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Engineering of *Deinococcus radiodurans* R1 for Bioprecipitation of Uranium from Dilute Nuclear Waste[▽]

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Genetic engineering of radiation-resistant organisms to recover radionuclides/heavy metals from radioactive wastes is an attractive proposition. We have constructed a *Deinococcus radiodurans* strain harboring *phoN*, a gene encoding a nonspecific acid phosphatase, obtained from a local isolate of *Salmonella enterica* serovar Typhi. The recombinant strain expressed an ~27-kDa active PhoN protein and efficiently precipitated over 90% of the uranium from a 0.8 mM uranyl nitrate solution in 6 h. The engineered strain retained uranium bioprecipitation ability even after exposure to 6 kGy of ^{60}Co gamma rays. The PhoN-expressing *D. radiodurans* offers an effective and eco-friendly in situ approach to biorecovery of uranium from dilute nuclear waste.

Nuclear waste contains a variety of heavy metals, radionuclides, organic solvents, and other toxic substances. The management of these wastes is a challenging problem (4, 6, 14). The treatment of environmental metal pollution by microbes or products thereof (bioremediation) generally offers a less expensive, in situ alternative to the commonly used physicochemical strategies (4–6, 14). Among the biotechnological processes, biosorption and bioaccumulation are limited by the availability of metal-interactive sites and metal toxicity, respectively (18). In comparison, bioprecipitation, especially as metal phosphates, offers a promising eco-friendly approach for metal recovery since it can also handle low concentrations of metals not amenable to chemical approaches (16).

Bacteria such as *Citrobacter* sp. and recombinant *Escherichia coli* expressing multiple copies of a nonspecific periplasmic acid phosphatase (PhoN) (10) have been reported to precipitate heavy metals efficiently (1, 19). PhoN hydrolyzes organic phosphates, and the inorganic phosphate, thus released, interacts with the metal and precipitates it on the cell surface as insoluble metal phosphate (12, 19). Other organisms capable of precipitating metals up to nine times their biomass have been known (10). However, the sensitivity of such bacteria to the adverse effects of radiation makes them unsuitable candidates for remediating radioactive waste.

The use of microbes for the treatment of radioactive wastes is determined largely by their ability to survive and catalyze the desired function(s) under high radiation stress (4). The bacteria belonging to the *Deinococcaceae* family have the extraordinary ability to withstand radiation doses of up to 10 to 15 kGy (2, 6, 8). There has been a worldwide research interest to unravel the secret(s) of the unusual radiation resistance of these bacteria (2, 11, 22, 23). Wild-type *Deinococcus* has also been shown to reduce uranium in the presence of humic acids under strict anaerobic conditions (8). In recent years, *Deinococcus radiodurans* R1 has been engineered and evaluated for its ability to bioremediate mixed radioactive wastes and especially to detoxify the mercury and toluene present in such wastes (4, 6, 14).

In this communication, we report on the cloning of a nonspecific acid phosphatase encoding the *phoN* gene obtained from a local isolate of *Salmonella enterica* serovar Typhi and its expression in *Deinococcus radiodurans* strain R1. The recombinant *Deinococcus* clone exhibited high uranium precipitation ability even after exposure to 6 kGy of ^{60}Co gamma irradiation.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *Deinococcus radiodurans* strain R1 was grown aerobically in TGY (1% Bacto Tryptone, 0.1% glucose, and 0.5% yeast extract) liquid medium at 32°C ± 1°C under agitation (180 ± 5 rpm). *E. coli* DH5α cells were grown in Luria-Bertani (LB) medium at 37°C ± 1°C under agitation (180 ± 5 rpm). Bacterial growth was assessed by measuring turbidity (optical density at 600 nm [OD_{600}]) or by determining the number of CFU on TGY agar plates (1.5% Bacto Agar) after 48 h of incubation at 32°C ± 1°C in the case of *D. radiodurans* and on LB after 24 h of incubation at 37°C ± 1°C in the case of *E. coli*.

The antibiotic concentration used for the selection of transformants was 3 μg ml^{-1} of chloramphenicol for *Deinococcus* and 100 μg ml^{-1} of ampicillin for *E. coli* (21). For screening of recombinant PhoN-expressing clones, LB-TGY containing phenolphthalein diphosphate (PDP) (1 mg ml^{-1}) and methyl green (MG) (50 μg ml^{-1}) was used in the case of *E. coli*. For *Deinococcus phoN* transformants, the MG concentration was reduced to 5 μg ml^{-1}, since higher concentrations were found to be toxic.

PCR amplification, cloning, and transformation. The various plasmids used in this study are listed in Table 1. Plasmid DNA was isolated from *Deinococcus* and *E. coli* cells as described earlier (7, 27). The primers for PCR amplification were designed on the basis of the published sequence of the *phoN* gene of *Salmonella enterica* serovar Typhimurium (GenBank accession no. X59036) and the *D. radiodurans groESL* promoter (DR0606) in their respective genomes. The primers used for cloning and DNA sequencing were obtained from Bangalore Genei Pvt. Ltd., Bangalore, India, and are listed in Table 1. PCR products were purified using an AuPrep PCR purification kit, Life Technologies Pvt. Ltd., India.

