Supplementary Online Material

Real Time-Polymerase Chain Reaction (RT-PCR). Total cellular RNA was extracted from 1 x 10^9 *D. radiodurans* cells harvested from three separate cultures growing exponentially in defined minimal medium [S1]. For RT-PCR comparisons, total cellular RNA was similarly isolated from 1 x 10^9 *D. radiodurans* cells growing exponentially in rich medium (TGY). RNA was prepared using TRIzol reagent (Invitrogen) and treated with Rnase-free DNaseI (Qiagen) at 37°C for 30 minutes. Upon inactivation of DNaseI by treatment at 65°C for 10 minutes, the total RNA was purified using RNeasy Minikit columns (Qiagen). The quality and quantity of RNA was determined spectrophotometrically at 260/280 nm and by agarose gel electrophoresis.

Fifteen gene-specific oligonucleotide primer-sets were designed for each of the TCA cycle genes (including the glyoxylate bypass genes), and 3 control primer sets were used for amplifying 23S, 16S, and 5S rRNA gene products (Table S1). The first-strand cDNA was synthesized in 100 µl of reaction buffer containing 5 µg of total cellular RNA, 30 µg of random hexamers (Invitrogen), 10 mM dithiothreitol, 500 µM dNTPs, and 2,000 U of Supescript™ II RNase H^- reverse transcriptase (Invitrogen) at 42°C for 60 min. Before adding the reverse transcriptase, 10 µl of each reaction was saved to serve as a negative control. The cDNAs and the negative controls were subsequently used as templates for RT-PCR. The PCR amplifications were done on microtiter plates as 50 µl reactions containing the appropriate primers at a final concentration of 0.4 µM, 0.5 µl of the synthesized cDNAs, and 20,000× diluted SYBR Green I fluorescent dye (Eugene, OR).

For Peer Review

Amplification was carried out by incubating the PCR mixture at 96°C for 15 s, 55°C for 30 s, and 72°C for 30 s for 45 cycles. Melting curve generation followed the amplification, starting at 55°C, with 0.5°C increments at 10 second intervals.

All assays were performed with the icycler iQ Real-Time Detection System (Bio-Rad, Herculus, CA). The fluorescent intensity of SYBR green I, a double-stranded DNA specific dye, was monitored at the end of each extension step, and copy numbers of the target cDNAs were estimated by the threshold cycles according to the standard curve. Standard curves were constructed with cDNA fragments of known size and copy number. For each gene, 3 cDNA templates derived from different independent defined minimal medium cell cultures were amplified, and each cDNA template was repeated 3 times in the same plate. Three independent PCR amplifications of the same design were performed in different plates for each gene, so a total of 27 data-points for each gene were obtained. t-distribution (TDIST) test was used to estimate statistically significant differences between the levels of expression-analyzed genes (Table S2).

D. radiodurans pre-cultured in defined minimal medium or TGY were inoculated at 5 x 10^6 CFU/ml into minimal or TGY medium, respectively, and monitored for growth. For both growth conditions, mid-exponential cells were harvested at OD_{600} ~0.6. All 15 TCA cycle gene expression levels were examined by RT-PCR (Table S2). For rRNA control amplifications, no significant changes in expression were observed (data not shown).

For Peer Review

ScholarOne Support 1-434/817-2040 ext 167

Transmission electron microscopy (Fig. 2). TEM was as described previously [S1] except in panels D-F where cells were fixed with paraformaldehyde, dehydrated, suspended in hydrophilic resin (KM4) and polymerized at -25°C, to avoid artificial contraction of DNA caused by osmium tetroxide. Sections were stained with uranyl acetate and lead citrate.

Growth with Mn oxides (Fig. 3). Growth of *Deinococcus* spp. on Mn was tested in defined minimal medium [S1] supplemented with 50 μM colloidal Mn(IV), δMnO_2, pyrolusite, 5 μM $MnCl_2$, or no added Mn. Colloidal Mn(IV) [S2], δMnO_2 [S3] and pyrolusite [S4] were prepared as described elsewhere. Cells used for the experiment were grown on spread plates of defined minimal medium with 5 μM $MnCl_2$. After 2-3 days of incubation at 30ºC, cells were harvested from the agar, and inoculated into defined minimal medium with no added metal. Initial cell density was adjusted to an OD_{600} of approximately 0.08 to 0.1. Each treatment was prepared in one volume from the original defined minimal medium suspension, and then transferred in 5 ml quantities to 15 ml, pre-sterilized, polystyrene culture tubes, and incubated at 30ºC and 100 rpm. Duplicate tubes were sacrificed at each sample time for OD_{600} measurement. References S1-S4 at bottom of Supplementary Material.

Growth and irradiation conditions (Fig. 4). 'R1 in 10 mM $MgSO_4$, +/- IR': Three replicate *D. radiodurans* (from 3 separate colonies of ATCC 13939) cultures were grown in 10 ml TGY for 18 h at 32°C. Cultures were diluted with TGY to OD_{600} 0.1 (~10^6 CFU/ml) followed by incubation at 32°C. Cells from 100 ml cultures were harvested at

ScholarOne Support 1-434/817-2040 ext 167

OD_{600} 0.5 (~6 x 10^7 CFU/ml), washed with 10 mM $MgSO_4$ and re-suspended in 10 mM $MgSO_4$ at the same cell density. $MgSO_4$-treated cultures were split into 50 ml portions, one irradiated to 5 kGy, the other serving as a non-irradiated control. 'R1 in TGY +/- IR': control *D. radiodurans* cultures not treated with $MgSO_4$. Irradiations were with ^{60}Co on ice [S1]. After irradiation, tubes were incubated without ionizing radiation at 32°C for 24, 48, 72 and 96 hours. For survival assays, cells were appropriately diluted in TGY, plated on TGY agar and incubated at 32°C for 5 days before CFU counting.

Protease secretion assays (Fig. 5). Tests for secretory proteases were carried out on indicator plates containing skimmed milk. For individual plates, bottom layer (30 ml): 0.3% Beef Extract (Sigma), 0.5% Tryptone Peptone (Difco), 1.5% Bacto-Agar (Difco); top layer (5 ml) poured after bottom layer solidified: 3.3% Skimmed Milk Powder (Difco), 0.3% Beef Extract (Sigma), 0.5% Tryptone Peptone (Difco), 1.2% Bacto-Agar (Difco). Inoculated plates were incubated for 48 hours at 32°C.

For Peer Review

ScholarOne Support 1-434/817-2040 ext 167

Supporting Online References

S1. Daly, M. J., Gaidamakova, E. K., Matrosova, V. Y., Vasilenko, A., Zhai, M., Venkateswaran, A., Hess, M., Omelchenko, M. V., Kostandarithes, H. M., Makarova, K. S., Wackett, L. P., Fredrickson, J. K. and Ghosal, D. (2004) Accumulation of Mn(II) in *Deinococcus radiodurans* facilitates gamma-radiation resistance. Science 306, 1025-1028.

