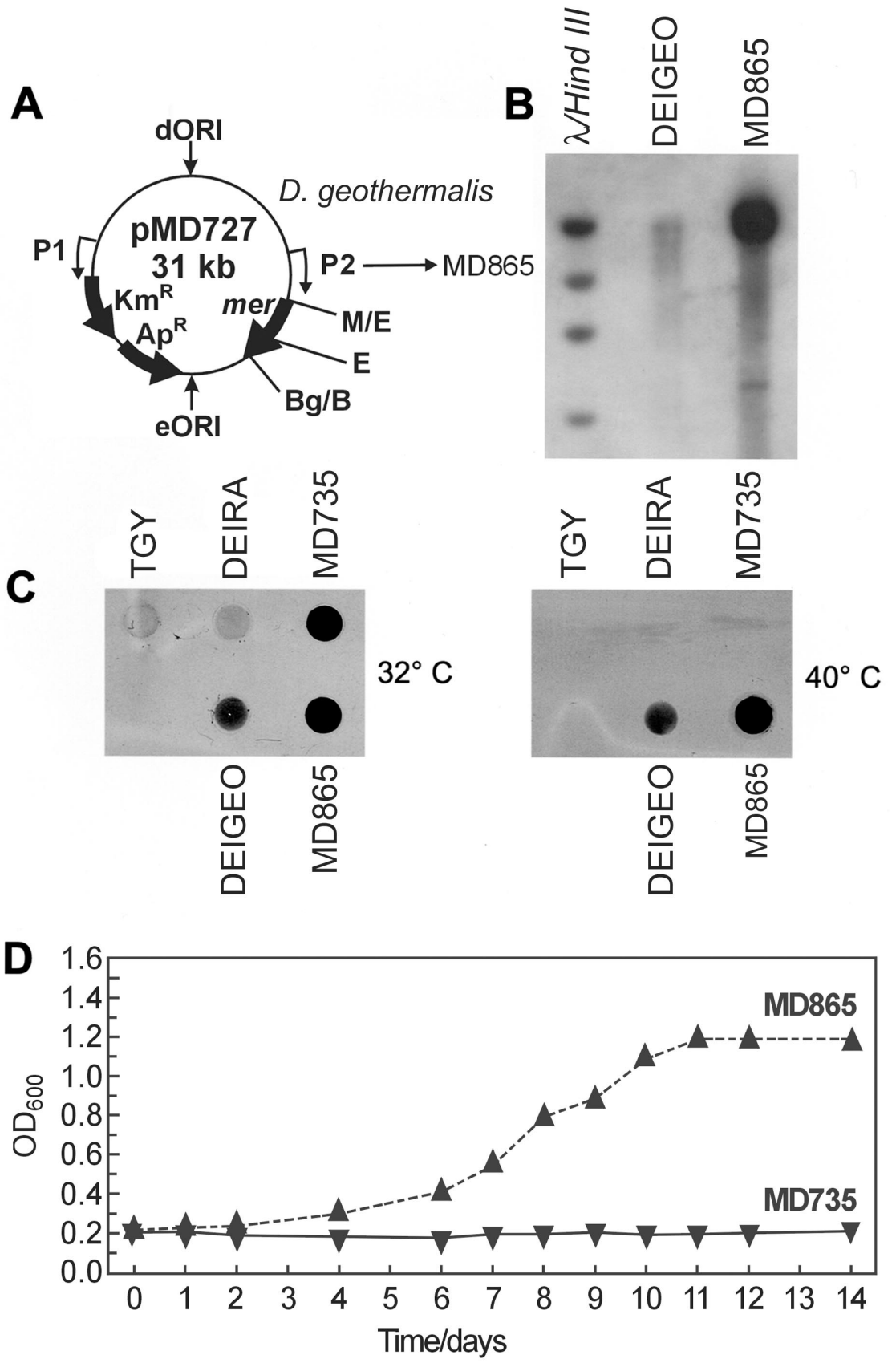
Reduction of metals. *D. geothermalis* reduced Fe(III)-NTA in the presence of lactate at 30°C (data not shown) and in the presence of lactate or pyruvate at 45°C (Fig. 3A). At 40°C *D. geothermalis* rapidly reduced Cr(VI) in TGY cultures under both aerobic and anaerobic conditions (Fig. 3B). AQDS (anthraquinone-2,6-disulfonate) is a quinone-containing organic compound that can be utilized as an electron acceptor for respiration and growth by a variety of dissimilatory metal-reducing bacteria (20). As an electron acceptor, AQDS is reduced to the corresponding dihydroquinone (AH_2DS) (20). Reduction of U(VI) by *D. geothermalis* at 40°C occurred only in the presence of AQDS (Fig. 3C). These results are very similar to the reduction capabilities reported for *D. radiodurans* at lower temperatures (14).

