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The RadioP1 – An Integrative Web Resource for Radioresistant Prokaryotes

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Abstract

The extremely radioresistant eubacterium *Deinococcus radiodurans* and the phenotypically related prokaryotes, whose genomes have been completely sequenced, are presently used as model species in several laboratories to study the lethal effects of DNA-damaging and protein-oxidizing agents, particularly the effects of ionizing radiation (IR). Unfortunately, providing relevant information about radioresistant prokaryotes (RP) in a neatly centralized and organized manner still remains a need. In this study, we designed RadioP1 Web resource (www.radiop.org.tn) to gather information about RP defined by the published literature with specific emphasis on (i) predicted genes that produce and protect against oxidative stress, (ii) predicted proteins involved in DNA repair mechanisms and (iii) potential uses of RP in biotechnology. RadioP1 allows the complete RP proteogenomes to be queried using various patterns in a user-friendly and interactive manner. The output data can be saved in plain text, Excel or HyperText Markup Language (HTML) formats for subsequent analyses. Moreover, RadioP1 provides for users a tool “START ANALYSIS”, including the previously described R-packages “drc” and “lethal”, to generate exponential or sigmoid survival curves with D_{10} and D_{50} values. Furthermore, when accessible, links to external databases are provided. Supplementary data will be included in the future when the sequences of other RP genomes will become available.

Keywords: Database, Prokaryotes, RadioP1, Radioresistant, Web resource

1. Introduction

To be considered as an RP, a microorganism should have a D_{10} —the ionizing-radiation (IR) dose necessary to effect a 90 % reduction in colony-forming units (CFU)—threshold that is greater than 1 kilogray (kGy), corresponding to efficient physiological, genetic and proteic protection and repair mechanisms ([1, 2] and references therein). In this context, to our knowledge, even when prokaryotic members belonging to a radioresistant-species-harboursing genus have contrasted optimum temperatures—for example, ranging from 10 to 47 °C—the least IR-resistant ones do not have D_{10} values inferior to 1 kGy [3]. Furthermore, as suggested from D_{10} and F_{10} —the ultraviolet (UV) dose necessary to effect a 90 % reduction in CFU—reported in literature [4-6], an RP is tolerant to both IR (e.g. α and β particles, γ - and x-rays, neutrons) and non-IR (UV light); and correlations were suggested [7]. In this context, it is important to note that UV may cause effects similar to those stimulated by IR [8].

The first RP to be described, designated *Micrococcus radiodurans*, was isolated from irradiated meat [9] and was subsequently reclassified as *D. radiodurans* [10]. IR resistance has been observed in a broad range of prokaryotic groups [11], including halophilic *Archaea* (*Halobacterium* sp.) [12], hyperthermophilic *Archaea* (*Pyrococcus abyssi*, *Pyrococcus furiosus*, *Thermococcus gammatolerans*, *Thermococcus marinus* and *Thermococcus radiotolerans*) [13-16], *Actinobacteria* (*Geodermatophilus dictyosporus*, *Geodermatophilus obscurus*, *Geodermatophilus poikilotrophus*, *Kineococcus radiotolerans*, *Rubrobacter radiotolerans* and *Rubrobacter xylanophilus*) [17-23], *Cyanobacteria* (*Arthrospira* sp. and *Chroococcidiopsis* sp.) [24, 25], the *Deinococcus-Thermus* group (many *Deinococcus* spp. and *Truepera radiovictrix*) ([3, 26-28] and references therein), *Proteobacteria* (*Acinetobacter radioresistens* and *Methylobacterium radiotolerans*) [29, 30] and *Sphingobacteria* (*Hymenobacter actinosclerus*) [31]. Recently, members of the genera *Knoellia*, *Lysobacter*, *Microvirga*, *Nocardioideis*, *Paracoccus*, *Pontibacter* and *Rufibacter* were suggested as RP [32]. However, the list of RP with available experimental D_{10}/F_{10} values (see examples in Table 1) and completely sequenced genomes is still limited.

As a complicated multifaceted phenotype, prokaryotic radioresistance is an important subject in radiation microbiology. A focus on just one contributing mechanism is unlikely to yield a complete understanding of the phenomenon [53]. The radioresistance of prokaryotes depends on their ability to protect enzymes including those needed to repair and replicate DNA from inactivation by oxidative protein damage (protein-centric view) and to fully amend their DNA—double-strand breaks (DSBs)—(DNA-centric view). Obviously, much is yet to be discovered from the mesmerizing radioresistance strategies posed by RP. There is an increasing need to compile the entire data about RP in a centralized and organized manner and to mine it regarding prokaryotic radioresistance. RadioP1 is addressing these requests by providing pertinent information as well as diverse analytical tools. This first version of RadioP1 is a preliminary step towards the establishment of a comprehensive RP database. The increase of the number of side aspects of radioresistance make us keen to collect and to make available for the scientific community the most up-to-date and relevant information.