The shuttle vector pRAD1 (21) was used for cloning of *Salmonella enterica* serovar Typhi *phoN* in *Deinococcus*. The *phoN* ORF was PCR amplified, using primers DAP-f and T3 (Table 1), from an existing plasmid (pASR1) in our laboratory (Table 1) that contains *S. enterica* serovar Typhi *phoN* with its native promoter cloned in the pBluescript SK(+) vector. The purified PCR-amplified DNA fragment was purified, digested, and ligated to the NruI-BamHI site of pRAD1. Upstream of the *phoN* ORF, the deinococcal *groESL* promoter, amplified using primers DAG-f and DAG-r (Table 1), was ligated at the XbAI/NdeI site, yielding plasmid pPN1. The recombinant plasmid was then used to transform competent *E. coli* DH5α cells, which were plated on LB agar containing

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TABLE 1. Bacterial strains, plasmids, and primers used in this study

Strain, plasmid, or primer	Description or sequence (5′ to 3′)	Source or reference
Strains		
E. coli DH5α	F^- *recA41 endA1 gyrA96 thi-1 hsdR17* (r_k^- m_k^+) *supE44 relA*λ *lacU169*	Lab collection
D. radiodurans R1	Wild-type strain	15
Plasmids		
pASR1	pBluescript SK(+) containing *S. enterica* serovar Typhi *phoN* gene with its native promoter	Unpublished work from this lab
pRAD1	*E. coli-D. radiodurans* shuttle vector; Ap^r Cm^r; 6.28 kb	21
pPN1	pRAD1 containing *S. enterica* serovar Typhi *phoN* gene with deinococcal *groESL* promoter	This study
Primers		
DAG-f	5′-GCCTCTAGACATGTTCAG-3′	This study
DAG-r	5′-GGTTTCAGCATATGGGGT-3′	This study
DAP-f	5′-GGTGAGTCCATATGAAAAG-3′	
T3	5′-AATTAACCCTCACTAAAGGG-3′	
P5	5′-GGAGCGGATAACAATTTCACACA-3′	This study
P6	5′-AACGCGGCTGCAAGAATGGTA-3′	This study

ampicillin (100 μg ml^{-1}). The transformants were screened for PhoN expression on LB agar containing PDP and MG. On these plates, positive clones turn distinctly greenish due to the deposition of methyl green on the colonies as the medium turns acidic on cleavage of phenolphthalein diphosphate by PhoN-positive clones. Plasmids were isolated from a few of the positive clones and used to transform competent *D. radiodurans* R1 cells as described earlier (15). The transformants were plated on TGY containing chloramphenicol (3 μg ml^{-1}). *Deinococcus* colonies expressing PhoN were subsequently screened on TGY agar containing PDP and MG.

DNA sequencing. DNA sequencing was carried out using a standard dideoxy DNA sequencing protocol (28). Sequencing grade primers P5 and P6 (Table 1) bound upstream and downstream of the multiple cloning sites in pRAD1, thus amplifying the insert (1,248 bp) present in these sites. Computational analyses of DNA were performed using Internet-based programs. Similarity searches were carried out using the BLAST algorithms available at http://www.ncbi.nlm.nih.gov/BLAST/. Multiple alignments comparing the sequences were performed using ClustalW (http://www.ebi.ac.uk/clustalw/). *D. radiodurans* genomic sequence data were obtained from the completed and annotated genome at The Institute for Genomic Research (http://www.tigr.org). Sequences from other bacteria were obtained from GenBank (http://www.ncbi.nlm.nih.gov).

Acid phosphatase expression: zymogram and activity assays. *Salmonella enterica* serovar Typhi, *E. coli*, and *Deinococcus* cultures were harvested in the early stationary phase, washed twice, and resuspended in chilled distilled water. To this, equal volumes of 2× nonreducing cracking buffer (13) were added, and samples were warmed to 50°C for 15 min. The suspension was clarified by centrifugation at 12, 000 × *g* for 10 min, and the protein concentration in the supernatant was estimated by a modified micro Lowry method (24). Equal amounts of protein (5 μg) were electrophoretically resolved by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis at 75 V for 30 min, followed by 100 V for 90 min. In the case of *Salmonella enterica* serovar Typhi, 5 to 50 μg protein was loaded. The gel was rinsed briefly with water to remove surface sodium dodecyl sulfate and renatured using 1% Triton X-100 in 100 mM acetate buffer of pH 5.0 (two washes of 20 min each), followed by a wash with 100 mM acetate buffer without Triton. The gel was developed using nitroblue tetrazolium (NBT)–5-bromo-4-chloro-3-indolylphosphate (BCIP) mix in 100 mM acetate buffer at pH 5.0. The assay was terminated by rinsing the gel in distilled water.

Acid phosphatase activity was estimated by the liberation of *p*-nitrophenol from disodium *p*-nitrophenyl phosphate (*p*NPP) as described earlier (3). PhoN activity of whole cells is reported as nmol of *p*-nitrophenol (*p*NP) liberated min^{-1} mg^{-1} bacterial protein. Protein concentration was determined by Lowry's method (17) using a protein estimation kit (Bangalore Genei Pvt. Ltd., India).