Growth characteristics of *D. geothermalis*. *D. geothermalis* was tested for its amino acid utilization and growth on various Embden-Meyerhof-Parnas substrates. Table 2 shows that, in the absence of irradiation, growth of *D. geothermalis* is independent of any amino acids and the bacterium can utilize ammonium sulfate and grow on tricarboxylic acid cycle intermediates. In the presence of chronic irradiation, growth of *D. geothermalis* is less dependent than that of *D. radiodurans* on Cys and Met, or other exogenously provided amino acids (data not shown). Therefore, the metabolism of *D. geothermalis* appears substantially more robust than that in *D. radiodurans*.

DISCUSSION

D. geothermalis is transformable with autonomous plasmids originally constructed for *D. radiodurans*. Thus, experimental advances in the genetic management of *D. radiodurans* over the last decade (5, 8, 24) could facilitate rapid development of *D. geothermalis* for fundamental and practical objectives. *D. geothermalis* is a thermophile (13) with substrate utilization-growth characteristics that are distinct from those of *D. radiodurans* (Table 2). Under nonirradiating conditions, *D. geothermalis* is not dependent on exogenous amino acids for growth and can utilize ammonium sulfate. These characteristics endow the species with the ability to grow in nutritionally restricted environments that do not support the growth of *D. radiodurans* (32). *D. geothermalis* is also able to grow over a broad temperature range extending to 55°C (13) and displays superior growth in the presence of chronic irradiation (50 Gy/h) in nutritionally restricted medium, compared to *D. radiodurans*. While these characteristics support the idea that *D. geothermalis* may be a more robust candidate than *D. radiodurans* for treatment of radioactive waste environments (32), until now

FIG. 2. Construction and characterization of Hg(II)-resistant-reducing *D. geothermalis*. (A) pMD727 (5) was transformed into *D. geothermalis*, giving strain MD865. (B) Southern blot hybridization of *Eco*RI-digested total DNA from *D. geothermalis* (wild type, *mer* negative) and MD865 (*D. geothermalis*/*mer*$^+$) with a radiolabeled *mer* probe. pMD727 contains a unique *Eco*RI (E) site. Molecular size standards: λ/*Hin*dIII, as in Fig. 1A and B. Wild-type strain abbreviations are as in Fig. 1. (C) Hg(0) volatilization assays at 32 and 40°C for *D. geothermalis*, *D. radiodurans*, MD865 and MD735 (*D. radiodurans*/*mer*$^+$), and TGY (growth medium, no cells). (D) Growth curves for MD865 and MD735 in TGY plus 50 μM merbromin [Hg(II)] at 50°C.


A
dORI
D. geothermalis
pMD727
31 kb
P1
P2
MD865
Km^R
Ap^R
mer
M/E
E
Bg/B
eORI
B
λ/Hind III
DEIGEO
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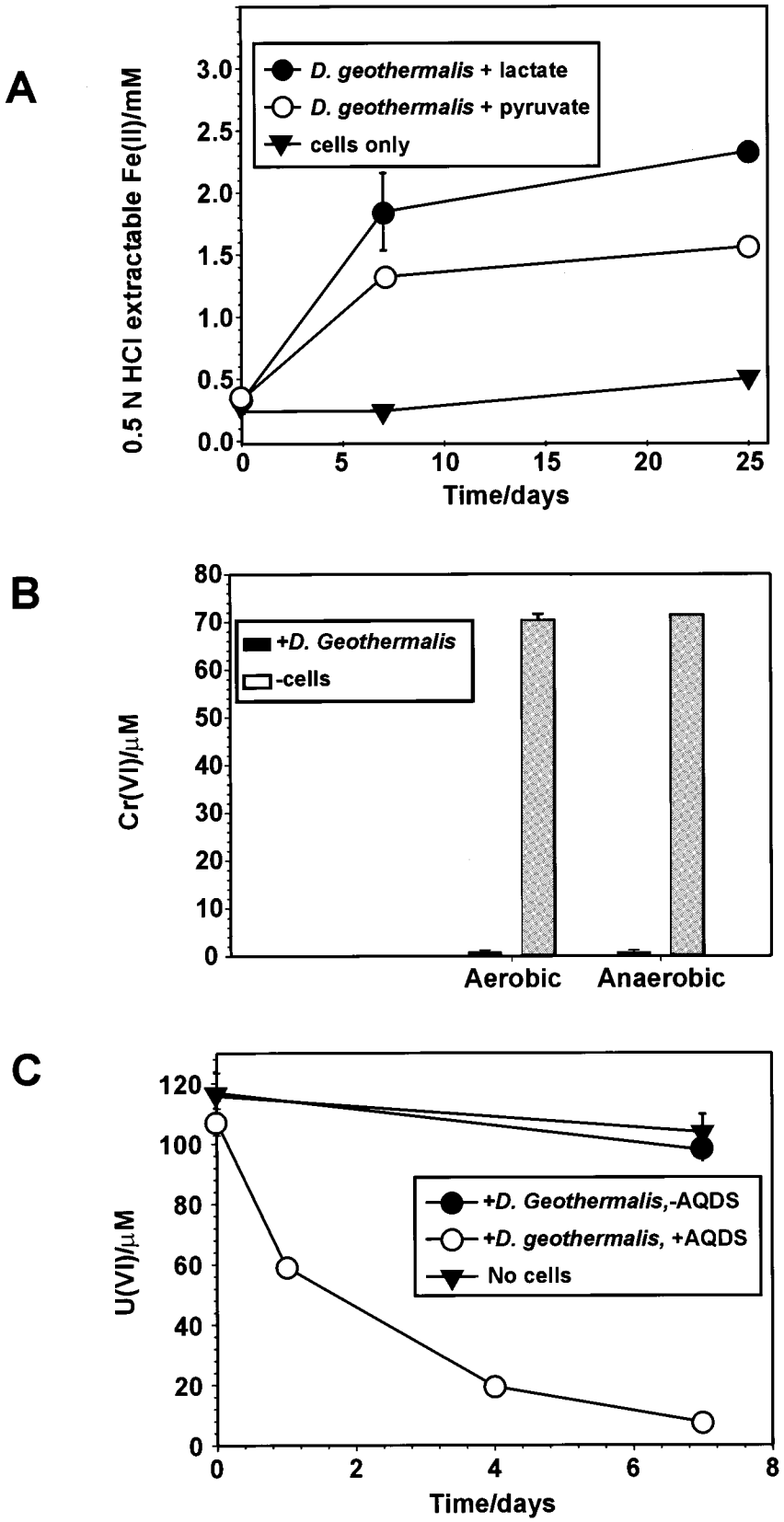