S2. Kostka, J. and Nealson, K. (1998) Isolation, cultivation and characterization of iron-and manganese-reducing bacteria. *In* Burlage, R., Atlas, R., Stahl, D., Geesey, G., and Sayler, G. (ed.), Techniques in Microbial Ecology. Oxford University Press.

S3. Burdige, D. J. and Nealson K. H. (1985) Microbial manganese reduction by enrichment cultures from coastal marine sediments. Appl. Environ. Microbiol. 50, 491-497.

S4. Fredrickson, J. K., Zachara, J. M., Kennedy, D. W., Liu, C., Duff, M. C., Hunter, D. B. and Dohnalkova, A. (2002) Influence of Mn oxides on the reduction of uranium(VI) by the metal-reducing bacterium *Shewanella putrefaciens*. Geochimica et Cosmochimica Acta, 66, 3247–3262.

S5. Liu, Y., Zhou, J., Omelchenko, M. V., Beliaev, A. S., Venkateswaran, A., Stair, J., Wu, L., Thompson, D. K., Xu, D., Rogozin, I. B., Gaidamakova, E. K., Zhai, M., Makarova, K. S., Koonin, E. V. and Daly, M. J. (2003) Transcriptome dynamics of *Deinococcus radiodurans* recovering from ionizing radiation. Proc. Natl. Acad. Sci. USA 100, 4191-4196.

For Peer Review

Table S1. Sequences of the primers used in the quantitative real-time PCR assays

Gene ID		Annotation	Primer sequences	Product Size/bp
DR0083	*sucB*	Dihydrolipoamide succinyltransferase	GCCTCAAGGAAGTGCAGAAC CTTCACGAACTGGTCCTGGT	107
DR0287	*sucA*	2-oxoglutarate dehydrogenase	GGTCATGGAGACGCTGAACT GGGTCGGAGATGGTAAAGC	102
DR0325	*mdh*	Malate dehydrogenase	CACCCAGTACCCTGACCTGT GGTGGGGATGTAGTCGTTCT	97
DR0757	*gltA*	Citrate synthase II	GACCTGGGCATCAAGAAGG CGGTTGTCCTGGCTGTACTC	98
DR0828	*aceA*	Isocitrate lyase	CCTTCGTGGAACTTCAGGAG GCGAGACGAGGTCGAAGTAG	104
DR0951	*sdhB*	Fumarate/succinate dehydrogenase type Fe-S protein	CCAGATCCAGACTCCCGTTA CGCAGGACCTTGACTTTCAG	105
DR0952	*sdhA*	Succinate	GGCCACATGATCCTTCAGAC	109

ScholarOne Support 1-434/817-2040 ext 167

		dehydrogenase flavoprotein	GCCCGTCTTCGATAATCAGG	
DR1155	*aceB*	Malate synthase	GCACCTGGACTTTGATGGAC CAGCTTGGGCACGTAGATGT	103
DR1247	*sucC*	Succinyl-CoA synthetase, beta chain	GTGAACAAGGTGCTGGTGAC CCATCAGGGTGTAGCTCTGG	103
DR1248	*sucD*	Succinyl CoA synthetase, alpha chain	CGTGGTCGTGATTGGTGA CCGAGATAAAGGCCACGAC	98
DR1540	*icd*	Isocitrate dehydrogenase	CTGATCCTCAAGGGTCTGGA ACTCGCTGGTCTTGACTTCG	100
DR1720	*acnA*	Aconitase	CAACGGTCAGGACGTGTTC GCCGTCGTAGACCTTCTTGA	109
DR2526	*lpd*	Lipoamide dehydrogenase, component E3	CAAGGTGGACCAGCACTACC CTTCTTCCTCGGCCTTGTG	98
DR2627	*fumC*	Fumarase	CACATCGGCTACGACAAGG ATTCGTCCTCGGTCACGTAG	106

For Peer Review

DRA0277	*aceB*	Malate synthase	CGTTCGAGATGGACGAGATT TGCGCAGCTTCTTGATGTAG	102
DRA5S		Ribosomal 5S RNA	ATAGCACTGTGGAACCA TGACCGACTTTTCCGGGA	100
DRA16S		Ribosomal 16S RNA	CGACTCCGTGAAGTTGGAAT TCTACTCCCATGGTGTGACG	102
DRA23S		Ribosomal 23S RNA	GCTATGTCCGGAACGGATAA GGTCTTCCGGGAGTCTTACC	99

For Peer Review

Table S2. Relative expression levels for TCA cycle and glyoxylate bypass genes of *D. radiodurans* (ATCC BAA-816) grown in defined minimal medium versus TGY determined by RT-PCR.

Gene ID		Annotation	[a]CV	[b]DMM vs. TGY	[c]T DIST
DR0083	*sucB*	Dihydrolipoamide succinyltransferase	0.23	1.04	no
DR0287	*sucA*	2-oxoglutarate dehydrogenase	0.18	0.62	yes
DR0325	*mdh*	Malate dehydrogenase	0.31	0.42	yes
DR0757	*gltA*	Citrate synthase II	0.34	0.66	no
DR0828	*aceA*	Isocitrate lyase	0.22	3.36	yes
DR0951	*sdhB*	Fumarate/succinate dehydrogenase type Fe-S protein	0.19	0.38	yes
DR0952	*sdhA*	Succinate dehydrogenase flavoprotein	0.08	0.55	yes
DR1155	*aceB*	Malate synthase	0.25	0.87	no
DR1247	*sucC*	Succinyl-CoA synthetase, beta chain	0.28	0.25	yes
DR1248	*sucD*	Succinyl CoA synthetase, alpha chain	0.27	0.92	no

For Peer Review

ScholarOne Support 1-434/817-2040 ext 167

DR1540	*icd*	Isocitrate dehydrogenase	0.52	0.22	yes
DR1720	*acnA*	Aconitase	0.20	0.64	yes
DR2526	*lpd*	Lipoamide dehydrogenase, component E3	0.10	0.92	no
DR2627	*fumC*	Fumarase	0.22	0.71	no
DRA0277	*aceB*	Malate synthase	0.07	0.37	yes

Footnotes for Table S2:

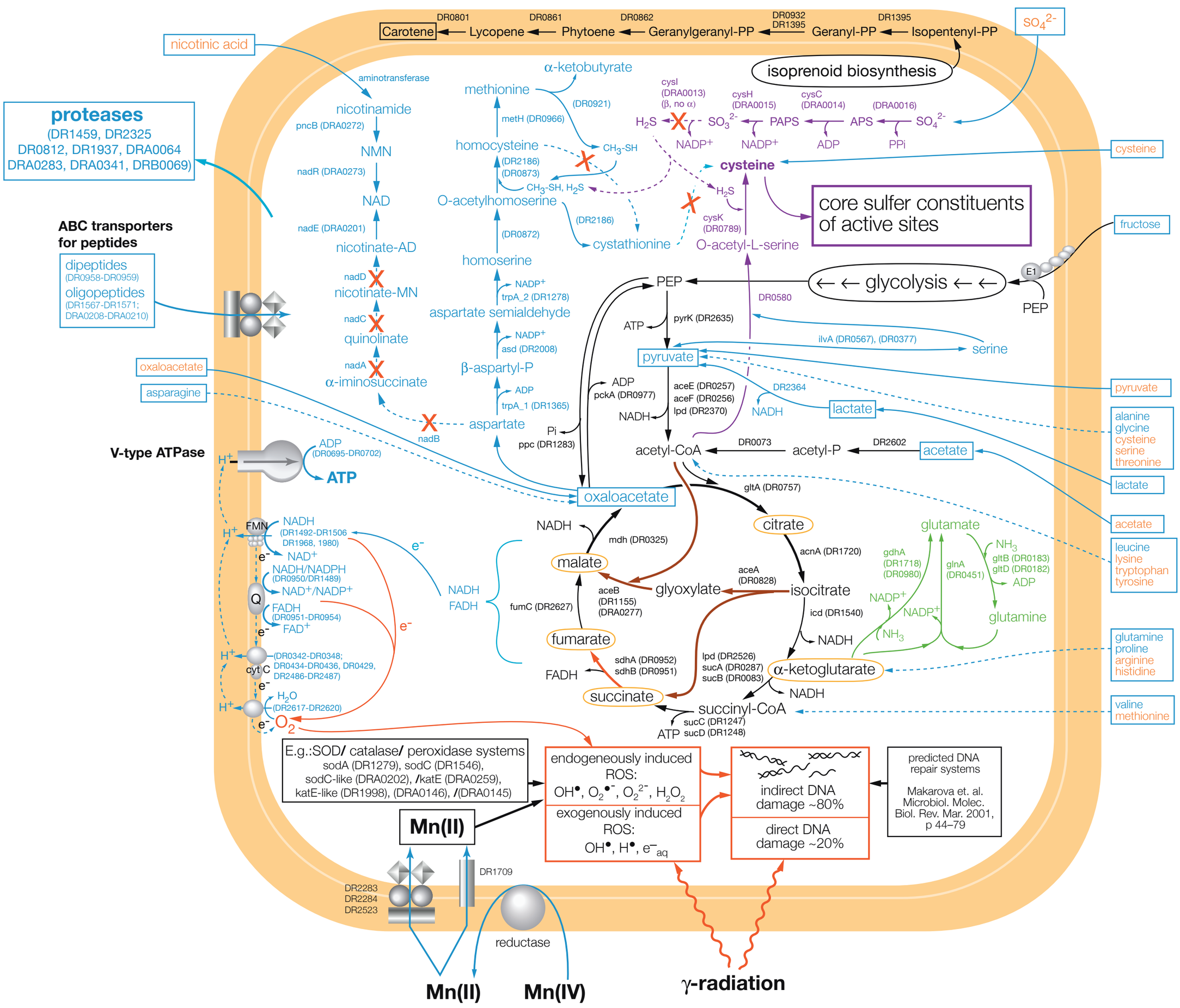
[a]CV, coefficient of variation.

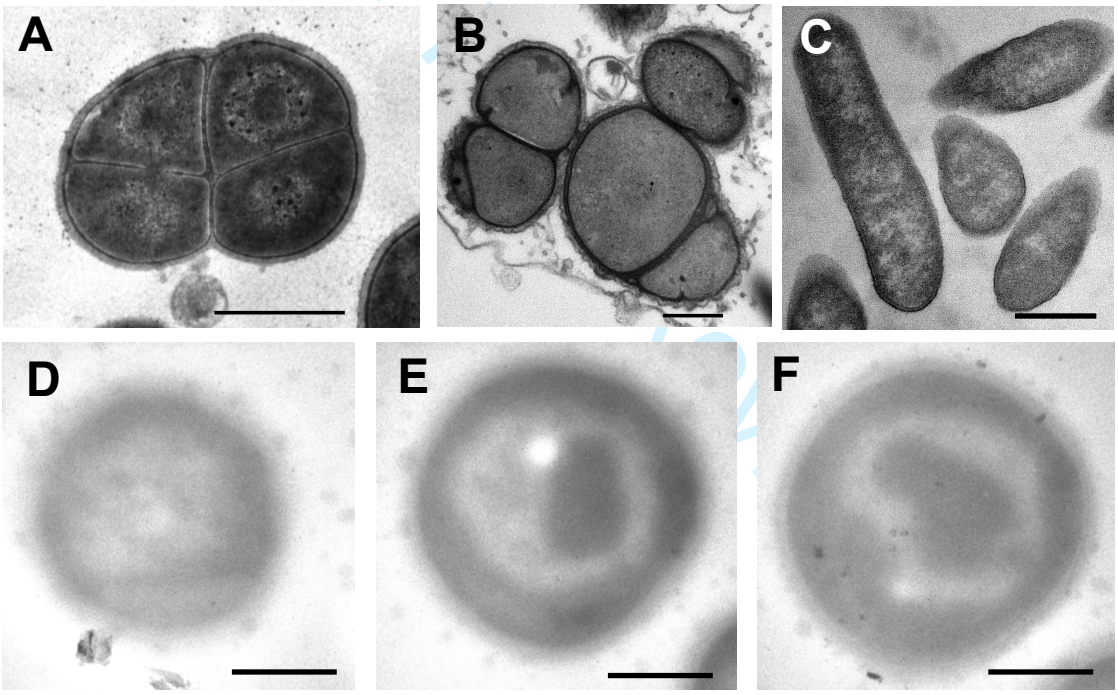
[b]defined minimal medium vs. TGY, gene expression ratio of cells in defined minimal medium versus rich (TGY) medium [S1].