Radiation response of transformants. Early-stationary-phase cultures of both *Deinococcus* and *E. coli* clones were washed twice and resuspended in fresh TGY and LB broth, respectively, at an OD_{600} of 3.0. The cultures were exposed to 6 to 21 kGy of ^{60}Co gamma rays at a dose rate of 5 kGy h^{-1} (^{60}Co Gamma Cell 5000 irradiation unit; HIRUP, Bhabha Atomic Research Centre, Mumbai, India). An aliquot of the culture kept outside the radiation source served as the sham control. The irradiated and control cells were washed, serially diluted, plated in triplicate on TGY plates containing chloramphenicol in the case of *Deinococcus* clones and on LB containing ampicillin in the case of *E. coli* clones, and incubated under optimum growth conditions. Colonies were counted, and the survival curve was plotted. Irradiated cells were also assayed for their PhoN activity as described earlier.

Uranium precipitation assays. Uranium precipitation assays were performed as previously described (20) with certain modifications. Overnight grown cultures (OD_{600} of 0.3) of *Deinococcus* and *E. coli* clones were independently incubated with 0.8 mM uranyl nitrate in 2 mM acetate buffer (pH 5.0), supplemented with 5 mM β-glycerophosphate at 25°C ± 2°C under static conditions. Aliquots were taken at different time intervals and subjected to centrifugation at 12,000 × *g* for 10 min. Residual uranium in the supernatant and uranium associated with the cell pellet were estimated using Arsenazo III by a modification of the method of Fritz and Bradford (9). The metal in the pellet fraction was estimated after the pellet was digested with concentrated HCl. A 0.1% solution of Arsenazo III was prepared by dissolution of 0.2 g of Arsenazo III in 180 ml of 0.01 N HCl and 20 ml of absolute ethanol (30 min of stirring) and filtration through Whatman No. 1 filter paper. Twenty microliters of the spent supernatant was diluted to a total volume of 600 μl. The samples were acidified (200 μl of 0.01 N HCl), followed by the addition of 200 μl of Arsenazo III. The resultant purple-colored metal-Arsenazo III complex was estimated spectrophotometrically at 655 nm using uranyl nitrate hexahydrate (Merck) as the standard. Metal precipitation was expressed as the percentage of the initial metal present in the supernatant.

For comparison of uranium precipitation by *Deinococcus* and *E. coli* cells harboring cloned *phoN*, cells containing equivalent PhoN activities (as measured by a *p*NPP assay and corresponding to OD_{600} values of 0.3 for *E. coli* and 1.5 for *D. radiodurans*) were incubated with 0.8 mM uranyl nitrate. In another experiment, *Deinococcus* and *E. coli* cells harboring *phoN* were irradiated at 1 and 6 kGy and the irradiated cells were then assessed for their uranium precipitation ability.

RESULTS

Cloning of *Salmonella enterica* serovar Typhi *phoN* in *D. radiodurans* R1. The *phoN* gene was PCR amplified from a previously cloned *phoN* gene of *Salmonella enterica* serovar Typhi (plasmid pASR1) already available in our laboratory (Table 1). The 0.887-kb PCR product was digested with NdeI and BamHI. The resultant 0.815-kb digested product was ligated to the NruI-BamHI site of the *E. coli-Deinococcus* shuttle vector pRAD1 (21). Upstream of the *phoN* ORF, the 0.256-kb deinococcal *groESL* promoter was ligated at the XbaI-NdeI site to obtain pPN1 (Table 1). The *E. coli* trans-

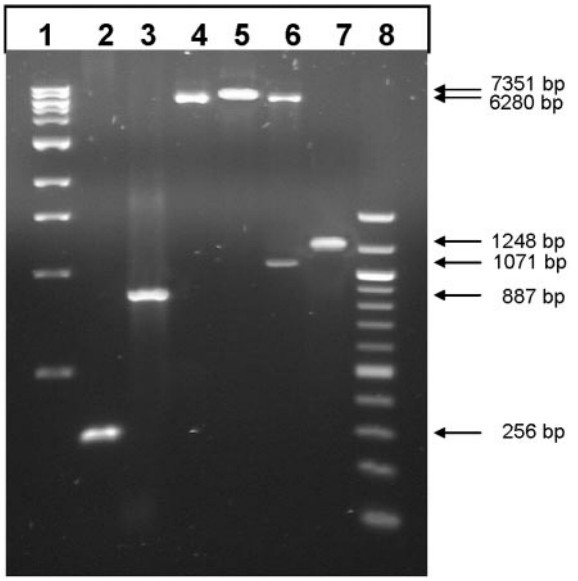

FIG. 1. DNA fragments were electrophoretically resolved on a 1% agarose gel at 8 V cm^{-1} in 0.5× Tris-borate-EDTA. The different lanes contained a 1-kb ladder (lane 1), *groESL* PCR product amplified using primers DAG-f and DAG-r (lane 2), *phoN* PCR product amplified using primers DAP-f and T3 (lane 3), pRAD1 digested with NdeI (lane 4), pPN1 digested with NdeI (lane 5), pPN1 digested with XbaI and BamHI (lane 6), PCR-amplified (using primers P5 and P6) cloned insert from pPN1 (lane 7), and a 100-bp ladder (lane 8).