FIG. 3. Metalloreduction by wild-type *D. geothermalis*. (A) Fe(III)-NTA reduction coupled to oxidation of organic substrates at 45°C in the absence of oxygen. (B) Cr(VI) reduction in aerobic or anaerobic conditions as measured by loss of Cr(VI) from solution at 40°C. (C) Reduction of U(VI) in the presence or absence of AQDS as measured by loss of U(VI) at 40°C.

TABLE 2. Growth characteristics of *D. radiodurans* and *D. geothermalis* grown in mm[a]

Substrate[c]	Growth of strain[b]	
	D. radiodurans	*D. geothermalis*
Fructose + C,H,L,A,M,P	+++	+++
Fructose + Met	+++	+++
Fructose −aa $+(NH_4)_2SO_4$	−	+++
Fructose −NAD +Met	−	+++
α-Ketoglutarate	−	++
Succinate	−	++
Fumarate	−	++
Oxaloacetate	+	++
Malate	−	+

[a] Deinococcal cells were grown in deinococcal MM (32) at 32°C. Deinococcal MM contained the indicated Embden-Meyerhof-Parnas substrate (2 mg/ml), NAD (1 μg/ml), methionine (Met) at 50 μg/ml, phosphate buffer (20 mM, pH 7.5), $CaCl_2$ (0.18 mM), and $MgSO_4$ (0.8 mM), and Mn^{2+} (5.4 μM $MnCl_2$) was added as the only transition metal cation.

[b] Growth on substrate: +++, good; ++, moderate; +, poor; −, absent.

[c] Abbreviations: Fructose + C,H,L,A,M,P, fructose plus Cys, His, Lys, Asp, Met, and Pro, each at 50 μg/ml. +Met, only methionine added at 50 μg/ml. −aa, no amino acids added. $+(NH_4)_2SO_4$, ammonium sulfate added to a final concentration of 15 mM. −NAD, no NAD added.

there has been no genetic system available to exploit this species.

Our data show that plasmid-based transformation systems developed for *D. radiodurans* (Fig. 1 and 2) can be used to functionally express cloned genes in *D. geothermalis* at temperatures as high as 50°C (Fig. 2) and in the presence of chronic irradiation. Plasmids introduced into *D. geothermalis* are also efficiently expressed following exposure to high-level acute irradiation (Fig. 1C), without any apparent plasmid loss or mutagenesis. The differential hybridization results with a chromosome- and a plasmid-derived probe in MD865 (*D. geothermalis*/pMD66) (Fig. 1B) support the idea that pMD66 exists in multiple copies in *D. geothermalis*. The survival of *D. geothermalis*/pMD66 plated on TGY-KAN was indistinguishable from that found for wild-type *D. geothermalis* on TGY. As in *D. radiodurans*, this suggests that multiple identical plasmid copies serve as a substrate for efficient repair by homologous recombination (10). Therefore, these studies establish *D. geothermalis* and *D. radiodurans* as the only two extremely radiation-resistant vegetative bacteria that are currently amenable to genetic engineering.

