Deinococcus radiodurans

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Content

I. PHYSIOLOGICAL STUDIES

1. Nutritional studies
2. Growth studies

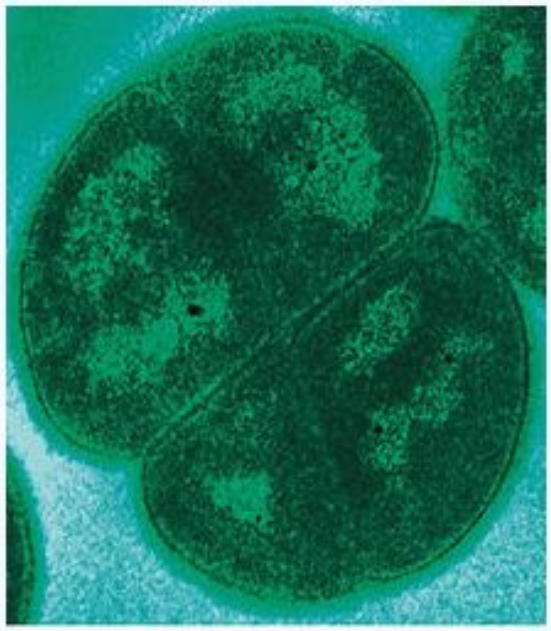
II. GENETIC SYSTEMS

1. Genetic methods
2. Genomic approaches
3. Proteome analysis

Introduction

Deinococcus radiodurans is an extremophilic bacterium, one of the most radiation-resistant organisms known. It can survive cold, dehydration, vacuum, acid and has been listed as the world's toughest bacterium in *The Guinness Book Of World Records*. Also, they are mesophiles.

Deinococcus radiodurans



A tetrad of *D. radiodurans*

Gram-positive bacteria (although its cell envelope is unusual and is reminiscent of the cell walls of Gram negative bacteria)

*Colonies- convex, smooth, pink to red in color (deinocrates - carotene)

*Size of cells- 1.5 to 3.5 μm.

*Do not form endospores, non-motile

*Obligate aerobic chemo-organo-heterotroph

* Habitat - rich in organic materials, such as soil, feces, meat, or sewage, but has also been isolated from dried foods, room dust, medical instruments and textiles

Scientific classification	
Domain:	Bacteria
Kingdom:	Eubacteria
Phylum:	Deinococcus-Thermus
Class:	Deinococci
Order:	Deinococcales
Family:	Deinococcaceae
Genus:	*Deinococcus*
Species:	***D. radiodurans***

Binomial name

Deinococcus radiodurans

Brooks & Murray, 1981

1. Nutritional studies: biochemical characteristics

	D. radiodurans R_1	*D. radiophilus* CCM2564	*D. proteolyticus* CCM2703
Gelatin liquefaction	+	++	++
Hydrolysis of casein	++	++	++
NO_3 reduction	−	−	−
Hydrolysis of fats	−	−	−
Growth with novobiocin 0.6 μg/ml	−	−	−
Production of acid			
from glucose	+	+	+
xylose	−	−	−
sucrose	+	−	+
Lysis of cells by lysozyme	−	++	−
lytic enzyme of "*A. lunatus*"[14]	++	+++	++
Oxidase	+++	+++	+++
Catalase	+++	+++	+++
GC content of DNA (%)	66	61	65

(+++)-strong, (++)-moderate, (+)- weak, (-)-negative

1. Nutritional studies:

TABLE 1. Basic metabolic pathways in *D. radiodurans*

Pathway	Genes in the pathway[a]	Genes missing[b]	Comments
Glycolysis	*glk, pgi, pfkA, fba/dhnA, tpi, gapA, pgk, pgm/yibO, eno, pykA*		Complete pathway
Gluconeogenesis	*ppsA, eno, pgm, pgk, gapA, tpi, fba/dhnA, fbp, pgi*	*fbp* (CE--)	Likely functional pathway
Pentose phosphate shunt and pentose biosynthesis	*zwf, gnd, tktA, talA, yhfD, rpiA, deoC*		Complete pathway
Entner-Doudoroff pathway	*zwf, edd, eda, gnd*	*eda* (CEB-)	Likely functional pathway
TCA cycle	*gltA, acnA, icd, sucA, sucB, sucC, sucD, frdA, frdB, fumA, fumC, mdh*	*fumA* (-E--)	Likely functional pathway
Glyoxalate bypass	*glcB, AceA*		Rare pathway present in *E. coli, M. tuberculosis*, and few other bacteria
Purine biosynthesis	*prsA, purF, purD, purN/purT, purL, purM, purK, purE, purC, purB, purH2, purH1, purA, guaB, guaA*		Complete pathway
Purine salvage	*purU, deoD, xapA, apt, xpt, hpt*	*xapA* (CEBR)	*D. radiodurans* has two *apt* genes of archaeal type
Pyrimidine biosynthesis	*curA, carB, pyrB, pyrC/ygeZ, pyrD, pyrE, pyrF, pyrH, ndk, pyrG*		Complete pathway
Pyrimidine salvage	*cdd, upp, udk, deoD, deoA, nrdF, nrdE, pfs/amn, tdk*		Complete pathway with two *nrdE* (one is of archaeal type with intein)
Thimidylate biosynthesis	*dcd, dut, thyA, tmk, ndk*	*dcd* (CE-R), *dut* (CEBR)	Pathway could be functional if unknown analogs of *dcd* and *dut* are present
Histidine biosynthesis	*prsA, hisG, hisI2, hisI, hisA, hisH, hisF, hisB2, hisC, hisB1, hisD*		Complete pathway
Branched-chain amino acid biosynthesis	*ilvA, ilvB, ilvN, ilvC, ilvD, leuA, leuC, leuD, leuB, ilvE*		Complete pathway
Glutamate and glutamine biosynthesis	*gltB, gdhA, glnA*		Complete pathway; *D. radiodurans* has two *glnA* genes, One is for the rare class III glutamine synthase; in R1 strain this gene has a frameshift
Aspartate and asparagine biosynthesis	*aspC, asnB, asnA, ansA*	*asnB* (-EBR), *asnA* (-E--)	Pathway could be functional if unknown analogs of *asnB* and *asnA* are present