Organism	D ₁₀ value (⁶⁰ Co, Gy)	F ₁₀ value (UV-C, J/m ²)	Reference
Archaea			
<i>Archaeoglobus fulgidus</i>	1,087	108	[4]
<i>Ignicoccus hospitalis</i> with <i>Nanoarchaeum equitans</i>	1,473	n.d.	[4]
<i>I. hospitalis</i>	1,482	n.d.	[4]
<i>Methanocaldococcus jannaschii</i>	1,036	n.d.	[4]
<i>Pyrococcus furiosus</i>	1,018	64	[4]
<i>Halobacterium salinarum</i>	5,000	280	[12, 33]
<i>Thermococcus stetteri</i>	6,000	30	[11]
Bacteria			
<i>Aquifex pyrophilus</i>	2,842	63	[4]
<i>Deinococcus aerius</i>	4,900	n.d.	[34]
<i>Deinococcus aetherius</i>	>8,000	n.d.	[35]
<i>Deinococcus alpinitundrae</i>	4,000	690	[3]
<i>Deinococcus altitudinis</i>	3,800	550	[3]
<i>Deinococcus claudionis</i>	3,600	310	[3]
<i>Deinococcus deserti</i>	>7,500	n.d.	[36]
<i>Deinococcus ficus</i>	11,000	n.d.	[37]
<i>Deinococcus geothermalis</i>	5,100–16,000	1800	[38-40]
<i>Deinococcus gobiensis</i>	12,700	>800	[41]
<i>Deinococcus grandis</i>	7,000–11,000	n.d.	[37, 42, 43]
<i>Deinococcus guangriensis</i>	9,800	n.d.	[44]
<i>Deinococcus indicus</i>	4,200	n.d.	[37]
<i>Deinococcus mumbaiensis</i>	17,000	n.d.	[45]
<i>Deinococcus murrayi</i>	9,100	n.d.	[40]
<i>Deinococcus piscis</i>	7,400	n.d.	[46]
<i>Deinococcus proteolyticus</i>	10,300	n.d.	[37]
<i>Deinococcus radiodurans</i>	6,700–16,000	660–2000	[3, 37-39, 47-50]
<i>Deinococcus radiomollis</i>	2,200	220	[3]
<i>Deinococcus radiophilus</i>	>16,000	n.d.	[51]
<i>Deinococcus radiopugnans</i>	5,300	n.d.	[52]
<i>Enterococcus faecium</i>	2,000	n.d.	[38]
<i>Geodermatophilus poikilotrophi</i>	9,000	6300–12600	[22]
n.d.: not determined			
For further details: www.radiop.org.tn			

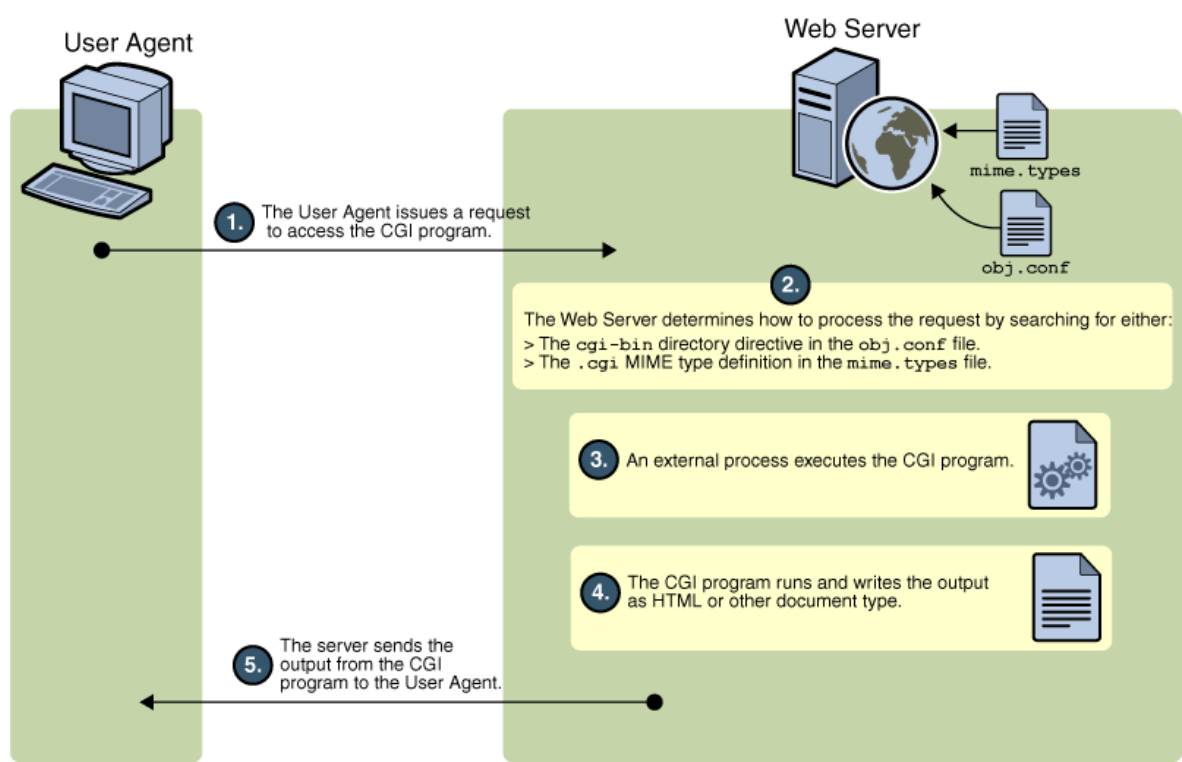
Table 1. F₁₀ and D₁₀ values for 90 % reduction of selected radioresistant prokaryotes (RP)

2. Source of data

Used information was obtained by searching the NCBI database [54]. Clusters of Orthologous Group (COG) [55] were used to classify orthologous gene records in RadioP1. Orthology was calculated with Basic Local Alignment Search Tool (BLAST), the best reciprocal hit approach and InParanoid program.