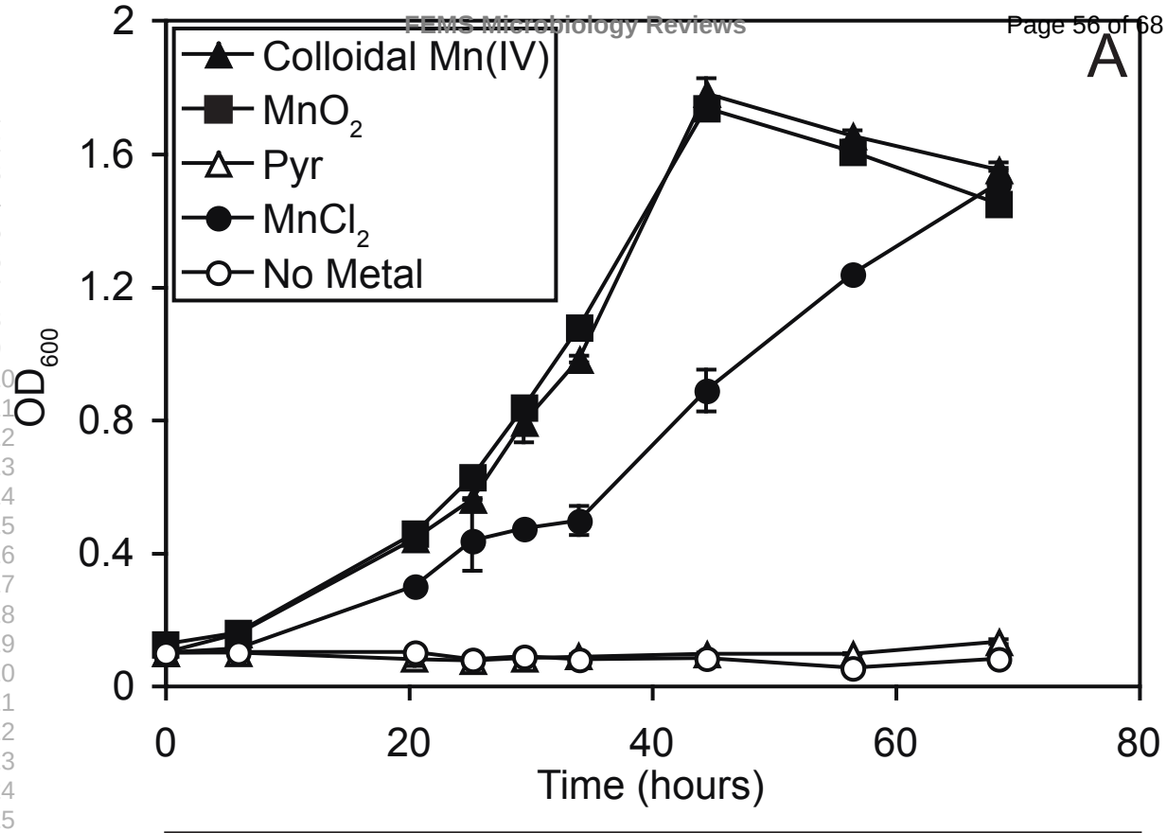
[c]TDIST, the results of statistical method (t-distribution test) implemented for estimating differences in the levels of expression. Yes, statistically significant change; No, no significant change. For RT-PCR methodology [S5] see above (Supplementary Online Material).