1. Nutritional studies:

TABLE 1. Basic metabolic pathways in *D. radiodurans*

Pathway	Genes in the pathway[a]	Genes missing[b]	Comments
Aromatic amino acid biosynthesis	*aroG/kdsA, aroB, aroD, aroE, aroK, aroA, aroC, pheA1, pheA2, tyrA2, tyrB, trpD1, trpE, trpD2, trpC2, trpC1, trpA, trpB*	*tyrB* (-E--)	*D. radiodurans* has both *aroG* and *kdsA*; *D. radiodurans* and *B. subtilis* have rare bifunctional protein: chorismate mutase (*tyrA*1) and 2-dehydro-3-deoxyphosphoheptonate aldolase (*aroG*); *D. radiodurans* has two *trpE* genes, one of which is fused to *trpG*; same fusion is also found in *Asospirillum* and *Rhizobium*; reverse fusion is in *Streptomyces*
Serine and glycine metabolism	*serA, serC, serB, glyA, gcvP, gsvT, gsvH, lpd*	*serC* (-EB-), *serB* (-E-R)	Pathway could be functional if unknown analogs of *serB* and *serC* are present
Threonine biosynthesis	*thrA, asd, thrB, thrC*		Complete pathway
Methionine biosynthesis	*metL1/thrA1, asd, metL2/thrA2, metA, metB, metC, metE/metH*	*metA* (-EB-)	Incomplete and unlikely to be a functional pathway
Cysteine biosynthesis	*cysD/cysH, cysC, cysN, cysI, cysJ, cysK/cysM, cysE*	*cysD/cysN, cysM, cysE* (CEBR), *cysJ* (CEB-)	Unlikely to be a functional pathway
Arginine biosynthesis	*argJ, argB, argC, argD, argE, argF, argG, argH, argI*		Likely functional pathway; circular type as in gram-positive bacteria; some genes were acquired from archaea (see Table 11)
Proline metabolism	*argB, argE, proB, proA, proC, putA*		Complete pathway
Lysine biosynthesis	*dapA, dapB, dapD, dapC, dapE, dapF, lysA*	*dapA, dapB, dapF* (CEBR), *dapD* (-EB-)	Unlikely to be a functional pathway; *dapC* may be substituted by other aminotransferase; the closest gene to *dapE* is more likely to be an ortholog of *B. subtilis rocB* and therefore is probably involved in degradation of amino acids rather than in lysine biosynthesis
Fatty acid biosynthesis	*accB, accC, accA, accD, acpP, fabB/fabF, fabH, fabD, fabG, fabI, fadA, BS_mmgB, caiD*		Complete pathway; *D. radiodurans* encodes four *accA*, four *accD*, four BS_*mmgB*, and five *caiD*
NAD biosynthesis	*nadB, nadA, nadC, nadD, nadE, pncB*	*nadB, nadA, nadC, nadD* (CEBR)	Unlikely to be a functional pathway
Riboflavin and FAD biosynthesis	*ribA, ribD, ribB, ribE, ribC, ribG*		Complete pathway

1. Nutritional studies:

Pathway	Genes in the pathway[a]	Genes missing[b]	Comments
Siroheme biosynthesis	*hemA, hemL, hemB, hemC, hemD, cysG2, cysG1*		*D. radiodurans* has two other genes related to this pathway; *hemF* and *hemY*
Cobalamin biosynthesis	*cysG2, cbiL, cbiH, cbiF, cbiJ, cbiE, cbiT, cbiC, cbiA, cobN, cobA, cbiP, cobD, cbiB, cobT, cobS, cobU*	*cbiL, cbiH, cbiJ, cbiE, cbiT, cbiC, cobN* (C--R)	Possible partly functional pathway
Biotin biosynthesis	*bioW, bioF, bioA, bioD, bioB, birA, bioH*	*bioW* (--B-), *bioA, bioD, bioB* (CEBR)	Pathway could be functional if unknown analogs of *bioD* and *bioW* are present; *bioA* aminotransferase can be substituted by paralogous enzyme, and any biotin synthase-related enzyme may replace *bioB*
Pyridoxal phosphate biosynthesis	*yaeM, ldh, serC, pdxA, pdxJ, BS_yaad, pdxH, pdxK*	*pdxA, pdxJ*, (CE--)	*D. radiodurans* has an ortholog of BS_*yaad* which is found so far only in archaea and eukaryotes
Thiamine biosynthesis	*thiC, thiD, thiK, thiE, thiL*	*thiK* (-EB-), *thiL* (CEBR)	Pathway could be functional if unknown analogs of *thiK* and *thiL* are present
Ubiquinone and menaquinone biosynthesis	*menF, menD, menC, menE, menB, menA, menG, ubiA, ubiX, ubiB, ubiH, ubiE, ubiG*	*menF, menD, menC, menE, menB, menA*, (CEBR), *menG* (CE-R)	Unlikely functional pathway of menaquinone biosynthesis; there are some paralogs of *menC*, but they are unlikely to be related to this pathway; synthesis of ubiquinone is likely to be present; only *ubiG* is missed, but it exists only in *E. coli, Rickettsia* and yeast
NAHD-ubiquinone oxidoreducatase	All 14 subunits in one operon		Complete pathway
H^+-ATPase	8 subunits in one operon		Complete pathway; vacuolar-type H^+-ATPase like in archaea, *Thermus*, spirochetes, and *Chlamydia*
Cytochrome *c* and *b*-dependent electron transport	*cccA/cccB, qcrB, ctaA, ctaE, ctaF, ctaD, ctaB, ctaC, ccdA, sdhC, ccmG, ccmF, ccmE, ccmD, ccmC, ccmB, ccmA, ccmH, cydB, cydA*	*ctaF, ccmA, ccmD* (-E--), *cydA, cydB* (CEBR)	Probably functional pathway; component of heme exporter (such proteins are definitely present and some of them can perform this function)

[a] The gene names and pathway classification follow the biochemical data and nomenclature described for *E. coli* and *S. enterica* serovar *Typhimurium* (152).
[b] The presence or absence in bacteria with large genomes is indicated in parentheses after the names of genes that are missing in *D. radiodurans*. Abbreviations are as follows: C, *Synechocystis* sp.; E, *E. coli*; B, *B. subtilis*; R, *M. tuberculosis*.

1. Nutritional studies: nutritional medium

Nutrient Agar with Glucose

Composition per liter:

Agar	15.0g
Pancreatic digest of gelatin	5.0g
Beef extract	3.0g
Glucose	10.0g

pH 6.8 ± 0.2 at 25°C

Source: Nutrient agar is available as a premixed powder from BD Diagnostic Systems.

Preparation of Medium: Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

Use: For the cultivation and maintenance of *Amycolata saturnea*, *Arthrobacter* species, *Corynebacterium* species, *Curtobacterium flaccumfaciens*, *Deinococcus radiodurans*, *Escherichia coli*, *Hafnia alvei*, *Micrococcus aurantiacus*, *Myxomicrobium multiplex*, *Nocardia petroleophila*, *Nocardia* species, *Pseudomonas* species, *Rhodococcus rhodochrous*, *Streptomyces piedadensis*, and *Xanthomonas* species.