3. Database construction

We built the database on a recent version of Linux operation system. MySQL workbench 5.2.38 was used to handle the database schema and to build the relational database. Perl scripts were developed to retrieve genome data and gene information from GenBank files collected from NCBI using file transfer protocol (FTP) and to store RP information in the appropriate database tables. A frontend user interface was developed using HTML5, JavaScript, Cascading Style Sheet (CSS) and Hypertext Preprocessor (PHP) program languages. Perl CGI (Common Gateway Interface) modules and PHP scripts were developed and used to link the Web interface to the database. These scripts allow all users to send requests via the Web interface to the server, run the jobs on the server and then return and display results on the Web interface (Figure 1).



Data source: <http://docs.oracle.com/cd/E19146-01/821-1828/6nmpm01g9/index.html>

Figure 1. Overview of the interactions between the Web interface and the database using CGI modules

The database schema (Figure 2) consists of 13 tables, allowing to search and to retrieve any stored biological data. Among the main tables is the species table (primary information: organism name and taxon ID), which is connected to the taxonomy and chromosome tables. from NCBI, and is linked to the taxonomy and chromosome tables. This later is connected to seqfile tables detailing the different file formats and paths related to each chromosome. The gene table, related to the chromosome table, stores information such as gene name, gene ID, symbol, first position, last position and strand. The gene table is linked to the orthology table.

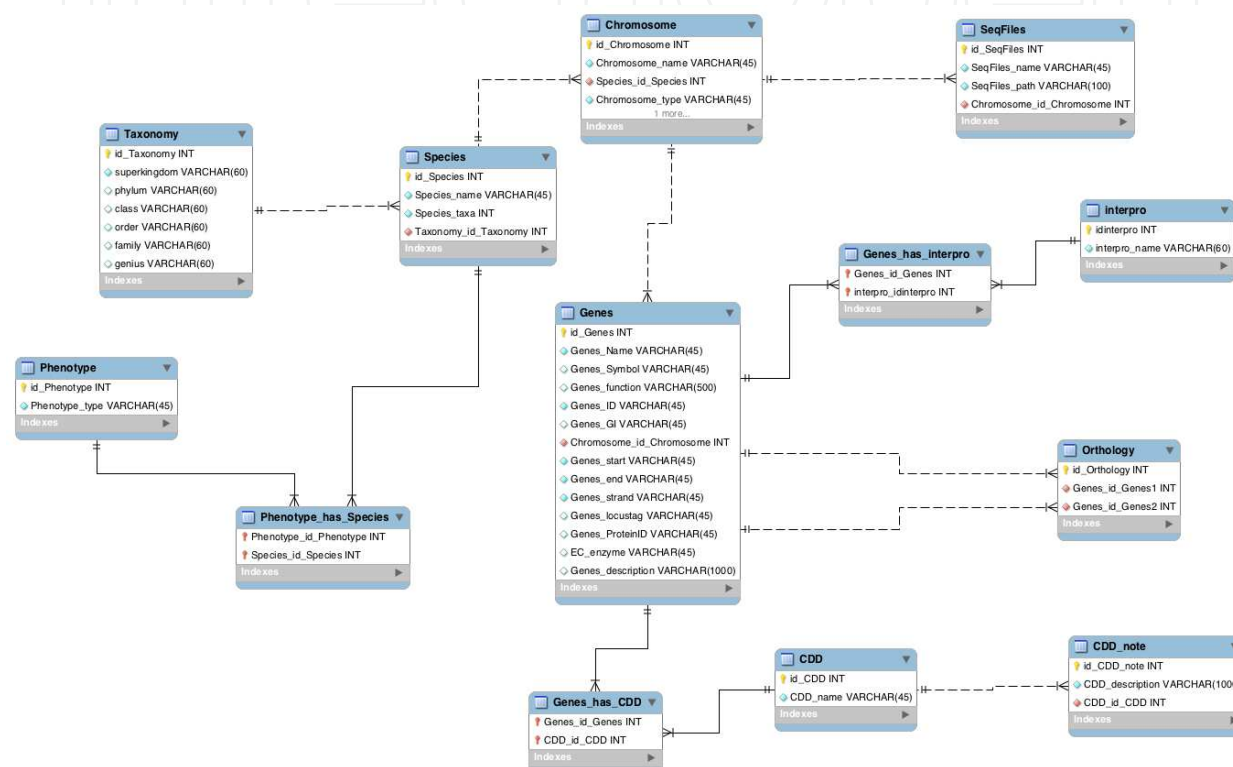


Figure 2. RadioP1 database structure

4. RadioP1 database user guide

RadioP1 is freely accessible through a Web browser at <http://www.radiop.org.tn>. There are at least three ways to use the database: browse, search and generate data.

5. Browse in the database

In the main page of RadioP1, a clickable list of currently available groups of IRRP—ionizing-radiation-resistant prokaryotes—is organized at the top-left side, allowing users to browse pages for each of the groups, IR-resistant archaea (IRRA) and IR-resistant bacteria (IRRB).