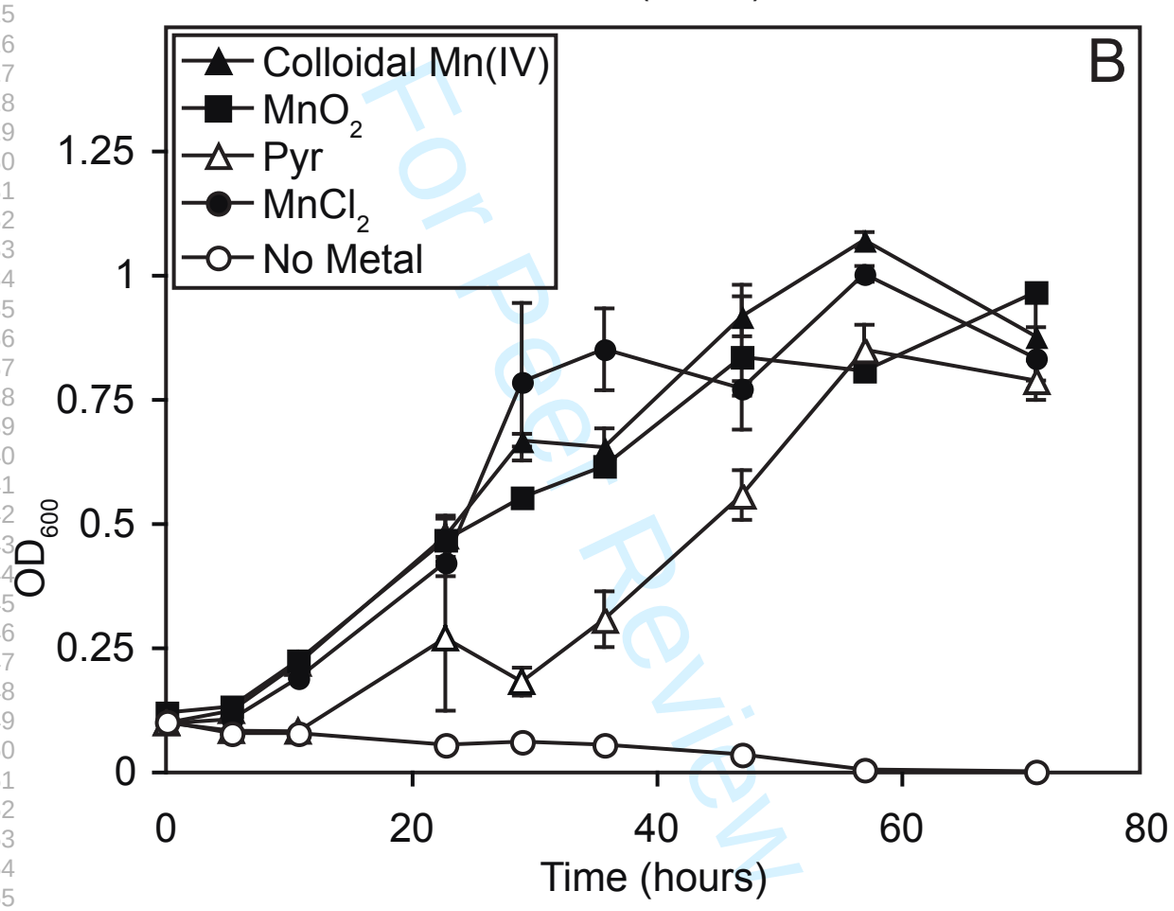
For Peer Review

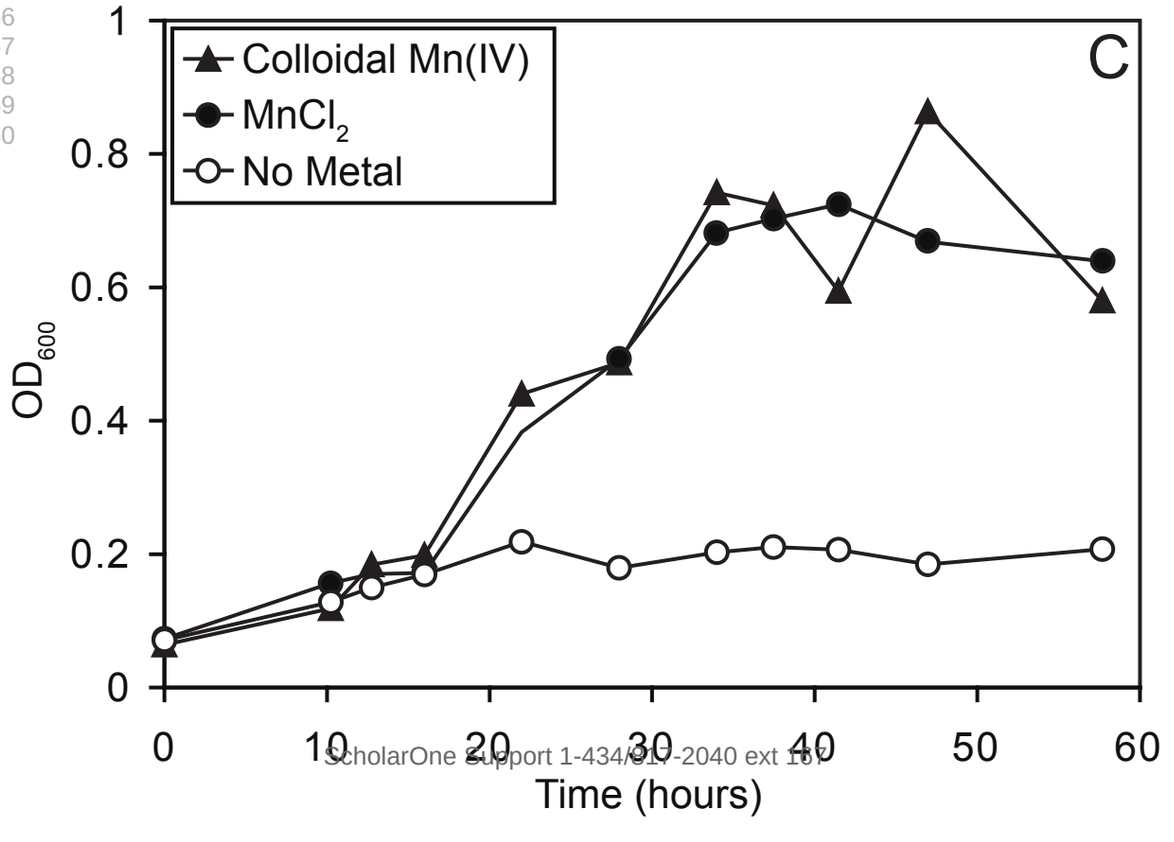
ScholarOne Support 1-434/817-2040 ext 167


Carotene
Lycopene
Phytoene
Geranylgeranyl-PP
Geranyl-PP
Isopentenyl-PP
DR0801
DR0861
DR0862
DR0932
DR1395
DR1395
isoprenoid biosynthesis
nicotinic acid
aminotransferase
nicotinamide
pncB (DRA0272)
NMN
nadR (DRA0273)
NAD
nadE (DRA0201)
nicotinate-AD
nadD
nicotinate-MN
nadC
quinolinate
nadA
α-iminosuccinate
nadB
aspartate
ADP
trpA_1 (DR1365)
β-aspartyl-P
asd (DR2008)
NADP+
aspartate semialdehyde
trpA_2 (DR1278)
homoserine
(DR0872)
O-acetylhomoserine
(DR2186)
homocysteine
(DR2186)
(DR0873)
methionine
metH (DR0966)
α-ketobutyrate
(DR0921)
cystathionine
CH3-SH
CH3-SH, H2S
cysI
(DRA0013)
(β, no α)
cysH
(DRA0015)
cysC
(DRA0014)
(DRA0016)
H2S
SO3 2-
PAPS
APS
SO4 2-
NADP+
ADP
PPi
cysK
(DR0789)
cysteine
O-acetyl-L-serine
core sulfer constituents of active sites
SO4 2-
cysteine
fructose
glycolysis
E1
PEP
DR0580
ilvA (DR0567), (DR0377)
serine
pyruvate
pyrK (DR2635)
ATP
ADP
pckA (DR0977)
NADH
aceE (DR0257)
aceF (DR0256)
lpd (DR2370)
DR2364
NADH
lactate
Pi
ppc (DR1283)
acetyl-CoA
DR0073
acetyl-P
DR2602
acetate
oxaloacetate
gltA (DR0757)
citrate
acnA (DR1720)
isocitrate
icd (DR1540)
NADH
α-ketoglutarate
NADH
succinyl-CoA
sucC (DR1247)
sucD (DR1248)
ATP
succinate
sdhA (DR0952)
sdhB (DR0951)
FADH
fumarate
fumC (DR2627)
malate
mdh (DR0325)
NADH
glyoxylate
aceB
(DR1155)
(DRA0277)
aceA
(DR0828)
lpd (DR2526)
sucA (DR0287)
sucB (DR0083)
glutamate
gdhA
(DR1718)
(DR0980)
glnA
(DR0451)
NH3
gltB (DR0183)
gltD (DR0182)
ADP
glutamine
NADP+
NH3
proteases
(DR1459, DR2325
DR0812, DR1937, DRA0064
DRA0283, DRA0341, DRB0069)
ABC transporters for peptides
dipeptides
(DR0958-DR0959)
oligopeptides
(DR1567-DR1571;
DRA0208-DRA0210)
oxaloacetate
asparagine
V-type ATPase
ADP
(DR0695-DR0702)
ATP
H+
FMN
NADH
(DR1492-DR1506
DR1968, 1980)
NAD+
NADH/NADPH
(DR0950/DR1489)
NAD+/NADP+
Q
FADH
(DR0951-DR0954)
FAD+
e-
cyt C
(DR0342-DR0348;
DR0434-DR0436, DR0429,
DR2486-DR2487)
H2O
(DR2617-DR2620)
O2
NADH
FADH
pyruvate
lactate
alanine
glycine
cysteine
serine
threonine
acetate
leucine
lysine
tryptophan
tyrosine
glutamine
proline
arginine
histidine
valine
methionine
E.g.:SOD/ catalase/ peroxidase systems
sodA (DR1279), sodC (DR1546),
sodC-like (DRA0202), /katE (DRA0259),
katE-like (DR1998), (DRA0146), /(DRA0145)
Mn(II)
endogeneously induced ROS:
OH•, O2•-, O2 2-, H2O2
exogenously induced ROS:
OH•, H•, e-aq
indirect DNA damage ~80%
direct DNA damage ~20%
predicted DNA repair systems
Makarova et. al. Microbiol. Molec. Biol. Rev. Mar. 2001, p 44–79
DR2283
DR2284
DR2523
DR1709
reductase
Mn(II)
Mn(IV)
γ-radiation