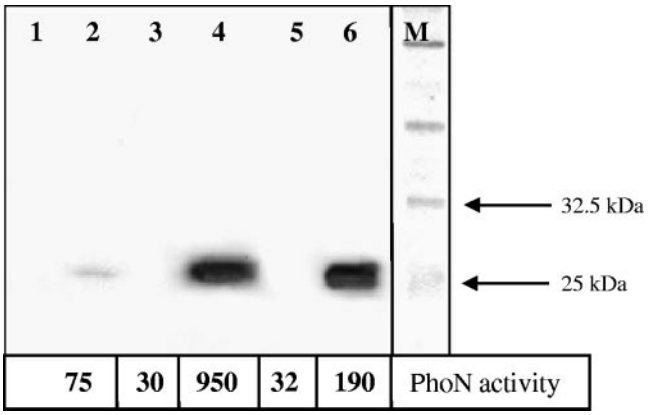

FIG. 2. Zymogram showing relative in-gel PhoN activities. The different lanes contained protein extracts from 5 μg *Salmonella enterica* serovar Typhi (lane 1), 50 μg *Salmonella enterica* serovar Typhi (lane 2), and 5 μg each of *E. coli* pRAD1 (lane 3), *E. coli* pPN1 (lane 4), *D. radiodurans* pRAD1 (lane 5), and *D. radiodurans* pPN1 (lane 6). Protein extracts were coelectrophoresed with prestained molecular weight standards (lane M). The values below each lane show the whole-cell PhoN activities of various clones expressed as nmol of *p*NP liberated min^{-1} mg^{-1} protein.

formants carrying plasmid pPN1 were first selected on ampicillin and then on plates containing a modified version of a histochemical medium, described previously (26), for selecting phosphatase-positive clones. A few phosphatase-positive *E. coli* colonies were grown, plasmids were isolated, and the inserts therein were confirmed by PCR amplification. A typical result is shown in Fig. 1. This plasmid was then used to transform *D. radiodurans* R1 and transformants screened on the modified histochemical plate containing chloramphenicol (3 μg μl^{-1}). DNA inserts (1,244 bp) from four *phoN*-positive *Deinococcus* clones were sequenced using pRAD1 primers P5 and P6 (Table 1), which, bind upstream and downstream, respectively, of the multiple cloning sites, and revealed no changes.

In vitro and in vivo acid phosphatase activity of *E. coli* and *Deinococcus* clones harboring PhoN. Cell extracts of both *E. coli* and *Deinococcus* showed distinct PhoN phosphatase activities, visualized in the zymograms as an ~27-kDa activity band (Fig. 2). This indicated good expression of an active enzyme from the deinococcal *groESL* promoter. The cell-based activity assays, performed using *p*NPP as the substrate, showed much less activity in the case of *Deinococcus* clones than in the case of *E. coli* clones, though nearly equal activities were seen in the zymogram (Fig. 2). On an equal protein amount basis (5 μg), protein extract from the parent *Salmonella enterica* serovar Typhi did not show detectable activity in the zymogram. However, when 10 times more protein was loaded, a low intensity band matching the expected molecular weight of PhoN (Fig. 2) was observed. This can be attributed to a single copy of the *phoN* gene in the *Salmonella* genome. Figure 2 clearly shows significant overexpression of PhoN in the *E. coli* and *Deinococcus* clones.

Radiation response of *Deinococcus* transformants carrying the *phoN* gene. The radiation resistances of different *Deinococcus* clones were determined by evaluating their D_{10} values (dose causing 90% lethality or allowing 10% survival). The D_{10} value for *E. coli* clones was ~200 Gy. In comparison, *Deinococcus* clones harboring the empty vector pRAD1 and *Deinococcus phoN* clones showed D_{10} values of 18.0 and 17.4 kGy, respectively (Fig. 3A), indicating that the cloned *S. enterica* serovar Typhi *phoN* gene did not affect the radioresistance of the host cell. There was also no change in the whole-cell PhoN activity of *D. radiodurans* clones after up to 12 kGy of exposure. However, beyond an 18-kGy dose a decrease of 20% was seen (Fig. 3B).

Uranium precipitation by transformed *E. coli* and *Deinococcus* cells. *E. coli* as well as *Deinococcus* clones harboring *phoN* precipitated uranium from uranyl nitrate solutions. On an equal cell number basis, *Deinococcus* clones were slow (~70% precipitation in 26 h) compared to *E. coli* clones (~70% precipitation in 2 h) (Fig. 4A). However, when cells carrying equal PhoN activities were used, the time courses of uranium precipitation were nearly identical and both *E. coli* and *D. radiodurans* clones could precipitate >90% uranium in approximately 6 h (Fig. 4B). Metal loss from the supernatant was shown to be in conformity with the precipitated metal in the pellet fraction (Fig. 5) in both *E. coli* and *D. radiodurans* clones.