6. Search the database

RadioP1 provides a search engine that is able to extract information from the database through: (i) text search, (ii) BLAST search and (iii) function category search..

The text and homology search contains three categories:

1. “SEARCH GENES”: This search category allows extracting annotation information—gene symbol, chromosome name, strand, predicted orthologous genes, etc.—using querying gene locus tags.. The querying results are displayed in a table with each hit represented by a row containing a corresponding gene ID and a summary of characteristics—gene name, symbol, strand and product. In addition, each listed row in the output table provides a link to the individual gene pages, which highlight the querying genes found in the page of NCBI [54]. Users can get results in HTML, plain text or Excel formats for further analyses.
2. “RETRIEVE SEQUENCES”: This search category enables extracting nucleic or proteic sequences using querying gene locus tags.
3. “HOMOLOGY DATA”: This search category enables extracting predicted orthologous gene clusters using querying gene locus tags.

The function category search contains four subclasses:

1. “OXIDATIVE STRESS PRODUCTION”:

When the generation of reactive oxygen species (ROS; superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl (HO^{\cdot}) radicals) produced by metabolism or irradiation exceeds the capacity of endogenous scavengers to neutralize them, cells become vulnerable to damage, a condition referred to as oxidative stress [56, 57]. Typically, during irradiation, ~80 % of DNA damage is caused indirectly by irradiation-induced ROS and the remaining ~20 % by direct interaction between c-photons and DNA [57]. HO^{\cdot} radicals are the primary product of the radiolysis of water and in the presence of oxygen, can also generate some $O_2^{\cdot-}$ and H_2O_2 by dismutation of $O_2^{\cdot-}$ [57]. In contrast, the primary ROS generated by metabolism are $O_2^{\cdot-}$ and H_2O_2 [56]. The total intracellular titer of cytochromes and flavins might serve as a marker for the proclivity of cells to survive radiation and other oxidizing conditions [58, 59]. For instance, the total number of c-type cytochromes in *D. radiodurans* and *Shewanella oneidensis* ($D_{10} = 70$ Gy [38]) is 7 and 39, respectively [58]. Searching RadioP1 by this function subcategory provides a way to find out predicted genes involved in ROS production and subsequently estimate cellular radioresistance level.

2. “OXIDATIVE STRESS PROTECTION”:

Unlike DNA DSB lesion yields ([6] and references therein), in IR-sensitive cells, yields of IR-induced protein oxidation can be ~100 times greater than in IR-resistant cells [60, 61]. Indeed, presently, it is demonstrated that proteins are major targets of IR damage and that shield against protein oxidation is an important mechanism for survival from IR exposure. IR resistance in some prokaryotes was highly correlated to the accumulation of high intracellular

concentration of Mn^{2+} , supporting the idea of a common model of Mn^{2+} -dependent ROS scavenging in the aerobes ([6, 62] and references therein). For example, the aerobic archaeon *H. salinarum* accumulates high intracellular concentration of Mn^{2+} , 155 ng/10⁹ cells [62, 63]. In contrast, hyperthermophilic anaerobic archaea *T. gammatolerans* and *P. furiosus* do not contain significant amounts of intracellular Mn^{2+} , 3 ng/10⁹ cells and 14 ng/10⁹ cells, respectively [62]. These low concentrations of Mn^{2+} in anaerobic hyperthermophiles were explained by the low levels of IR-generated ROS under anaerobic conditions combined with efficient detoxification systems [62]. In RadioP1, using the “OXIDATIVE STRESS PROTECTION” function subcategory, a summary table is provided to users giving insights about radioprotectors of each RP.

3. “DNA REPAIR GENES”:

During irradiation, DNA double-strand breaks (DSBs) are considered as the most lethal damage, although they are the least frequent form of cellular DNA damage—compared to single-strand breaks and DNA base damages [60]. For example, in *D. radiodurans*, PprA protein has an important role in DNA DSBs repair [64]. Exploring RadioP1 by the “DNA REPAIR GENES” function subcategory allows users to generate a list of genes—orthologs of genes in Table 2—for which a functional knockout may change the level of radioresistance of mutant cells.

Gene name (locus tag, D ₁₀ of a <i>D. radiodurans</i> strain lacking the gene)	Reference
<i>recA</i> (DR_2340, 0.1 kGy)	[65]
<i>polA</i> (DR_1707, 1 kGy)	[66]
<i>recQ</i> (DR_1289, 6 kGy)	[67]
<i>recD</i> (DR_1902, 6 kGy)	[68]
<i>crtB</i> (DR_0862, 9 kGy)	[69]
<i>crtI</i> (DR_0861, 9 kGy)	[69]
<i>sbcC</i> (DR_1922, 15 kGy)	[70]
<i>sbcD</i> (DR_1921, 15 kGy)	[70]
<i>polX</i> (DR_0467, 15 kGy)	[71]
<i>pprA</i> (DR_A0346, 2 kGy)	[64]
<i>ddrB</i> (DR_0070, 8 kGy)	[65]
<i>ddrA</i> (DR_0423, 12 kGy)	[65]
<i>ddrC</i> (DR_0003, > 14 kGy)	[65]
<i>ddrD</i> (DR_0326, > 14 kGy)	[65]