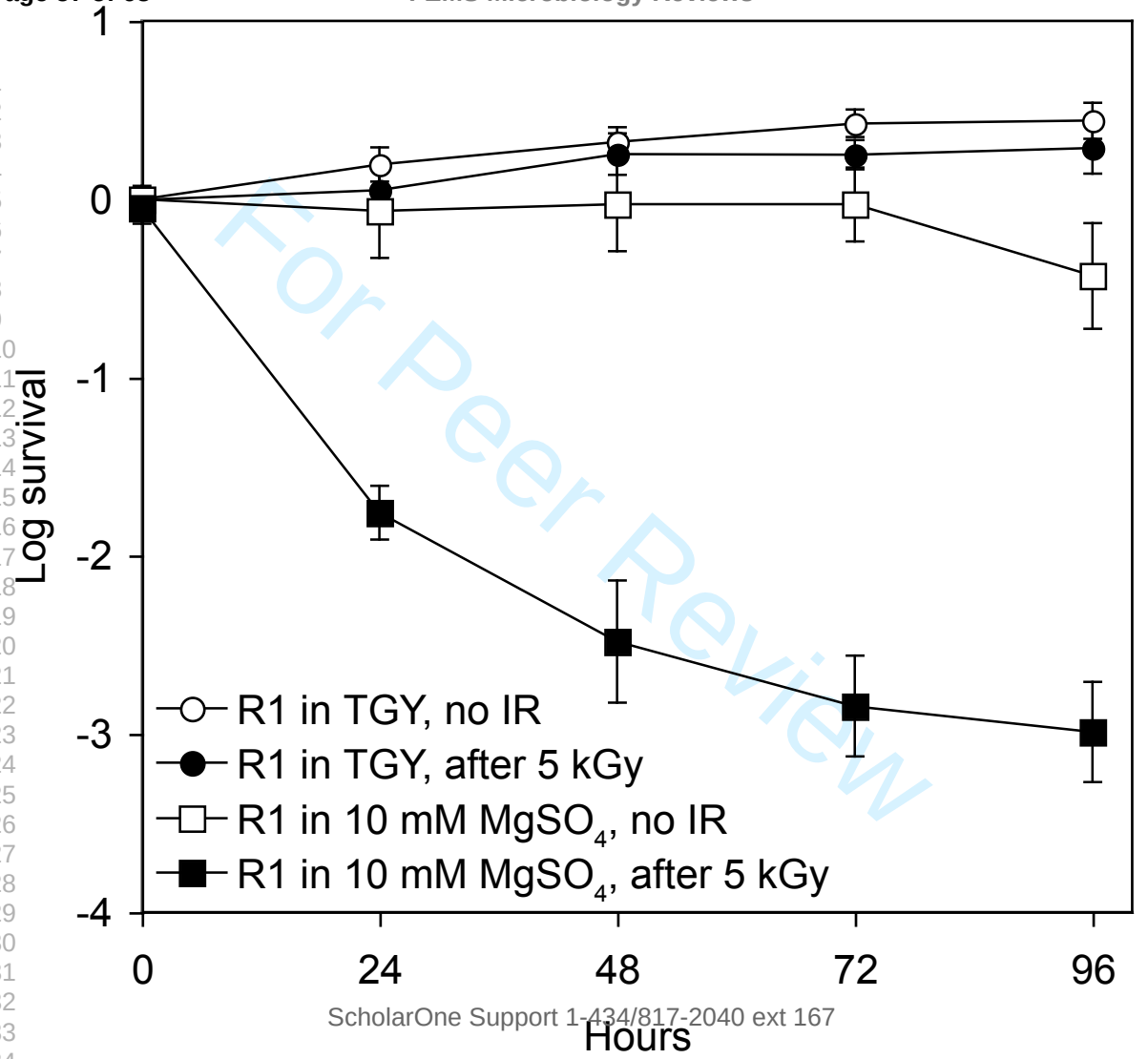

A
B
C
D
E
F



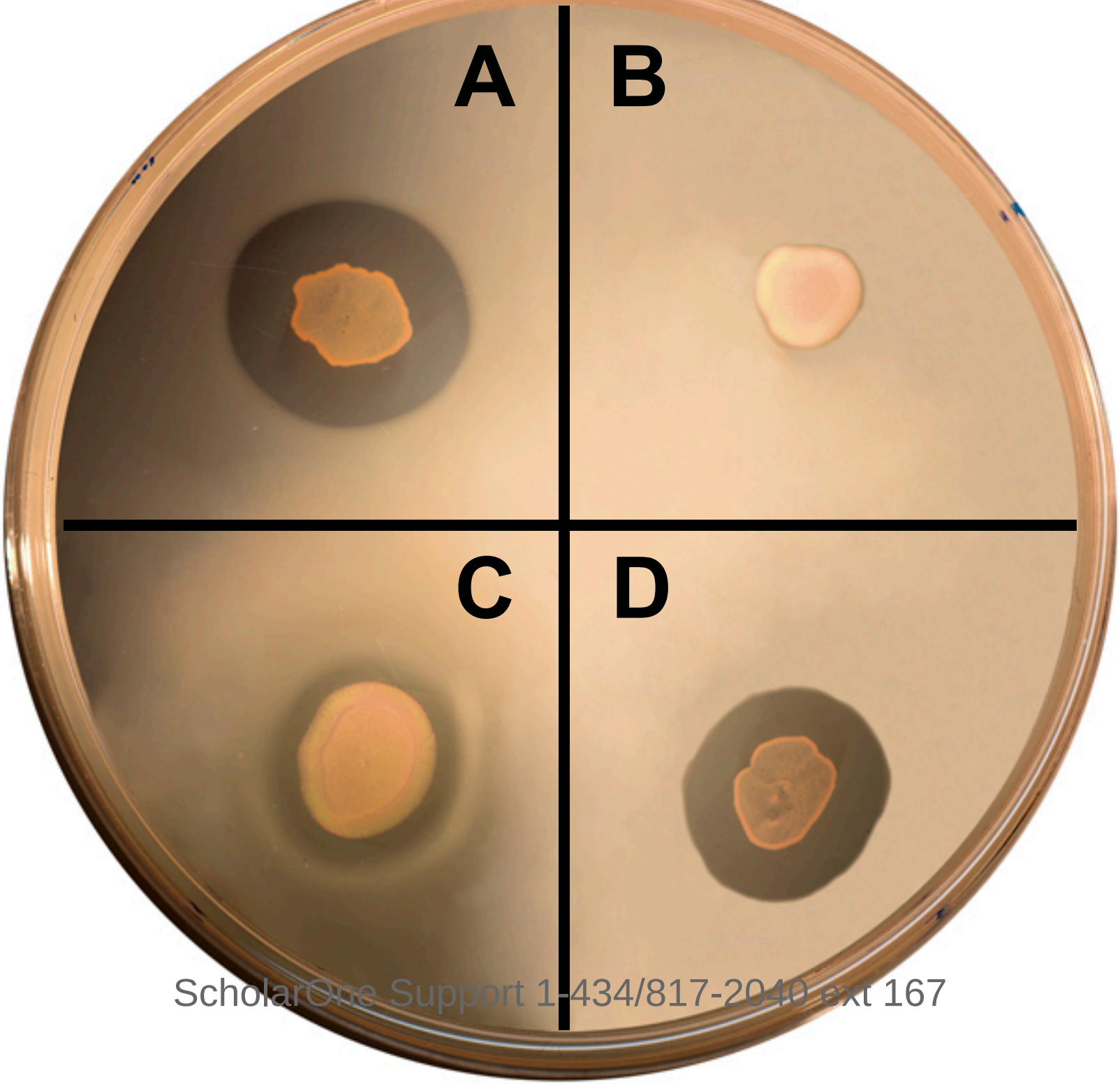




For Peer Review



For Peer Review



ScholarOne Support 1-434/817-2040 ext 167

Supplementary Online Material

Real Time-Polymerase Chain Reaction (RT-PCR). Total cellular RNA was extracted from 1 x 10^9 *D. radiodurans* cells harvested from three separate cultures growing exponentially in defined minimal medium [S1]. For RT-PCR comparisons, total cellular RNA was similarly isolated from 1 x 10^9 *D. radiodurans* cells growing exponentially in rich medium (TGY). RNA was prepared using TRIzol reagent (Invitrogen) and treated with Rnase-free DNaseI (Qiagen) at 37°C for 30 minutes. Upon inactivation of DNaseI by treatment at 65°C for 10 minutes, the total RNA was purified using RNeasy Minikit columns (Qiagen). The quality and quantity of RNA was determined spectrophotometrically at 260/280 nm and by agarose gel electrophoresis.

Fifteen gene-specific oligonucleotide primer-sets were designed for each of the TCA cycle genes (including the glyoxylate bypass genes), and 3 control primer sets were used for amplifying 23S, 16S, and 5S rRNA gene products (Table S1). The first-strand cDNA was synthesized in 100 µl of reaction buffer containing 5 µg of total cellular RNA, 30 µg of random hexamers (Invitrogen), 10 mM dithiothreitol, 500 µM dNTPs, and 2,000 U of Supescript™ II RNase H^- reverse transcriptase (Invitrogen) at 42°C for 60 min. Before adding the reverse transcriptase, 10 µl of each reaction was saved to serve as a negative control. The cDNAs and the negative controls were subsequently used as templates for RT-PCR. The PCR amplifications were done on microtiter plates as 50 µl reactions containing the appropriate primers at a final concentration of 0.4 µM, 0.5 µl of the synthesized cDNAs, and 20,000× diluted SYBR Green I fluorescent dye (Eugene, OR).

For Peer Review

ScholarOne Support 1-434/817-2040 ext 167

Amplification was carried out by incubating the PCR mixture at 96°C for 15 s, 55°C for 30 s, and 72°C for 30 s for 45 cycles. Melting curve generation followed the amplification, starting at 55°C, with 0.5°C increments at 10 second intervals.

All assays were performed with the icycler iQ Real-Time Detection System (Bio-Rad, Herculus, CA). The fluorescent intensity of SYBR green I, a double-stranded DNA specific dye, was monitored at the end of each extension step, and copy numbers of the target cDNAs were estimated by the threshold cycles according to the standard curve. Standard curves were constructed with cDNA fragments of known size and copy number. For each gene, 3 cDNA templates derived from different independent defined minimal medium cell cultures were amplified, and each cDNA template was repeated 3 times in the same plate. Three independent PCR amplifications of the same design were performed in different plates for each gene, so a total of 27 data-points for each gene were obtained. t-distribution (TDIST) test was used to estimate statistically significant differences between the levels of expression-analyzed genes (Table S2).