1. Nutritional studies: nutritional medium

Corynebacterium Agar

Composition per liter:

Agar	15.0g
Beef extract	10.0g
Peptone	10.0g
NaCl	5.0g

pH 7.2 ± 0.2 at 25°C

Preparation of Medium: Add components to distilled/deionized water and bring volume to 1.0L. Mix thoroughly. Adjust pH to 7.2. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Pour into sterile Petri dishes or leave in tubes.

Use: For the cultivation and maintenance of *Brevibacterium helvolum, Brevibacterium linens, Brochothrix thermosphacta, Cellulomonas cellasea, Corynebacterium ammoniagenes, Corynebacterium callunae, Corynebacterium glutamicum,* other *Corynebacterium* species, *Curtobacterium flaccumfaciens, Deinococcus radiodurans, Microbacterium laevaniformans, Mycobacterium vaccae, Rhodococcus equi, Rhodococcus fascians, Sporolactobacillus inulinus,* and *Streptococcus mutans.*

1. Nutritional studies: nutrition medium

Known nutrient broth (TU 42-14-83-78) [1] for the cultivation of a wide range of microorganisms and distilled water having the following composition, g/l:

Pancreatic hydrolysate sprat	of 10.05
Sodium chloride	4,95

pH 7,2±0,2.

A disadvantage of the known nutrient broth is the low productivity of cultivation of bacteria Deinococcus radiodurans.

Known nutrient medium [2], used for the cultivation of microorganisms Deinococcus radiodurans VKPM B-8209, including g/l:

Yeast extract	5,0
Peptone	15,0
NaCl	5,0
Agar	15,0
Distilled water	to 1 l

A disadvantage of the known nutrient medium is expensive components (product of animal origin such as peptone, yeast extract, agar). However, the performance of culturing bacteria Deinococcus radiodurans using Dan the second environment is not high enough.

* Also called L medium

1. Nutritional studies: nutrition medium

Closest to the present invention is selected as a prototype nutrient medium [3], used for the cultivation of microorganisms Deinococcus radiodurans, the following composition, g/l:

Triptan	5,0
Glucose	1,0
Yeast extract	3,0
Distilled water	to 1 l

The disadvantage of this environment is the use for nutrition expensive ingredients (tripton - tripeny hydrolyzed protein substrate of animal origin, yeast extract - Baker's yeast autolysate). However, the performance of culturing bacteria Deinococcus radiodurans using this environment is not high enough.

Media requirements - simple in composition, increase the yield of biomass.

This objective is achieved in that a nutrient medium containing a nutrient basis and distilled water, according to the invention as nutrition contains soy flour in the following ratio of components, g/l:

Soy flour	50,0
Distilled water	to 1 l

Unlike the prototype, the proposed environment more simple and cheap and provides optimal conditions for the growth of Deinococcus radiodurans.

1. Nutritional studies: nutrition medium selection

3 strains of microorganisms: *Deinococcus radiodurans VKPM B-8209, Deinococcus radiodurans BKM-1422, Deinococcus radiodurans BKM-1467.*

Different concentrations of soy flour – 10g, 50g, 100g per liter

Strains of *Deinococcus radiodurans* was grown in 250-ml wide-mouthed Erlenmeyer flasks with 50 ml of this liquid media, which contributed 50 μl of overnight culture of bacteria.

Regime: 150 rpm on the circular shaker at 30°C for 48 hours

Evaluation of growth:
1) Visual: pink-orange color of medium (intensity of color)
2) Quantitative: number of CFU/ml (standard technique of parallel dilutions in saline solution and in-depth inoculation on solid agar medium [30°C for 24-48 hours])

1. Nutritional studies: nutrition medium selection

Concentration of soybean flour	Number of colony forming units (CFU/ml)		
	Deinococcus radiodurans VKPM B-8209	*Deinococcus radiodurans* BKMB-1422	*Deinococcus radiodurans* BKMB-1467
10 g/l	$1,1 * 10^8$	$1,2 * 10^8$	$0,9 * 10^8$
50 g/l	$1,5 * 10^9$	$1,7 * 10^9$	$1,6 * 10^9$
100 g/l	$1,3 * 10^9$	$1,3 * 10^9$	$1,4 * 10^9$

1. Nutritional studies: nutrition medium selection

The name of the media	Number of colony forming units (CFU/ml)		
	Deinococcus radiodurans VKPM B-8209	*Deinococcus radiodurans* BKMB-1422	*Deinococcus radiodurans* BKMB-1467
TGY	$5,0 * 10^8$	$7,7 * 10^8$	$7,1 * 10^8$
L media	$1,0 * 10^8$	$1,6 * 10^8$	$2,1 * 10^8$
Nutrient broth (TU 42-14-83-78)	$3,5 * 10^7$	$5,5 * 10^7$	$6,3 * 10^7$
Soy environment	$1,3 * 10^9$	$1,7 * 10^9$	$1,3 * 10^9$

2. Growth studies

Cultivation -> Solid state/submerged

Bioreactor design ->Airlift bioreactor

Fermentation mode -> Batch, Continuous, Semi-continuous

Culture monitoring -> Primarily Visual evaluation (pink-orange to red color and turbidity), microscopy, evaluation of cell density.

Basic parameters->
*aerobic cultivation
* pH neutral 6,8-7,2 +/- 0.2 range
* Temperature 25°C- 37°C
*Stirring intensity and etc

2. Genomic approaches

The genome of *D. radiodurans* consists of four major parts. The complete sequence of the R1 strain has 3,284,156 base pairs made up of two circular chromosomes (2,648,638 and 412,348 base pairs), a major plasmid (177,466 base pairs), and a small plasmid (45,704 base pairs).

No current research shows whether or not these plasmids contribute specifically to functionality or virulence. However, it is known that multiple copies of each gene are found on all the chromosomes and plasmids, which most likely contributes to its amazing repair capabilities associated with its radiation resistance.