Table 2. Selected important DNA protection and repair genes for ionizing-radiation-resistant prokaryotes (IRRP) based on data of *Deinococcus radiodurans*

4. “USE IN BIOTECHNOLOGY”:

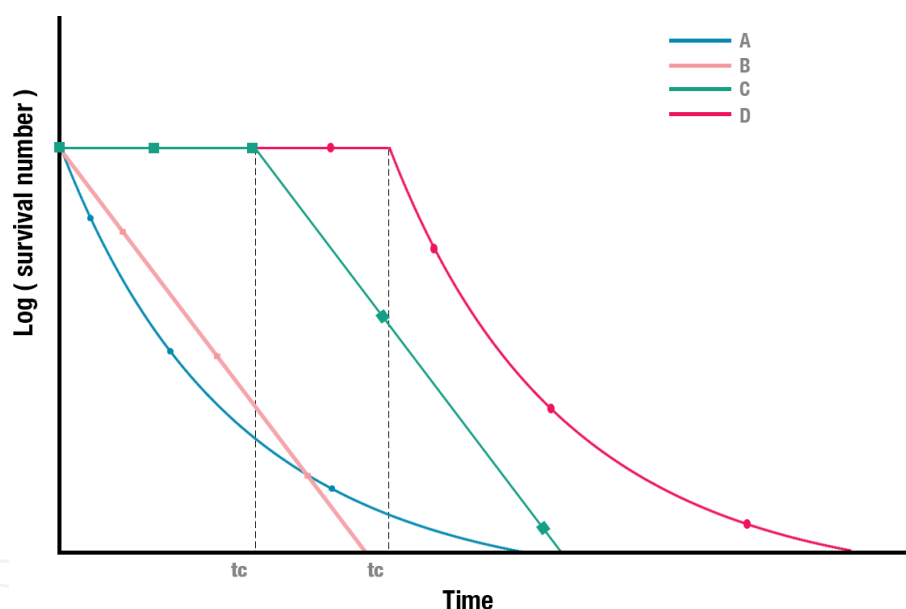
RP provide inestimable opportunities in therapeutics for multiple diseases [72], biotechnology [73], pharmaceuticals [74] and bioengineering—bioremediation—of toxic and radioactive compounds [75-81]. The function “USE IN BIOTECHNOLOGY” in RadioP1 was designed to

present the diversity of IRRP genomes in terms of genes with potential applications in biotechnology.

7. Generate survival plots and D_x (D_{10} and D_{50}) values

Cell survival models aim to describe the relationship between the absorbed dose and the fraction of surviving cells—cell survival curve. Distinct cell survival models were described [82-86]: the linear—single-hit single-target, the linear-quadratic (LQ) and the repairable-conditionally repairable damage (RCR) models. Other models include those based on target theory first described by Lea [87] and those described by Tobias [88], Curtis [89] and Sontag [90].

For instance, for UV-C-irradiated prokaryotes, as summarized previously [91], the mathematical dose-response models which describe the probability of a specific biological response at a given dose can be represented as follows (Figure 3):



Shoulder curve (A), linear (B), two-stage curve (C) and sigmoid (D)

$$N = N_0 e^{-\alpha(t-t_c)} \text{ for } t \geq t_c \text{ (A)}$$

$$N = N_0 e^{-\alpha t} \text{ (B)}$$

$$N = N_0 [(1-F_0)e^{-\alpha_1 t} + F_0 e^{-\alpha_2 t}] \text{ (C)}$$

$$N = N_0 [(1-F_0)e^{-\alpha_1 (t-t_c)} + F_0 e^{-\alpha_2 (t-t_c)}] \text{ for } t \geq t_c \text{ (D)}$$

Where:

* N and N_0 represent the microorganisms surviving at time t and those initially present at time $t = 0$ respectively.

* α is a parameter proportional to the applied UV-C intensity and depends on the sensitivity of the microorganism to the UV-C ray exposure.

* t_c is the time during which microorganisms are substantially not inactivated.

* F_0 represents the most resistant fraction, characterized by a lower sensitivity to the UV-C rays exposure, in a population of microorganisms, compared to the fraction $(1 - F_0)$ less resistant to such exposure.

Figure 3. Summary of prokaryotic survival curves

RadioP1 provides a tool “START ANALYSIS” for users to generate exponential survival curves [92, 93]. In addition, it integrated the previously described R-packages “drc” [94] for sigmoid curves and “lethal” [95] that computes lethal doses (LD) with confidence intervals [22]. All curves are supplied with D_{10} and D_{50} values.

8. Future directions

RadioP1 is a specialized database aimed at making a comprehensive repository of identified RP with experimentally determined D_{10} . It is complemented by data extraction and analysis tools to help further analysis of RP. Researchers are kindly requested and encouraged to invigorate RadioP1 by depositing their new results— D_{10} —of RP at RadioP1. Submission might either be performed through the “Submit new RP with a D_{10} ” form accessible under the IRRP main page or by e-mail to corresponding authors. In the future, we intend to include more detailed information about RP in the area of evolutionary biology, biotechnology and therapeutics. Additional data sources like Kyoto Encyclopedia of Genes and Genomes (KEGG) and COGs will be integrated to extract further information about gene functions, clusters and pathways, helping users to categorise genes of interest into functional units and perform more efficient analysis on RP genomes.