The presence of pMD66 in *D. geothermalis* as a covalently closed circle was confirmed by plasmid rescue in *E. coli* (Table 1) (12), and restriction enzyme mapping and Southern analysis confirmed its predicted structure and stability in *D. geothermalis* (Fig. 1A). When total DNA containing pMD66 was purified from *D. geothermalis* and transformed back into wild-type *D. geothermalis*, there was only a small increase in the number of transformants over that with pMD66 purified from *E. coli*. In contrast, there was a large increase in transformation frequency observed in *D. radiodurans* with pMD66 purified from *D. radiodurans* or *D. geothermalis* over that with pMD66 purified from *E. coli* (Table 1). Therefore, the plasmid transformation capabilities of *D. geothermalis* appear to be significantly less than those of for *D. radiodurans*. While the reasons for this difference are unclear, the fact that pMD66 purified from *D. geothermalis* could be used to transform *D. radiodurans* at high efficiency, but not *D. geothermalis*, suggests that transport of DNA into *D. geothermalis* is inefficient. Wild-type *D. murrayi* is naturally resistant to KAN and, therefore, was not tested for transformability with pMD66/68. However, *D. murrayi* is sensitive to chloramphenicol and could be a suitable host for plasmids encoding Cm^r, but we found it to be nontransformable with high concentrations of pMD300/308 (10) purified from *E. coli* or *D. radiodurans* and did not investigate this species further.

To demonstrate the utility of *D. geothermalis* for bioremediation purposes, we introduced the highly characterized Hg(II) resistance operon (*mer*) of *E. coli* (3) into *D. geothermalis* on an autonomously replicating *D. radiodurans* plasmid (Fig. 2A). Ionic Hg(II) is a prevalent contaminant of radioactive DOE waste sites, where the highest concentration level in contaminated areas has been reported as 10 μM (28). When present in *D. radiodurans*, the *mer* operon confers Hg(II) resistance and endows cells with the ability to reduce highly toxic Hg(II) to much less toxic elemental Hg(0) (5). Similarly, we show that strain MD865 (*D. geothermalis*/*mer*$^+$) is (i) resistant to the bactericidal effects of ionic Hg(II) at concentrations (50 μM; Fig. 2D) well above the highest concentration reported for Hg(II)-contaminated DOE waste sites, (ii) able to reduce toxic Hg(II) to much less toxic elemental and volatile Hg(0) (Fig. 2C), and (iii) able to functionally express the *mer* operon in highly irradiating environments (50 Gy/h) at temperatures as high as 50°C. It is notable that the mesophilic *E. coli* Mer proteins (3) were functional in *D. geothermalis* growing at 50°C. While mechanisms underlying thermophilicity appear to be complex and currently are not well characterized (23), there is some precedent for the interchangeability of genes from mesophiles and thermophiles. For example, the aspartate aminotransferase gene (*aspATSs*) of the hyperthermophile *Sulfolobus solfataricus* has been functionally expressed at mesophilic temperatures in *E. coli* (2). We believe that numerous other metal resistance functions from other bacteria, specific for other metals, could be cloned into *D. geothermalis* by this approach.

It was recently shown that under strict anaerobic conditions *D. radiodurans* can reduce Fe(III)-NTA coupled to the oxidation of lactate to CO_2 and acetate (14). *D. radiodurans* could also reduce U(VI) or Tc(VII) in the presence of AQDS and could directly reduce Cr(VI) in both anaerobic and aerobic conditions (14). The enzymatic reduction of multivalent metals and radionuclides can have a major impact on their solubility and, hence, mobility in the environment. Such changes in solubility make microbial metal reduction a suitable process for immobilizing metals and radionuclides within contaminated environments in situ (8, 25). Localized contaminated sediments and soils at DOE sites can have temperature levels that exceed those that can be tolerated by *D. radiodurans*. We show that the *D. geothermalis* suite of metal-reducing capabilities appears to be very similar to that reported in detail for *D. radiodurans* (14) but functional at higher temperatures (Fig. 3).

We are not aware of expression of any cloned genes in *D. geothermalis* previous to this report. Our demonstration that plasmids developed for *D. radiodurans* are functional in *D. geothermalis* strongly supports the idea that bioremediating gene constructs developed for *D. radiodurans* could be transferred to *D. geothermalis*. This could yield metabolically proficient, extremely radiation-resistant, and thermophilic bacteria suit-

able for the treatment of high-temperature mixed radioactive wastes.

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