1. Genetic methods: Horizontal gene transfer

TABLE 6. Examples of horizontally transferred genes in *D. radiodurans*

Protein	Gene name	Taxons where homologs are found	Best BLAST hit: species, gene identifier, and e-value	Comments
Topoisomerase IB	DR0690	*Eucarya* and double-stranded DNA viruses	Orf virus, gil521138, 2×10^{-11}	Belongs to eukaryotic type I topoisomerases; performs ATP-independent breakage of single-stranded DNA, followed by passage and rejoining; the first finding of a topoisomerase of this family in bacteria
Yellow protein (*Drosophila*) or royal jelly protein (honeybee)	DR1790	*Insecta*	*Drosophila subobscura*, gil2222667, 1×10^{-14}	Required for cuticular pigmentation in *Drosophila* and important component of royal jelly of honeybee
Acyl coenzyme A-binding protein (ACBP)	DR0166	*Eucarya*	*Caenorhabditis elegans*, gil2088729, 2×10^{-17}	Binds medium- and long-chain acyl coenzyme A esters with very high affinity
Ro RNA-binding protein	DR1262	*Eucarya*	*Xenopus laevis*, gil1173109, 4×10^{-86}	Ribonucleoproteins complexed with several small RNA molecules; involved in UV-resistance in *Deinococcus*
LEA14-like desiccation-induced protein	DR1372	*Plantae* and *Archaea*	*Lycopersicon esculentum*, gil1684830, 1×10^{-3}	Protein induced in leaves by desiccation, ethylene, or abscisic acid
Desiccation-induced protein	DRB0118	*Craterostigma plantagineum* (plants)	*Craterostigma plantagineum*, gil118926, 4×10^{-19}	Protein induced in leaves by desiccation or abscisic acid
LEA76/LEA26-like desiccation-induced protein	DR1172	*Eucarya* (mostly plants)	*C. elegans*, gil2353333, 2×10^{-26}	In plants, protein induced in leaves by desiccation, ethylene, or abscisic acid
Protein kinase of RIO1 family	DR2209	*Eucarya* and *Archaea*	*Schizosaccharomyces pombe*, gil2661615, 1×10^{-12}	Protein kinase SudD, RIO1 family member, is a suppressor of *bimD* genes, which are involved in cell cycle control in *Emericella nidulans*

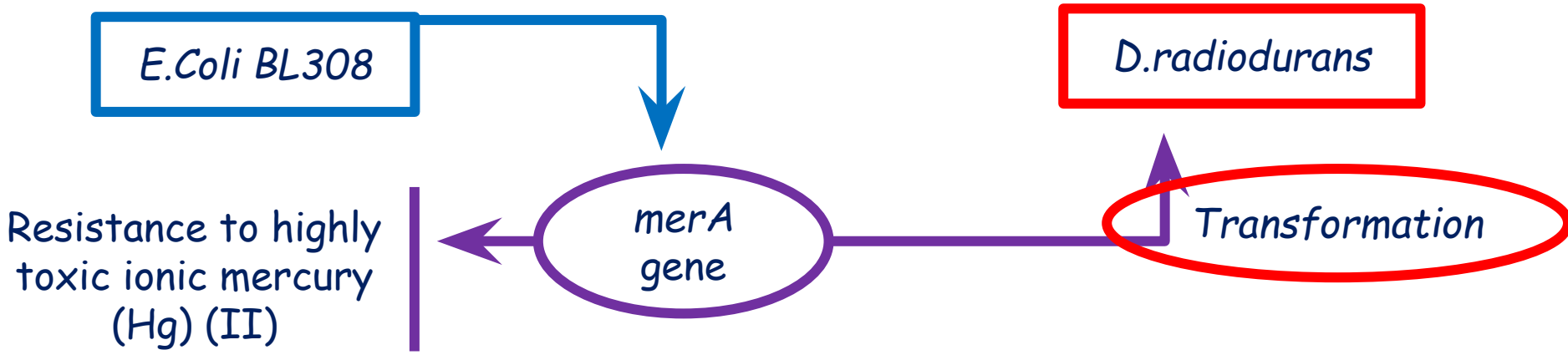
1. Genetic methods: Horizontal gene transfer

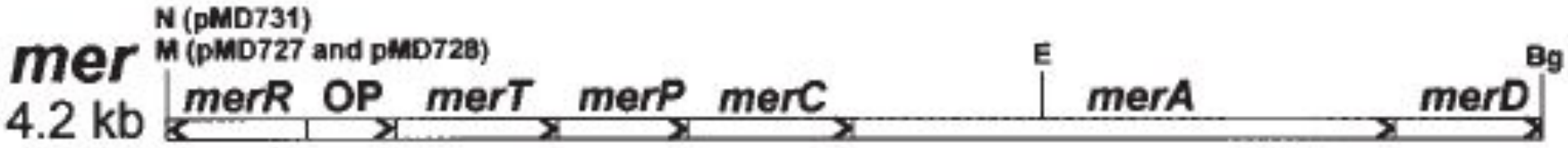
TABLE 6. Examples of horizontally transferred genes in *D. radiodurans*

Protein	Gene name	Taxons where homologs are found	Best BLAST hit: species, gene identifier, and e-value	Comments
Peroxidase	DRA0145	*Polyporaceae* spp. (fungi)	*Polyporaceae* spp., gil2160705, 4×10^{-34}	
Tryptophan-2,3-dioxygenase	DRA0339	*Eucarya*	*Drosophila simulans*, gil881370, 5×10^{-18}	Converts L-tryptophan to L-formylkynurenine; binds heme; can utilize other substrates
L-Kynurenine hydrolase	DRA0338	Orthologs only in *Eucarya*	*Saccharomyces cerevisiae*, gil1532216, 4×10^{-37}	Belongs to pyridoxal-dependent aminotransferase family; hydrolyzes L-kynurenine to anthranilate and L-alanine
Serine carboxypeptidase	DR0964	*Eucarya*	*Homo sapiens*, >gil2098347, 9×10^{-7}	
Tungsten formylmethanofuran dehydrogenase, subunit E (FwdE)	DRA0267	*Archaea*	*Pyrococcus horikoshii*, >gil3257655, 2×10^{-13}	Involved in methanogenesis; operon encoding all subunits of this enzyme contains six genes, *fwdEFACDB*, most of which are absent in this genome
Homolog of a tymocyte protein cThy28kD	DR0566	*Eucarya, Archaea*, and cyanobacteria	*Synechocystis* spp., gil1653325, 2×10^{-28}	Bacterial proteins show significantly greater similarity to each other and to eukaryotic homologs than to archaeal homologs, which suggests horizontal transfer between bacteria and eukaryotes
Uncharacterized protein	DR0376	Cyanobacteria and *Aquifecales*	*Synechocystis* spp., gil2708801, 4×10^{-44}	Probable enzymatic domain with a conserved glutamate; *Synechocystis* encodes at least 35 proteins of this family; *Deinococcus* has 3 of them

1. Genetic methods: Transformation

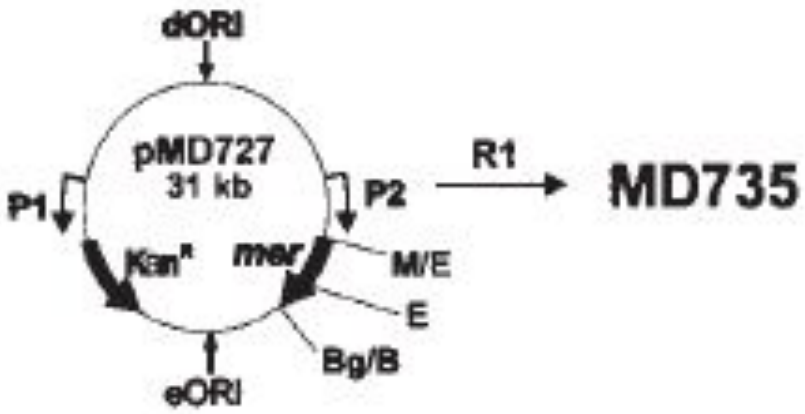
Engineering *Deinococcus radiodurans* for metal remediation in radioactive mixed waste environments