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References

- [1] Sghaier H: DNA Repair: Lessons from the Evolution of Ionizing- Radiation-Resistant Prokaryotes – Fact and Theory. In: *Selected Topics in DNA Repair*. Edited by Clark CC, University of California. San Diego: InTech; 2011: 145–156.
- [2] Sghaier H, Ghedira K, Benkahla A, Barkallah I: Basal DNA repair machinery is subject to positive selection in ionizing-radiation-resistant bacteria. *BMC Genomics* 2008, 9:297.
- [3] Callegan RP, Nobre MF, McTernan PM, Battista JR, Navarro-Gonzalez R, McKay CP, da Costa MS, Rainey FA: Description of four novel psychrophilic, ionizing radiation-sensitive *Deinococcus* species from alpine environments. *Int J Syst Evol Microbiol* 2008, 58(Pt 5):1252–1258.
- [4] Beblo K, Douki T, Schmalz G, Rachel R, Wirth R, Huber H, Reitz G, Rettberg P: Survival of thermophilic and hyperthermophilic microorganisms after exposure to UV-C, ionizing radiation and desiccation. *Arch Microbiol* 2011, 193(11):797–809.
- [5] Daly MJ: Modulating radiation resistance: insights based on defenses against reactive oxygen species in the radioresistant bacterium *Deinococcus radiodurans*. *Clin Lab Med* 2006, 26(2):491–504.

- [6] Daly MJ: Death by protein damage in irradiated cells. *DNA Repair (Amst)* 2011, 11(1): 12–21.
- [7] Arrange AA, Phelps TJ, Benoit RE, Palumbo AV, White DC: Bacterial sensitivity to UV light as a model for ionizing radiation resistance. *J Microbiol Methods* 1993, 18(2): 127–136.
- [8] Nikogosyan DN: Two-quantum UV photochemistry of nucleic acids: comparison with conventional low-intensity UV photochemistry and radiation chemistry. *Int J Radiat Biol* 1990, 57(2):233–299.
- [9] Anderson AW, Nordan HC, Cain RF, Parrish G, Duggan D: Studies on a radio-resistant *Micrococcus*. I. Isolation, morphology, cultural characteristics, and resistance to gamma radiation. *Food Technol* 1956, 10:575–577.
- [10] Brooks BW, Murray RGE: Nomenclature for “*Micrococcus radiodurans*” and other radiation-resistant cocci: *Deinococcaceae* fam. nov. and *Deinococcus* gen. nov., including five species. *Int J Syst Bacteriol* 1981, 31:353–360.
- [11] Kopylov VM, Bonch-Osmolovskaya EA, Svetlichnyi VA, Miroshnicheko ML, Skobin VS: Gamma-irradiation resistance and UV sensitivity of extremely thermophilic archaeobacteria and eubacteria. *Mikrobiologiya* 1993(62):90–95.
- [12] Kottemann M, Kish A, Iloanusi C, Bjork S, DiRuggiero J: Physiological responses of the halophilic archaeon *Halobacterium* sp. strain NRC1 to desiccation and gamma irradiation. *Extremophiles* 2005, 9(3):219–227.
- [13] DiRuggiero J, Santangelo N, Nackerdien Z, Ravel J, Robb FT: Repair of extensive ionizing-radiation DNA damage at 95 degrees C in the hyperthermophilic archaeon *Pyrococcus furiosus*. *J Bacteriol* 1997, 179(14):4643–4645.
- [14] Jolivet E, Matsunaga F, Ishino Y, Forterre P, Prieur D, Myllykallio H: Physiological responses of the hyperthermophilic archaeon “*Pyrococcus abyssi*” to DNA damage caused by ionizing radiation. *J Bacteriol* 2003, 185(13):3958–3961.
- [15] Jolivet E, Corre E, L'Haridon S, Forterre P, Prieur D: *Thermococcus marinus* sp. nov. and *Thermococcus radiotolerans* sp. nov., two hyperthermophilic archaea from deep-sea hydrothermal vents that resist ionizing radiation. *Extremophiles* 2004, 8(3):219–227.
- [16] Jolivet E, L'Haridon S, Corre E, Forterre P, Prieur D: *Thermococcus gammatolerans* sp. nov., a hyperthermophilic archaeon from a deep-sea hydrothermal vent that resists ionizing radiation. *Int J Syst Evol Microbiol* 2003, 53(Pt 3):847–851.
- [17] Yoshinaka T, Yano K, Yamaguchi H: Isolation of highly radioresistant bacterium, *Arthrobacter radiotolerans* nov. sp. *Agr Biol Chem* 1973, 37(10):2269–2275.