Postirradiation uranium precipitation activities. *E. coli* and *Deinococcus* clones with equivalent PhoN activities were subjected to different doses of ionizing ^{60}Co gamma radiation (1 to 6 kGy) and immediately allowed to precipitate uranium from dilute (<1 mM) uranyl nitrate solution. Under these conditions, *Deinococcus phoN* clones could precipitate uranium much more efficiently than the corresponding *E. coli phoN* clones. *Deinococcus phoN* clones retained nearly 90% of their uranium-precipitating ability after up to 6 kGy of irradiation,

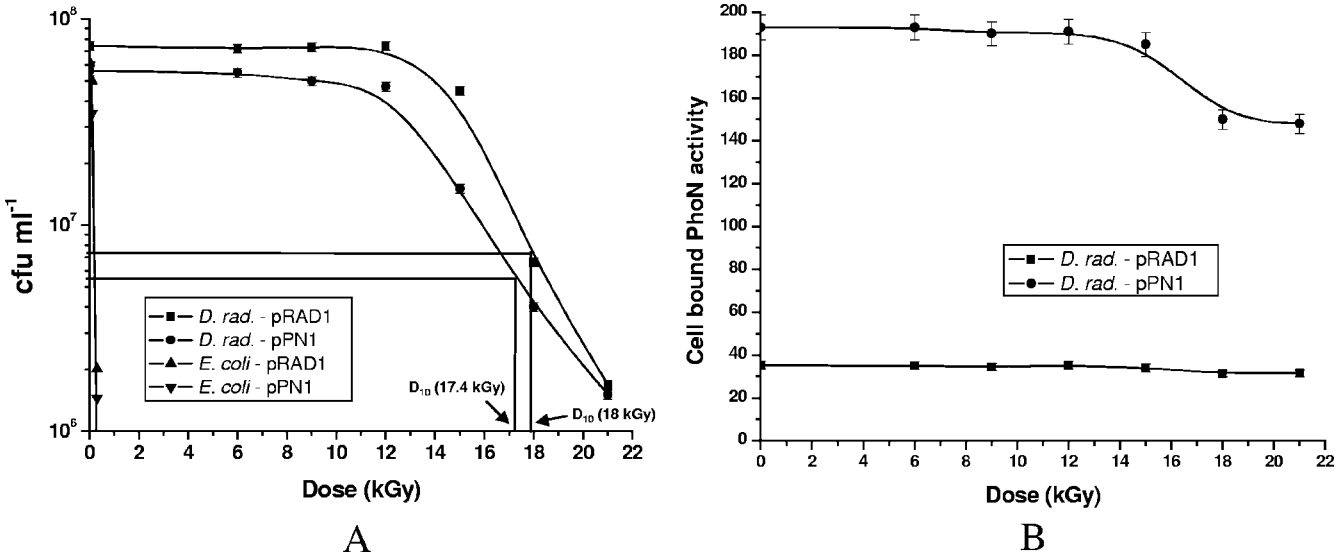

FIG. 3. Radioresistance of *phoN* transformants. (A) Stationary-phase cultures adjusted to an OD_{600} of 3.0 were exposed to different doses of ^{60}Co gamma radiation. Survival was measured in terms of the number of CFU ml^{-1}. (B) Postirradiation PhoN activity of *D. radiodurans* (*D. rad.*) clones expressed as nmol of *p*NP liberated min^{-1} mg^{-1} protein.

whereas *E. coli* clones showed limitations even at 1 kGy and were severely inhibited at higher doses (Fig. 6).

DISCUSSION

Radioactive waste is an inevitable problem arising from the use of radioactive materials in the nuclear industry. Based on its radioactive properties, nuclear waste is classified as low-level waste, intermediate-level waste, or high-level waste (25). The problems associated with low-level and intermediate-level wastes are due largely to their bulk volume. The recovery of desirable metals from such solutions poses a major challenge. The use of radioresistant microbes to precipitate metals in a radioactive environment offers a very feasible and, therefore, attractive approach in such a context.

Deinococcus radiodurans can survive extremely high doses of ionizing radiations (2). Attempts to genetically engineer this bacterium for bioremediation of radioactive wastes have met with success. Recombinant *Deinococcus* strains capable of degrading toluene and detoxifying mercury from aqueous solutions in radioactive environments have already been reported (4, 14). The present work has successfully demonstrated the construction of a recombinant *Deinococcus radiodurans* strain capable of bioprecipitating uranium from aqueous solutions containing less than 1 mM uranium, subsequent to exposure to a very high dose of ^{60}Co gamma rays.

The engineered *E. coli* and *D. radiodurans* strains transformed with the *phoN* gene exhibited the desired gain of function and expressed an ~27-kDa PhoN protein with high acid