D. radiodurans pre-cultured in defined minimal medium or TGY were inoculated at 5 x 10^6 CFU/ml into minimal or TGY medium, respectively, and monitored for growth. For both growth conditions, mid-exponential cells were harvested at OD_{600} ~0.6. All 15 TCA cycle gene expression levels were examined by RT-PCR (Table S2). For rRNA control amplifications, no significant changes in expression were observed (data not shown).

For Peer Review

Transmission electron microscopy (Fig. 2). TEM was as described previously [S1] except in panels D-F where cells were fixed with paraformaldehyde, dehydrated, suspended in hydrophilic resin (KM4) and polymerized at -25°C, to avoid artificial contraction of DNA caused by osmium tetroxide. Sections were stained with uranyl acetate and lead citrate.

Growth with Mn oxides (Fig. 3). Growth of *Deinococcus* spp. on Mn was tested in defined minimal medium [S1] supplemented with 50 μM colloidal Mn(IV), δMnO_2, pyrolusite, 5 μM $MnCl_2$, or no added Mn. Colloidal Mn(IV) [S2], δMnO_2 [S3] and pyrolusite [S4] were prepared as described elsewhere. Cells used for the experiment were grown on spread plates of defined minimal medium with 5 μM $MnCl_2$. After 2-3 days of incubation at 30ºC, cells were harvested from the agar, and inoculated into defined minimal medium with no added metal. Initial cell density was adjusted to an OD_{600} of approximately 0.08 to 0.1. Each treatment was prepared in one volume from the original defined minimal medium suspension, and then transferred in 5 ml quantities to 15 ml, pre-sterilized, polystyrene culture tubes, and incubated at 30ºC and 100 rpm. Duplicate tubes were sacrificed at each sample time for OD_{600} measurement. References S1-S4 at bottom of Supplementary Material.

Growth and irradiation conditions (Fig. 4). 'R1 in 10 mM $MgSO_4$, +/- IR': Three replicate *D. radiodurans* (from 3 separate colonies of ATCC 13939) cultures were grown in 10 ml TGY for 18 h at 32°C. Cultures were diluted with TGY to OD_{600} 0.1 (~10^6 CFU/ml) followed by incubation at 32°C. Cells from 100 ml cultures were harvested at

ScholarOne Support 1-434/817-2040 ext 167

OD_{600} 0.5 (~6 x 10^7 CFU/ml), washed with 10 mM $MgSO_4$ and re-suspended in 10 mM $MgSO_4$ at the same cell density. $MgSO_4$-treated cultures were split into 50 ml portions, one irradiated to 5 kGy, the other serving as a non-irradiated control. 'R1 in TGY +/- IR': control *D. radiodurans* cultures not treated with $MgSO_4$. Irradiations were with ^{60}Co on ice [S1]. After irradiation, tubes were incubated without ionizing radiation at 32°C for 24, 48, 72 and 96 hours. For survival assays, cells were appropriately diluted in TGY, plated on TGY agar and incubated at 32°C for 5 days before CFU counting.

Protease secretion assays (Fig. 5). Tests for secretory proteases were carried out on indicator plates containing skimmed milk. For individual plates, bottom layer (30 ml): 0.3% Beef Extract (Sigma), 0.5% Tryptone Peptone (Difco), 1.5% Bacto-Agar (Difco); top layer (5 ml) poured after bottom layer solidified: 3.3% Skimmed Milk Powder (Difco), 0.3% Beef Extract (Sigma), 0.5% Tryptone Peptone (Difco), 1.2% Bacto-Agar (Difco). Inoculated plates were incubated for 48 hours at 32°C.