- [18] Ferreira AC, Nobre MF, Moore E, Rainey FA, Battista JR, da Costa MS: Characterization and radiation resistance of new isolates of *Rubrobacter radiotolerans* and *Rubrobacter xylanophilus*. *Extremophiles* 1999, 3(4):235–238.
- [19] Chen MY, Wu SH, Lin GH, Lu CP, Lin YT, Chang WC, Tsay SS: *Rubrobacter taiwanensis* sp. nov., a novel thermophilic, radiation-resistant species isolated from hot springs. *Int J Syst Evol Microbiol* 2004, 54(Pt 5):1849–1855.
- [20] Phillips RW, Wiegel J, Berry CJ, Fliermans C, Peacock AD, White DC, Shinkets LJ: *Kineococcus radiotolerans* sp. nov., a radiation-resistant, gram-positive bacterium. *Int J Syst Evol Microbiol* 2002, 52(Pt 3):933–938.
- [21] Gtari M, Essoussi I, Maaoui R, Sghaier H, Boujmil R, Gury J, Pujic P, Brusetti L, Chouaia B, Crotti E *et al*: Contrasted resistance of stone-dwelling *Geodermatophilaceae* species to stresses known to give rise to reactive oxygen species. *FEMS Microbiol Ecol* 2012, 80(3):566–577.
- [22] Montero-Calasanz MC, Hofner B, Göker M, Rohde M, Spröer C, Hezbri K, Gtari M, Schumann P, Klenk HP: *Geodermatophilus poikilotrophi* sp. nov.: a multitolerant actinomyces isolated from dolomitic marble. *Biomed Res Int* 2014, 2014:11.
- [23] Montero-Calasanz MC, Hezbri K, Göker M, Sghaier H, Rohde M, Spröer C, Schumann P, Klenk HP: Description of gamma radiation-resistant *Geodermatophilus dictyosporus* sp. nov. to accommodate the not validly named *Geodermatophilus obscurus* subsp. *dictyosporus* (Luedemann, 1968). *Extremophiles* 2015, 19(1):77–85.
- [24] Billi D, Friedmann EI, Hofer KG, Caiola MG, Ocampo-Friedmann R: Ionizing-radiation resistance in the desiccation-tolerant cyanobacterium *Chroococcidiopsis*. *Appl Environ Microbiol* 2000, 66(4):1489–1492.
- [25] Badri H, Monsieus P, Coninx I, Wattiez R, Leys N: Molecular investigation of the radiation resistance of edible cyanobacterium *Arthrospira* sp. PCC 8005. *Microbiologyopen* 2015:doi: 10.1002/mbo1003.1229.
- [26] Albuquerque L, Simoes C, Nobre MF, Pino NM, Battista JR, Silva MT, Rainey FA, da Costa MS: *Truepera radiovictrix* gen. nov., sp. nov., a new radiation resistant species and the proposal of *Trueperaceae* fam. nov. *FEMS Microbiol Lett* 2005, 247(2):161–169.
- [27] Dong N, Li HR, Yuan M, Zhang XH, Yu Y: *Deinococcus antarcticus* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* 2015, 65(Pt 2):331–335.
- [28] Srinivasan S, Lee JJ, Lim SY, Joe MH, Im SH, Kim MK: *Deinococcus radioresistens* sp. nov., a UV and gamma radiation-resistant bacterium isolated from mountain soil. *Antonie Van Leeuwenhoek* 2015, 107(2):539–545.
- [29] Ito H, Iizuka H: Taxonomic studies on a radio-resistant *Pseudomonas*. Part XII. Studies on the microorganisms of cereal grain. *Agric Biol Chem* 1971, 35(10):1566–1571.

- [30] Nishimura Y, Uchida K, Tanaka K, Ino T, Ito H: Radiation sensitivities of *Acinetobacter* strains isolated from clinical sources. *J Basic Microbiol* 1994, 34(5):357–360.
- [31] Collins MD, Hutson RA, Grant IR, Patterson MF: Phylogenetic characterization of a novel radiation-resistant bacterium from irradiated pork: description of *Hymenobacter actinosclerus* sp. nov. *Int J Syst Evol Microbiol* 2000, 50(Pt 2):731–734.
- [32] Yu LZ, Luo XS, Liu M, Huang Q: Diversity of ionizing radiation-resistant bacteria obtained from the Taklimakan Desert. *J Basic Microbiol* 2015, 55(1):135–140.
- [33] Shahmohammadi HR, Asgarani E, Terato H, Saito T, Ohyama Y, Gekko K, Yamamoto O, Ide H: Protective roles of bacterioruberin and intracellular KCl in the resistance of *Halobacterium salinarium* against DNA-damaging agents. *J Radiat Res* 1998, 39(4):251–262.
- [34] Yang Y, Itoh T, Yokobori S, Itahashi S, Shimada H, Satoh K, Ohba H, Narumi I, Yamagishi A: *Deinococcus aeri* sp. nov., isolated from the high atmosphere. *Int J Syst Evol Microbiol* 2009, 59(Pt 8):1862–1866.
- [35] Yang Y, Itoh T, Yokobori S, Shimada H, Itahashi S, Satoh K, Ohba H, Narumi I, Yamagishi A: *Deinococcus aetherius* sp. nov., isolated from the stratosphere. *Int J Syst Evol Microbiol* 2010, 60(Pt 4):776–779.
- [36] de Groot A, Chapon V, Servant P, Christen R, Saux MF, Sommer S, Heulin T: *Deinococcus deserti* sp. nov., a gamma-radiation-tolerant bacterium isolated from the Sahara Desert. *Int J Syst Evol Microbiol* 2005, 55(Pt 6):2441–2446.
- [37] Shashidhar R, Kumar SA, Misra HS, Bandekar JR: Evaluation of the role of enzymatic and nonenzymatic antioxidant systems in the radiation resistance of *Deinococcus*. *Can J Microbiol* 2010, 56(3):195–201.
- [38] Daly MJ, Gaidamakova EK, Matrosova VY, Vasilenko A, Zhai M, Venkateswaran A, Hess M, Omelchenko MV, Kostandarithes HM, Makarova KS *et al*: Accumulation of Mn(II) in *Deinococcus radiodurans* facilitates gamma-radiation resistance. *Science* 2004, 306(5698):1025–1028.
- [39] Makarova KS, Omelchenko MV, Gaidamakova EK, Matrosova VY, Vasilenko A, Zhai M, Lapidus A, Copeland A, Kim E, Land M *et al*: *Deinococcus geothermalis*: the pool of extreme radiation resistance genes shrinks. *PLoS One* 2007, 2(9):e955.
- [40] Ferreira AC, Nobre MF, Rainey FA, Silva MT, Wait R, Burghardt J, Chung AP, da Costa MS: *Deinococcus geothermalis* sp. nov. and *Deinococcus murrayi* sp. nov., two extremely radiation-resistant and slightly thermophilic species from hot springs. *Int J Syst Bacteriol* 1997, 47(4):939–947.
- [41] Yuan M, Zhang W, Dai S, Wu J, Wang Y, Tao T, Chen M, Lin M: *Deinococcus gobiensis* sp. nov., an extremely radiation-resistant bacterium. *Int J Syst Evol Microbiol* 2009, 59(Pt 6):1513–1517.