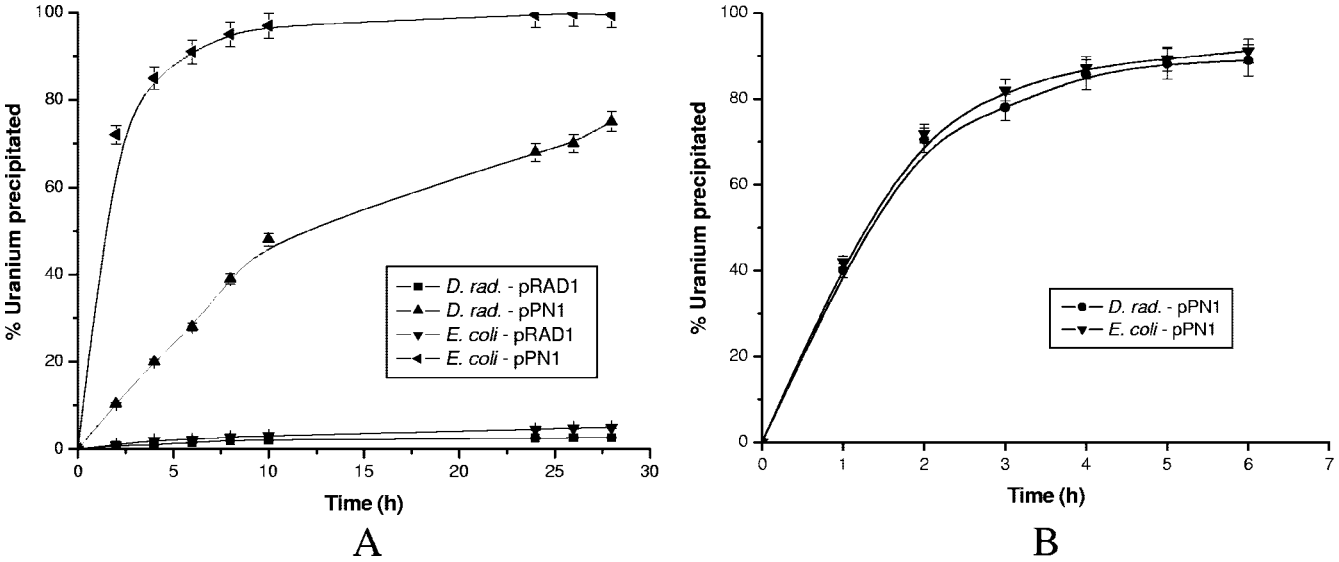

FIG. 4. Comparison of uranium precipitation by *E. coli* and *Deinococcus* (*D. rad.*) *phoN* transformants. (A) Comparable cell numbers (OD_{600} of 0.3). (B) Cells with equivalent PhoN activities.

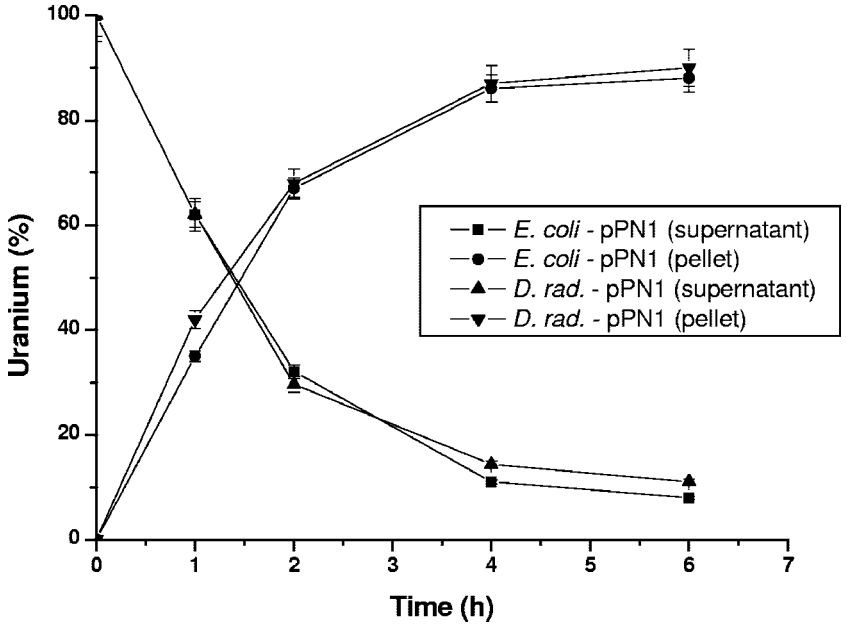

FIG. 5. Uranium bioprecipitation by *E. coli* and *Deinococcus* (*D. rad.*) *phoN* clones. The percent loss of uranium from the supernatant and the corresponding gain in pellet are shown.

phosphatase activity (Fig. 2). While the in-gel activities visualized in the zymogram were comparable in the two bacteria, the whole-cell-based in vivo PhoN activity was severalfold less in *D. radiodurans* than in *E. coli* transformants (Fig. 2). The reason(s) for this is not clear but may have to do with the multilayered cell envelope that the gram-positive *D. radiodurans* possesses, which may reduce accessibility of the substrate to the periplasmic PhoN compared to the enzyme in *E. coli*. Alternatively, the *Salmonella* signal peptide may interfere with optimal localization of PhoN in deinococcal membranes.

