SUPPORTING INFORMATION

Evaluating the Effects of Bioremediation on Genotoxicity of Polycyclic Aromatic Hydrocarbon-Contaminated Soil Using Genetically Engineered, Higher Eukaryotic cell Lines

Jing Hu, Jun Nakamura[*]*, Stephen D. Richardson*[1]*, and Michael D. Aitken*[*]

Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC 27599-7431, U.S.A.

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[*] Corresponding authors:
Jun Nakamura (T: 1-919-966-6140; F: 1-919-966-6123; Email: ynakamur@email.unc.edu);
Michael D. Aitken (T: 1-919-966-1024; F: 1-919-966-7911; Email: mike_aitken@unc.edu).
[1] Current address: Solutions-IES, Raleigh, NC

Table S1 Concentrations of individual PAHs in the soil before and after two bioremediation processes (ng/mg dry soil) (n = 3).

Compound	BFS[a]	BTS[a] (at Day 7)	CPS[a]	CTR[a] (at Port A)	BIO[a] (at Port A)
NAP	22.3±2.5	16.5±0.1	12.2±0.2	6.4±0.1	10.4±0.9
ACE	22.2±2.2	1.8±0.2	11.3±2.5	2.5±0.4	2.8±0.4
FLU	15.2±1.7	2.5±0.2	6.3±1.6	2.1±0.7	1.7±0.2
PHN	226±17	50.1±14.4	129±45	41.7±5.8	27.2±0.3
ANT	9.1±1.0	2.0±0.3	11.9±1.2	4.3±1.2	2.3±0.5
FLA	55.8±6.9	11.5±1.9	42.9±0.5	17.6±3.8	9.1±2.3
PYR	80.9±5.2	25.4±4.6	63.4±7.1	24.7±5.9	17.1±3.8
BaA	36.4±4.3	12.1±0.3	18.6±2.8	12.4±4.2	5.8±1.3
CHR	34.6±3.8	17.8±2.3	27.4±2.8	18.2±3.9	7.2±0.2
BbF	13.4±0.7	8.3±0.8	11.8±0.3	7.4±2.4	4.8±0.8
BkF	10.8±1.4	6.8±0.8	8.7±1.1	5.4±1.4	3.2±0.6
BaP	13.7±1.3	8.4±1.5	13.8±1.6	11.4±3.2	7.2±1.6
DBA	1.9±0.1	1.3±0.1	0.78±0.02	0±0	0±0
BgP	23.1±2.8	13.5±0.9	10.6±0.8	7.2±2.9	6.4±1.4
Total PAHs	566±50	178±20	369±54	161±38	105±12

[a] BFS: untreated bioreactor feed soil; BTS: bioreactor treated soil; CPS: untreated column packing soil; CTR: control-column treated soil; BIO: biostimulated-column treated soil.

Table S2 Table of LD_{50} for BPDE, MMS and H_2O_2 as positive control (μg/L) (n = 3).

	LD_{50}(DT40)	LD_{50}(*Rad54*$^{-/-}$)
BPDE	49.6±8.5	27.0±2.7
MMS	$7.1\times10^3\pm1.5\times10^3$	$1.7\times10^3\pm1.7\times10^3$
H_2O_2	61.2±8.5	34.7±3.4

Table S3 Partial correlation coefficients and corresponding *p*-values among LD_{50}, $1/C_{tPAHs}$ and $1/C_{residue}$.

	LD_{50}(DT40)	LD_{50}(*Rad54*$^{-/-}$)
$1/C_{tPAHs}$ (Control Variable: $1/C_{residue}$)	0.464 (p=0.08)	0.482 (p=0.07)
$1/C_{residue}$ (Control Variable: $1/C_{tPAHs}$)	0.789[a] (p=7×10^{-4})	0.836[a] (p=1×10^{-4})

[a] Partial correlation is significant at p<0.05.

LD_{50} calculation method

LD_{50} is calculated based on the dose-response relation as follows:

$$\ln(R_{survival}) = a + b \cdot C_{exposure-residue} \quad \text{(Eq.1)}$$

where, $C_{exposure\text{-}residue}$ is the exposure concentration of residue (μg/mL); $R_{survival}$ is the cell survival relative to vehicle control (%); a and b are fitting parameters.

For each residue sample, cells were exposed to 6 concentrations, thus generating 6 survival percentage values. The exposure concentration and the obtained cell survival percentage data were used to fit Eq. 1 to obtain the values of fitting parameters a and b. After a and b values were obtained, $LD_{50\text{-}residue}$ was calculated as follows:

$$LD_{50-residue} = \left(\ln 0.5 - a\right) / b \quad \text{(Eq. 2)}$$

$LD_{50\text{-}residue}$ obtained from Eq. 2 is in terms of residue dose (μg residue/mL). It was converted to $LD_{50\text{-}soil}$ in terms of soil dose (mg soil/mL) as follows:

$$LD_{50-soil} = LD_{50-residue} / C_{residue/soil}$$

where, $C_{residue/soil}$ is the residue mass produced per unit soil (μg residue/mg soil).

Test of benzo[*a*]pyrene metabolic activation by DT40 cell lines

The DT40 system has not been tested previously for its ability to activate compounds that require metabolic activation before exerting a genotoxic effect. Therefore, we evaluated the potential for metabolic activation by exposing DT40 parental cell line and its mutant *Rev3*$^{-/-}$ to benzo[*a*]pyrene (BaP). According to unpublished data from Dr. Nakamura's lab, *Rev3*$^{-/-}$ is sensitive to benzo[a]pyrene diolepoxide (BPDE), BaP's ultimate carcinogenic metabolite.

The DT40 and *Rev3*$^{-/-}$ were exposed to BaP using the method as described in Ridpath et al. (2011). The results are shown in Figure S1. A paired-sample t-test was applied to determine the significant differences of cell survival rate between the DT40 and *Rev3*$^{-/-}$. The survival rate of *Rev3*$^{-/-}$ was significantly lower (p<0.05) than that of the DT40 parental cell line. Therefore, BaP could cause DNA damage response in *Rev3*$^{-/-}$, which indicates that DT40 cells may have metabolic activation capacity for PAHs.

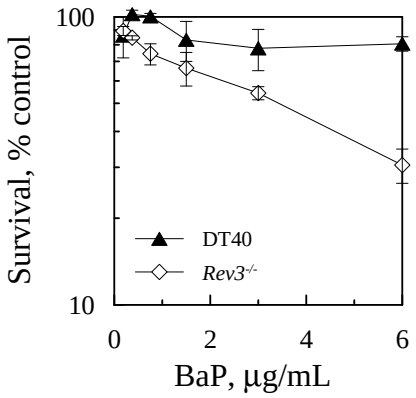

Figure S1. Cell survival of DT40 parental cells and three mutants (*Rad54*$^{-/-}$, *Rev3*$^{-/-}$ and *XPA*$^{-/-}$) exposed to benzo[*a*]pyrene.

Ridpath, J. R.; Takeda, S.; Swenberg, J. A.; Nakamura, J. Convenient, multi-well plate-based DNA damage response analysis using DT40 mutants is applicable to a high-throughput genotoxicity assay with characterization of modes of action. *Environ. Mol. Mutagen.* **2011,** *52* (2), 153-160