For Peer Review

Supporting Online References

S1. Daly, M. J., Gaidamakova, E. K., Matrosova, V. Y., Vasilenko, A., Zhai, M., Venkateswaran, A., Hess, M., Omelchenko, M. V., Kostandarithes, H. M., Makarova, K. S., Wackett, L. P., Fredrickson, J. K. and Ghosal, D. (2004) Accumulation of Mn(II) in *Deinococcus radiodurans* facilitates gamma-radiation resistance. Science 306, 1025-1028.

S2. Kostka, J. and Nealson, K. (1998) Isolation, cultivation and characterization of iron-and manganese-reducing bacteria. *In* Burlage, R., Atlas, R., Stahl, D., Geesey, G., and Sayler, G. (ed.), Techniques in Microbial Ecology. Oxford University Press.

S3. Burdige, D. J. and Nealson K. H. (1985) Microbial manganese reduction by enrichment cultures from coastal marine sediments. Appl. Environ. Microbiol. 50, 491-497.

S4. Fredrickson, J. K., Zachara, J. M., Kennedy, D. W., Liu, C., Duff, M. C., Hunter, D. B. and Dohnalkova, A. (2002) Influence of Mn oxides on the reduction of uranium(VI) by the metal-reducing bacterium *Shewanella putrefaciens*. Geochimica et Cosmochimica Acta, 66, 3247–3262.

S5. Liu, Y., Zhou, J., Omelchenko, M. V., Beliaev, A. S., Venkateswaran, A., Stair, J., Wu, L., Thompson, D. K., Xu, D., Rogozin, I. B., Gaidamakova, E. K., Zhai, M., Makarova, K. S., Koonin, E. V. and Daly, M. J. (2003) Transcriptome dynamics of *Deinococcus radiodurans* recovering from ionizing radiation. Proc. Natl. Acad. Sci. USA 100, 4191-4196.

For Peer Review

ScholarOne Support 1-434/817-2040 ext 167

Table S1. Sequences of the primers used in the quantitative real-time PCR assays

Gene ID		Annotation	Primer sequences	Product Size/bp
DR0083	*sucB*	Dihydrolipoamide succinyltransferase	GCCTCAAGGAAGTGCAGAAC CTTCACGAACTGGTCCTGGT	107
DR0287	*sucA*	2-oxoglutarate dehydrogenase	GGTCATGGAGACGCTGAACT GGGTCGGAGATGGTAAAGC	102
DR0325	*mdh*	Malate dehydrogenase	CACCCAGTACCCTGACCTGT GGTGGGGATGTAGTCGTTCT	97
DR0757	*gltA*	Citrate synthase II	GACCTGGGCATCAAGAAGG CGGTTGTCCTGGCTGTACTC	98
DR0828	*aceA*	Isocitrate lyase	CCTTCGTGGAACTTCAGGAG GCGAGACGAGGTCGAAGTAG	104
DR0951	*sdhB*	Fumarate/succinate dehydrogenase type Fe-S protein	CCAGATCCAGACTCCCGTTA CGCAGGACCTTGACTTTCAG	105
DR0952	*sdhA*	Succinate	GGCCACATGATCCTTCAGAC	109

ScholarOne Support 1-434/817-2040 ext 167

		dehydrogenase flavoprotein	GCCCGTCTTCGATAATCAGG	
DR1155	*aceB*	Malate synthase	GCACCTGGACTTTGATGGAC CAGCTTGGGCACGTAGATGT	103
DR1247	*sucC*	Succinyl-CoA synthetase, beta chain	GTGAACAAGGTGCTGGTGAC CCATCAGGGTGTAGCTCTGG	103
DR1248	*sucD*	Succinyl CoA synthetase, alpha chain	CGTGGTCGTGATTGGTGA CCGAGATAAAGGCCACGAC	98
DR1540	*icd*	Isocitrate dehydrogenase	CTGATCCTCAAGGGTCTGGA ACTCGCTGGTCTTGACTTCG	100
DR1720	*acnA*	Aconitase	CAACGGTCAGGACGTGTTC GCCGTCGTAGACCTTCTTGA	109
DR2526	*lpd*	Lipoamide dehydrogenase, component E3	CAAGGTGGACCAGCACTACC CTTCTTCCTCGGCCTTGTG	98
DR2627	*fumC*	Fumarase	CACATCGGCTACGACAAGG ATTCGTCCTCGGTCACGTAG	106

For Peer Review

ScholarOne Support 1-434/817-2040 ext 167

DRA0277	*aceB*	Malate synthase	CGTTCGAGATGGACGAGATT TGCGCAGCTTCTTGATGTAG	102
DRA5S		Ribosomal 5S RNA	ATAGCACTGTGGAACCA TGACCGACTTTTCCGGGA	100
DRA16S		Ribosomal 16S RNA	CGACTCCGTGAAGTTGGAAT TCTACTCCCATGGTGTGACG	102
DRA23S		Ribosomal 23S RNA	GCTATGTCCGGAACGGATAA GGTCTTCCGGGAGTCTTACC	99

For Peer Review

Table S2. Relative expression levels for TCA cycle and glyoxylate bypass genes of *D. radiodurans* (ATCC BAA-816) grown in defined minimal medium versus TGY determined by RT-PCR.

Gene ID		Annotation	[a]CV	[b]DMM vs. TGY	[c]T DIST
DR0083	*sucB*	Dihydrolipoamide succinyltransferase	0.23	1.04	no
DR0287	*sucA*	2-oxoglutarate dehydrogenase	0.18	0.62	yes
DR0325	*mdh*	Malate dehydrogenase	0.31	0.42	yes
DR0757	*gltA*	Citrate synthase II	0.34	0.66	no
DR0828	*aceA*	Isocitrate lyase	0.22	3.36	yes
DR0951	*sdhB*	Fumarate/succinate dehydrogenase type Fe-S protein	0.19	0.38	yes
DR0952	*sdhA*	Succinate dehydrogenase flavoprotein	0.08	0.55	yes
DR1155	*aceB*	Malate synthase	0.25	0.87	no
DR1247	*sucC*	Succinyl-CoA synthetase, beta chain	0.28	0.25	yes
DR1248	*sucD*	Succinyl CoA synthetase, alpha chain	0.27	0.92	no

For Peer Review

ScholarOne Support 1-434/817-2040 ext 167

DR1540	*icd*	Isocitrate dehydrogenase	0.52	0.22	yes
DR1720	*acnA*	Aconitase	0.20	0.64	yes
DR2526	*lpd*	Lipoamide dehydrogenase, component E3	0.10	0.92	no
DR2627	*fumC*	Fumarase	0.22	0.71	no
DRA0277	*aceB*	Malate synthase	0.07	0.37	yes

Footnotes for Table S2:

[a]CV, coefficient of variation.

[b]defined minimal medium vs. TGY, gene expression ratio of cells in defined minimal medium versus rich (TGY) medium [S1].

[c]TDIST, the results of statistical method (t-distribution test) implemented for estimating differences in the levels of expression. Yes, statistically significant change; No, no significant change. For RT-PCR methodology [S5] see above (Supplementary Online Material).

For Peer Review