Notwithstanding this difference, *E. coli* and *D. radiodurans* recombinant clones containing equivalent PhoN activities exhibited efficient and equal uranium precipitation capabilities (Fig. 4B). Both clones removed more than 90% of the uranium from solutions containing 0.8 mM uranyl nitrate in about 6 h. This is much superior to the uranium precipitation ability reported for native *Citrobacter* strains (strain N14, PhoN activity of 400 to 500 U and 37 to 61% uranium precipitation in 6 h; strain dc5c, PhoN activity of 850 U and 58% uranium precipitation in 6 h) under similar experimental conditions (10, 20) or

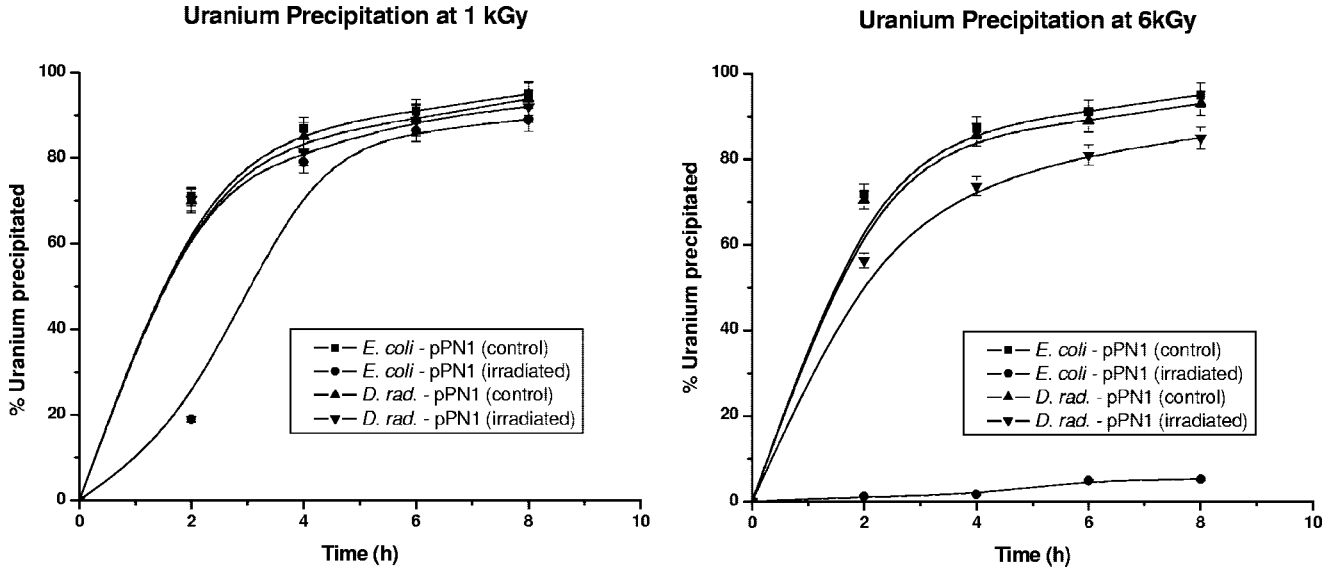

FIG. 6. Postirradiation uranium precipitation ability of *E. coli* and *Deinococcus* (*D. rad*) clones harboring *phoN*. *E. coli phoN* and *Deinococcus phoN* cultures with equivalent PhoN activities (as determined by the *p*-NPP method) were used.

for an engineered and immobilized *E. coli* strain expressing PhoN (PhoN activity of 100 to 150 U and 98% uranium precipitation in 400 h) (1).

While *E. coli* cells (both wild type and transformants) showed considerable radiosensitivity, the transfer of *phoN* to *Deinococcus radiodurans* did not compromise its radioresistance (Fig. 3A). The recombinant *D. radiodurans* clones retained their uranium precipitation ability even after 6 kGy of ^{60}Co gamma irradiation (Fig. 6). In contrast, *E. coli* clones could not function optimally even at low doses (1 kGy) and showed severe inhibition of PhoN activity at higher doses (3 to 6 kGy).

Exploiting recombinant *Deinococcus phoN* transformants for bioprecipitation of uranium thus seems feasible. The inherent radioresistance of this microbe facilitates the use of an engineered strain for sustained in situ bioremediation of radioactive waste and allows uranium recovery from low concentrations and high-radiation environments typical of dilute nuclear waste. It is worth mentioning that once the recombinant cells are loaded with sufficient uranyl phosphate, they sink to the bottom of the container and can be collected with ease to recover uranium from loaded cells.