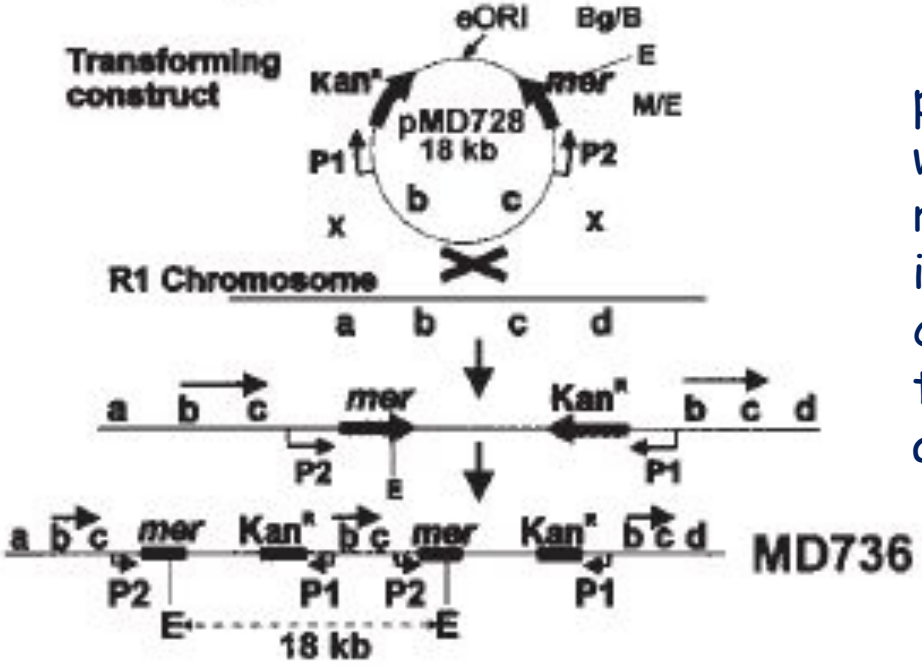


4.2-kb *mer* operon of pBD724 encodes six proteins: MerR, activation/repression of the *mer* operon; MerT, mercuric ion transport protein; MerP, periplasmic mercuric ion binding protein; MerC, transmembrane protein; MerA, mercuric reductase; and MerD, putative secondary regulatory protein. OP, operator/promoter sequence; M, *Mfe*I; N, *Nco*I; E, *Eco*RI; Bg, *Bgl*II.

1. Genetic methods: Transformation

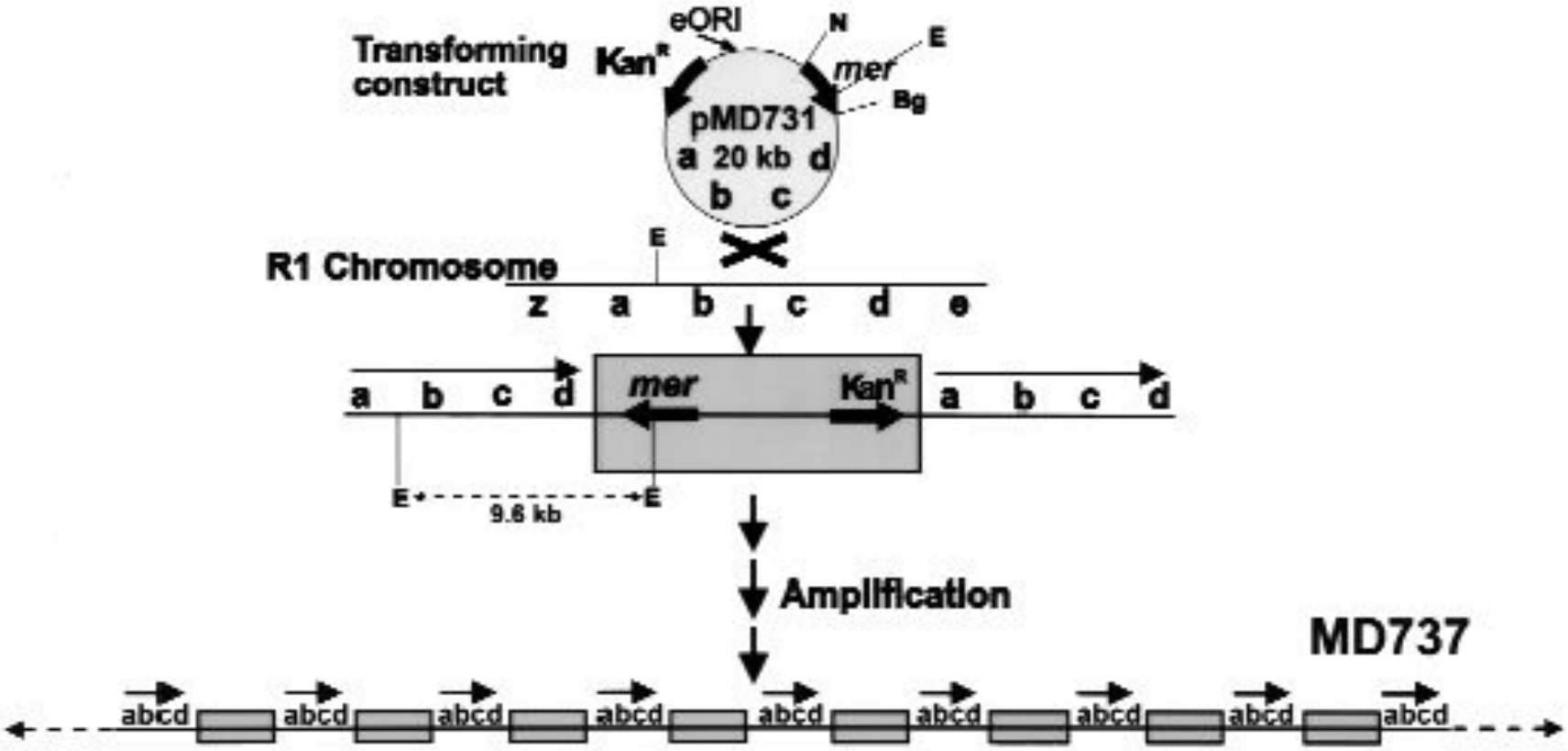


pMD727 was transformed into *D. radiodurans* strain R1 by selection with kanamycin (Kan), giving MD735. dORI, deinococcal origin of replication18; eORI, *E. coli* origin of replication18. P1 and P2 are two different constitutive deinococcal promoters. KanR, kanamycin resistance gene *aphA*; *mer*, mercury operon. Bg/B, *Bgl*II/*BamH*I fusion; M/E, *Mfe*I/*Eco*RI fusion.



pMD728 was transformed into strain R1 with *Km* selection, giving MD736. Two rounds of recombinative duplication are illustrated, yielding two vector copies on a chromosome. bc, duplicated chromosomal target sequence; X, *Xba1*; all other abbreviations and symbols, as in A.

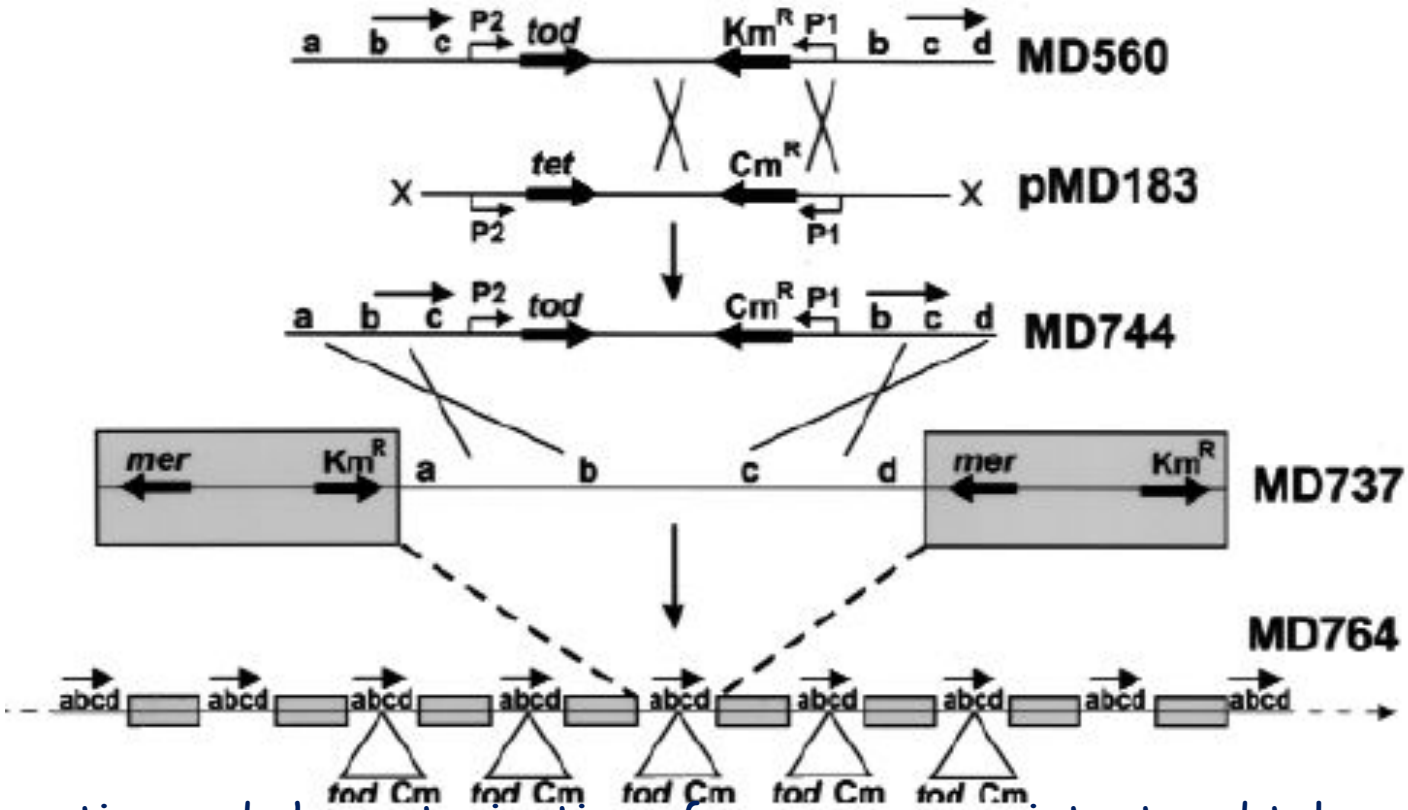
1. Genetic methods: Transformation



pMD731 was transformed into strain R1 with *Km* selection, giving MD737. Several rounds of recombinative duplication are illustrated, yielding many insertions per chromosome. abcd, duplicated chromosomal target sequence; all other abbreviations and symbols, as in A and B above.

1. Genetic methods: Transformation

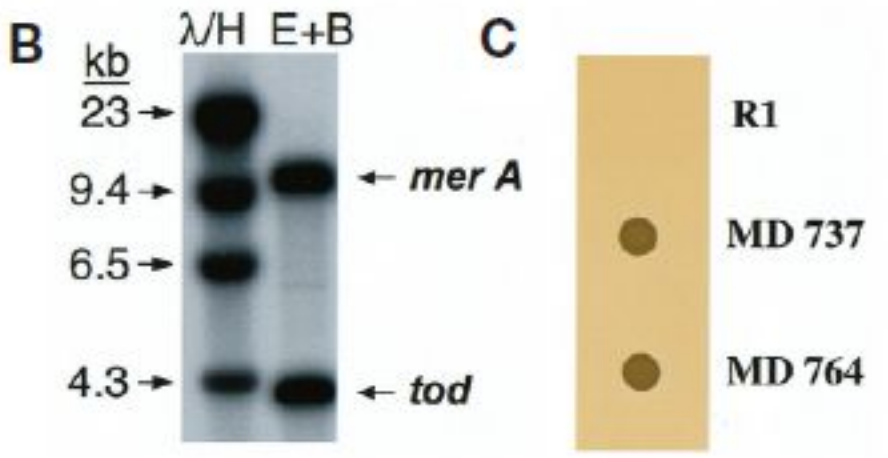
Final objective: to engineer *D. radiodurans* for treatment of mixed radioactive wastes by developing a strain to detoxify both mercury and toluene.