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REFERENCES

1. **Basnakova, G., E. R. Stephens, M. C. Thaller, G. M. Rossolini, and L. E. Macaskie.** 1998. The use of *Escherichia coli* bearing a *phoN* gene for the removal of uranium and nickel from aqueous flows. Appl. Microbiol. Biotechnol. **50:**266–272.
2. **Battista, J. R.** 1997. Against all odds: the survival strategies of *Deinococcus radiodurans*. Annu. Rev. Microbiol. **51:**203–224.
3. **Bolton, P. G., and A. C. R. Dean.** 1972. Phosphatase synthesis in *Klebsiella* (*Aerobacter*) *aerogenes* growing in continuous cultures. J. Biochem. **127:**87–96.
4. **Brim, H., S. C. McFarlan, J. K. Fredrickson, K. W. Minton, M. Zhai, L. P. Wackett, and M. J. Daly.** 2000. Engineering *Deinococcus radiodurans* for metal remediation in radioactive mixed waste environments. Nat. Biotechnol. **18:**85–90.
5. **Brim, H., A. Venkateshwaran, H. M. Kostandarithes, J. K. Fredrickson, and M. J. Daly.** 2003. Engineering *Deinococcus geothermalis* for bioremediation of high-temperature radioactive waste environments. Appl. Environ. Microbiol. **69:**4575–4582.
6. **Daly, M. J.** 2000. Engineering radiation-resistant bacteria for environmental biotechnology. Curr. Opin. Biotechnol. **11:**280–285.
7. **Daly, M. J., L. Ouyang, P. Fuchs, and K. W. Minton.** 1994. In vivo damage and RecA-dependent repair of plasmid and chromosomal DNA in the radiation-resistant bacterium *Deinococcus radiodurans*. J. Bacteriol. **176:** 3508–3517.
8. **Fredrickson, J. K., H. M. Kostandarithes, S. W. Li, A. E. Plymale, and M. J. Daly.** 2000. Reduction of Fe(III), Cr(VI), U(VI) and Tc(VII) by *Deinococcus radiodurans* R1. Appl. Environ. Microbiol. **66:**2006–2011.
9. **Fritz, J. S., and E. C. Bradford.** 1958. Detection of thorium and uranium. Anal. Chem. **30:**1021–1022.
10. **Jeong, B. C., C. Hawes, K. M. Bonthrone, and L. E. Macaskie.** 1997. Localization of enzymically heavy metal accumulation by *Citrobacter* sp. and metal accumulation in vitro by liposomes containing entrapped enzyme. Microbiology **143:**2497–2507.
11. **Joshi, B., R. Schmid, K. Altendorf, and S. K. Apte.** 2004. Protein recycling is a major component in *Deinococcus radiodurans* strain R1. Biochem. Biophys. Res. Commun. **320:**1112–1117.
12. **Kier, L. D., R. M. Weppelman, and B. N. Ames.** 1977. Resolution and purification of three periplasmic phosphatases of *Salmonella typhimurium*. J. Bacteriol. **130:**399–410.
13. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685.
14. **Lange, C. C., L. P. Wackett, K. W. Minton, and M. J. Daly.** 1998. Engineering a recombinant *Deinococcus radiodurans* for organopollutant degradation in radioactive mixed waste environments. Nat. Biotechnol. **16:**929–933.
15. **Lennon, E., and K. W. Minton.** 1990. Gene fusions with *lacZ* by duplication insertion in the radioresistant bacterium *Deinococcus radiodurans*. J. Bacteriol. **172:**2955–2961.
16. **Lokhande, M., P. V. Achuthan, U. Jambunathan, A. Ramanujam, A. S. Rao, and S. K. Mahajan.** 2001. Studies on the recovery of uranium using enzyme mediated and chemical methods, p. 224–225. *In* Proceedings of the Nuclear and Radiochemistry Symposium. Department of Chemistry, University of Pune, Pune, India.
17. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
18. **Macaskie, L. E.** 1990. An immobilised cell bioprocess for the removal of heavy metals from aqueous flows. J. Chem. Tech. Biotechnol. **49:**357–379.
19. **Macaskie, L. E., K. M. Bonthrone, and D. A. Rouch.** 1994. Phosphatase-mediated heavy metal accumulation by a *Citrobacter* sp. and related enterobacteria. FEMS Microbiol. Lett. **121:**141–146.
20. **Macaskie, L. E., K. M. Bonthrone, P. Yong, and D. T. Goddard.** 2000. Enzymically mediated bioprecipitation of uranium by a *Citrobacter* sp.: a concerted role for exocellular lipopolysaccharide and associated phosphatase in biomineral formation. Microbiology **146:**1855–1867.
21. **Meima, R., and M. E. Lidstrom.** 2000. Characterization of the minimal replicon of a cryptic *Deinococcus radiodurans* SARK plasmid and development of versatile *Escherichia coli-D. radiodurans* shuttle vectors. Appl. Environ. Microbiol. **66:**3856–3867.
22. **Misra, H. S., N. P. Khairnar, A. Barik, I. K. Priyadarsini, H. Mohan, and S. K. Apte.** 2004. Pyrroloquinoline-quinone: a reactive oxygen species scavenger in bacteria. FEBS Lett. **578:**26–30.
23. **Misra, H. S., N. P. Khairnar, S. Kota, S. Shrivastava, V. P. Joshi, and S. K. Apte.** 2006. An exonuclease I-sensitive DNA repair pathway in *Deinococcus radiodurans*: a major determinant of radiation resistance. Mol. Microbiol. **59:**1308–1316.
24. **Peterson, G. L.** 1977. A simplification of the protein assay method of Lowry et al., which is more generally applicable. Anal. Biochem. **83:**346–356.
25. **Ramana, M. V., D. G. Thomas, and S. Varughese.** 2001. Estimating nuclear waste production in India. Curr. Sci. **81:**1458–1462.
26. **Riccio, M. L., G. M. Rossolini, G. Lombardi, A. Chiesurin, and G. Satta.** 1997. Expression cloning of different bacterial phosphatase-encoding genes by histochemical screening of genomic libraries onto an indicator medium containing phenolphthalein diphosphate and methyl green. J. Appl. Microbiol. **82:**177–185.
27. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
28. **Sanger, F., S. Nickien, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.

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ERRATUM

Engineering of *Deinococcus radiodurans* R1 for Bioprecipitation of Uranium from Dilute Nuclear Waste

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Volume 72, no. 12, p. 7873–7878, 2006. Page 7873, column 2, last paragraph, line 6, and page 7874, column 2, last paragraph, line 7: "NruI" should read "NdeI."