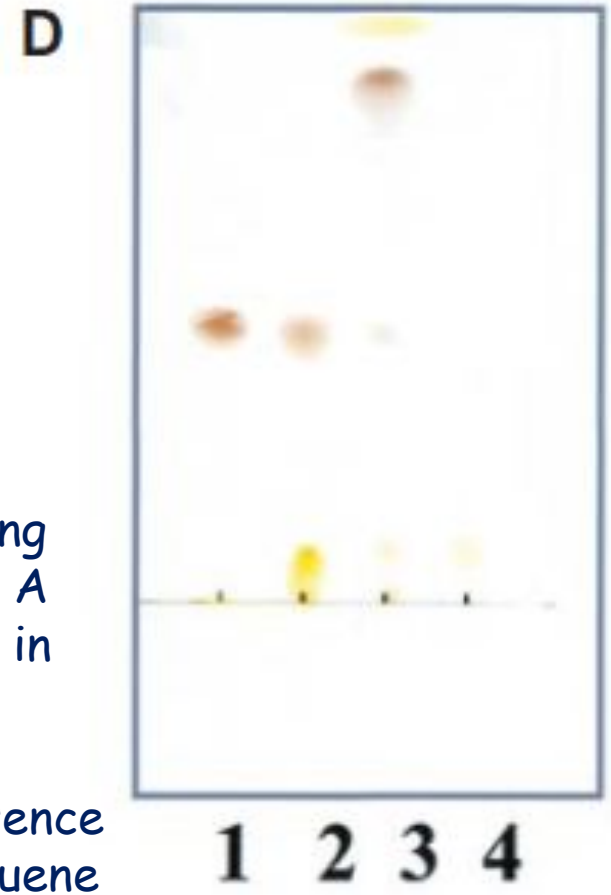


Construction and characterization of a mercury resistant and toluene metabolizing *D. radiodurans*. MD56021 is a previously constructed *D. radiodurans* strain cloned with the *tod* genes of *Pseudomonas putida*, encoding TDO. The *aphA* gene in MD560 was replaced with *cat* by transformation and Cm selection, using *Xba*I linearized pMD18325, forming MD744. MD744 genomic DNA was transformed into MD737 with double *Cm* and KanR selection, giving MD764.

1. Genetic methods: Transformation

Final objective: to engineer *D. radiodurans* for treatment of mixed radioactive wastes by developing a strain to detoxify both mercury and toluene.





(B) Southern blotting of genomic DNA from MD764 using both a *merA*- and a *tod*-specific radiolabeled probe. (C) A TGY agar plate containing 30 mM Merbromin and grown in the irradiator were spotted with 2 x 10^5 cells of the indicated strains. (D) The production of *cis*-toluene dihydrodiol from toluene by MD764 growing in the presence of 50 mM Merbromin, monitored by TLC. Lane 1: *cis*-toluene dihydrodiol; lane 2: organic extract of MD764 supernatant (20h); lane 3: organic extract of MD764 supernatant (40 h); lane 4: organic extract of MD737 supernatant (20 h).

1. Genetic methods: mobile elements

Inteins. Two inteins, protein splicing elements that are typically inserted in genes involved in DNA metabolism and other nucleotide-utilizing enzymes.

One of these is inserted in the ribonucleotide reductase and is similar to the inteins inserted in orthologous enzymes from *B. subtilis*, pyrococci, and chilo iridiscent virus.

The second intein is inserted between the P-loop motif and the Mg2+-binding (Walker B) motif of a SWI2/SNF2 family ATPase, which is involved in chromatin remodeling; this is the first documented instance of an intein interrupting a protein of this family.

1. Genetic methods: mobile elements

Overall, 52 IS elements were detected in the *D. radiodurans* genome (Table 7). The three most abundant ISs are IS4_DR (13 copies), IS2621_DR (11 copies), and IS200_DR (8 copies). IS elements are unevenly distributed on the chromosomes and plasmids. The number of copies per 10,000 nucleotides in the plasmid and the megaplasmid is more than 10 times greater than the number found in chromosomes I and II.

There is, however, little direct evidence for any active transposition in *D.radiodurans*. In the entire genome, there is only one example of gene disruption by an IS element, where *IS2621* is inserted into the gene for alkaline serine exoprotease A (aqualysin I).

TABLE 7. Distribution of insertion sequences in the *D. radiodurans* genome

Name	Family	Length (bp)	Copy no. in:				Total length (bp)
			Plasmid	DR177	DR412	DR_MAIN	
IS*2621*	IS*4*	1,322	0	6	1	6	17,186
IS*2621* (5′ fragment)		25	0	1	2	4	NA
IS*4*_DR	IS*4*	1,207	4	6	0	3	15,942
IS*605*_DR	IS*605*	~1,060	0	0	0	8	8,480
TCL9	Tc1/mariner	1,048	0	1	0	4	5,250
TCL121	Tc1/mariner	1,073	0	2	0	1	3,210
TCL23	Tc1/mariner	1,069	1	1	0	1	3,207
AXL_DR	Tc1/mariner	912	1	0	0	1	1,824
IS*3*_DR	IS*3*	1,304	0	1	0	0	1,300
TNPA2_DR	TNPA	~600	0	0	0	1	600
VCL_DR	IS*15*	~500	1	0	0	0	1,500
DNIV_DR	DNA invertase	~600	1	0	0	0	600
TNPA1_DR	TNPA	~3,000	1	0	0	0	3,000
Total			9	17	1	25	62,099
No. of copies per 10,000 nucleotides			1.97	0.96	0.02	0.09	

1. Genetic methods: mobile elements

TABLE 8. Number of repeats in bacterial genomes

Species	Genome size (Mb)	No. of IS elements	No. of SNRs
D. radiodurans	3.3	52	295
B. subtilis	4.2	0	36
E. coli	4.6	37	263
M. tuberculosis	4.4	32	252
Synechocystis spp.	3.6	NA[a]	118
A. fulgidus	2.2	13	NA

[a] NA, not applicable.

TABLE 9. Distribution of SNRs in the *D. radiodurans* genome

Name	Length (bp)	Copy no. in:			
		Plasmid	DR177	DR412	DR_MAIN
SRE	160	0	3	4	32
SNR1	139	0	0	1	39
SNR2	114	0	0	8	76
SNR4	147	0	1	2	4
SNR5	215	0	0	1	27
SNR7	140	0	2	0	14
SNR8	131	0	0	1	19
SNR9	105	0	0	1	6
SNR10	60	0	0	0	6
Total no.		0	6	18	223
No. of copies per 10,000 nucleotides		0	0.3	0.4	0.8

Also, it was identified several families of small noncoding repeats (SNRs) in the *D.radiodurans* intergenic regions. A comparison to other bacterial genomes showed that, like IS elements, SNRs are more abundant in *D. radiodurans* than in *E. coli*. However, the location bias observed for IS elements appears to be reversed for SNRs. There are no SNRs in the plasmid, that contains five IS elements. In contrast, chromosome II, that contains only one IS element, has 18 SNRs.

1. Genetic methods: Gene inactivation

Inactivation of Proteins Presumed to Be Involved in the Desiccation Tolerance

Mutational inactivation of the genes designated DR1172 and DRB0118 in *Deinococcus radiodurans* R1 greatly sensitizes this species to desiccation, but not to ionizing radiation. These genes encode proteins that share features with the desiccation-induced LEA76 proteins of many plants and the PCC13-62 protein of *Craterostigma plantagineum*, suggesting that *D. radiodurans* may serve as a useful model for the study of desiccation tolerance in higher organisms.

Inactivation method- *In vitro* transposition was performed using the protocol developed by New England Biolabs Beverly,
-> circular pGTC101 was combined with the TnsABC* transposase supplied with the system and target DNA. The vector pGTC101 carries the transposon *Tn*DrCat. When combined with this transposase, the transposon excises from pGTC101 and inserts randomly into the target DNA. The transposition reaction mixture was transformed into targeted cells by electroporation.
-> then colonies with insertion are selected, and the desiccation impact is tested.

2. Proteome analysis

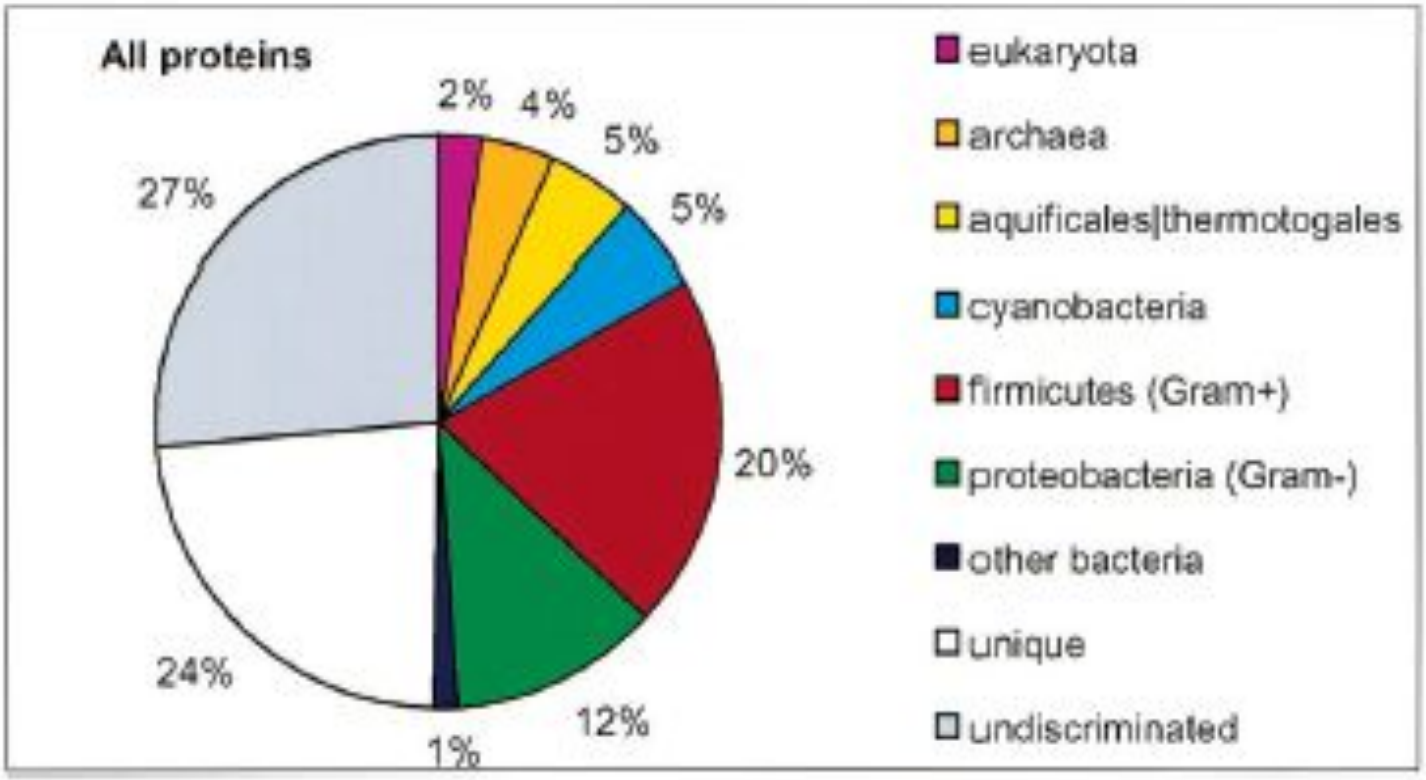


FIG. 8. Taxonomic affinities of *Deinococcus* proteins. We defined a hit to a particular lineage as the best one if it had a BLAST E-value for a protein from this lineage 100 times lower than to any protein from another lineage. Hits to *Thermus-Deinococcus* group species were disregarded.

2. Proteome analysis

Deinococcus radiodurans contains two thioredoxins (Trx and Trx1) and a single thioredoxin reductase (TrxR) as part of its response to oxidative stress. Thioredoxin reductase is a member of the family of pyridine nucleotide-disulfide oxidoreductase flavoenzymes.

Steps:

1. The TrxR gene (DR1982) was obtained by the polymerase chain reaction (PCR) using *D. radiodurans* strain R1 genomic DNA as a template (ATCC). The following were used as forward and reverse oligonucleotide primers for PCR, respectively: 5′-*GCG CCA TGG GTA TGA CGG CAC CTA CTG*-3′ and 5′-*GCG GGA TCC TCA GTC GGC AGCC*-3′.

2. 1kbp fragment was purified by gel extraction, digested with NcoI and BamHI and cloned into a modified pET-30b vector, by which *E.coli* is transformed for cloning.

2. Proteome analysis

3. E.coli was cultures on LB agar plates with kanamycin, Then single colonies were selected and grown overnight in LB media with also kanamycin.

4. The frozen cell pellets were thawed and resuspended in sonication buffer (50 mM Tris–HCl pH 8.0, 1 mM AEBSF, 20 mg ml1 Dnase and 20 mg ml1 lysozyme).

The thawed cells were mechanically disrupted by sonication and cell debris was removed by centrifugation. The resulting supernatant was loaded onto a POROS MC50 metal-chelation column (Applied Biosystems,USA) pre-equilibrated with a buffer containing 5 mM imidazole, 0.5 M NaCl, 50 mM Tris– HCl pH 8.0. The column was washed with a 60 mM imidazole, 0.5 M NaCl, 50 mM Tris–HCl pH 8.0 buffer to remove non-specific binding and the protein eluted with a 0–1 M imidazole gradient. Fractions (10 ml) were collected and the purity of the protein was checked on Coomassie-stained SDS–PAGE.

Reference

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Reference

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Links- https://en.wikipedia.org/wiki/Deinococcus_radiodurans
http://russianpatents.com/patent/241/2418061.html

Thanks for attention