- [42] Chanal A, Chapon V, Benzerara K, Barakat M, Christen R, Achouak W, Barras F, Heulin T: The desert of Tataouine: an extreme environment that hosts a wide diversity of microorganisms and radiotolerant bacteria. *Environ Microbiol* 2006, 8(3):514–525.
- [43] Oyaizu H, Stackebrandt E, Schleifer KH, Ludwig W, Pohla H, Ito H, Hirata A, Oyaizu Y, Komagata K: A radiation-resistant rod-shaped bacterium, *Deinobacter grandis* gen. nov., sp. nov., with peptidoglycan containing ornithine. *Int J Syst Bacteriol* 1987, 37:62–67.
- [44] Sun J, Shen P, Chao H, Wu B: Isolation and identification of a new radiation-resistant bacterium *Deinococcus guangriensis* sp.nov. and analysis of its radioresistant character. *Wei Sheng Wu Xue Bao* 2009, 49(7):918–924.
- [45] Shashidhar R, Bandekar JR: *Deinococcus mumbaiensis* sp. nov., a radiation-resistant pleomorphic bacterium isolated from Mumbai, India. *FEMS Microbiol Lett* 2006, 254(2):275–280.
- [46] Shashidhar R, Bandekar JR: *Deinococcus piscis* sp. nov., a radiation-resistant bacterium isolated from a marine fish. *Int J Syst Evol Microbiol* 2009, 59(Pt 11):2714–2717.
- [47] Battista JR, Earl AM, Park MJ: Why is *Deinococcus radiodurans* so resistant to ionizing radiation? *Trends Microbiol* 1999, 7(9):362–365.
- [48] Bauermeister A, Bentchikou E, Moeller R, Rettberg P: Roles of PprA, IrrE, and RecA in the resistance of *Deinococcus radiodurans* to germicidal and environmentally relevant UV radiation. *Arch Microbiol* 2009, 191(12):913–918.
- [49] Shukla M, Chaturvedi R, Tamhane D, Vyas P, Archana G, Apte S, Bandekar J, Desai A: Multiple-stress tolerance of ionizing radiation-resistant bacterial isolates obtained from various habitats: correlation between stresses. *Curr Microbiol* 2007, 54(2):142–148.
- [50] Battista JR: Against all odds: the survival strategies of *Deinococcus radiodurans*. *Annu Rev Microbiol* 1997, 51:203–224.
- [51] Lewis NF: Studies on a radio-resistant coccus isolated from Bombay duck (*Harpodon nehereus*). *J Gen Microbiol* 1971, 66(1):29–35.
- [52] Davis NS, Silverman GJ, Keller WH: Combined effects of ultrahigh vacuum and temperature on the viability of some spores and soil organisms. *Appl Microbiol* 1963, 11:202–210.
- [53] Harris DR, Pollock SV, Wood EA, Goiffon RJ, Klingele AJ, Cabot EL, Schackwitz W, Martin J, Eggington J, Durfee TJ *et al*: Directed evolution of ionizing radiation resistance in *Escherichia coli*. *J Bacteriol* 2009, 191(16):5240–5252.
- [54] Wheeler DL, Chappey C, Lash AE, Leipe DD, Madden TL, Schuler GD, Tatusova TA, Rapp BA: Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res* 2000, 28(1):10–14.

- [55] Tatusov RL, Galperin MY, Natale DA, Koonin EV: The COG database: a tool for genome-scale analysis of protein functions and evolution. *Nucleic Acids Res* 2000, 28(1): 33–36.
- [56] Imlay JA: Pathways of oxidative damage. *Annu Rev Microbiol* 2003, 57:395–418.
- [57] Halliwell B, Gutteridge JMC: Free Radicals in Biology and Medicine, 4 edn. Oxford: Oxford University Press; 2007.
- [58] Ghosal D, Omelchenko MV, Gaidamakova EK, Matrosova VY, Vasilenko A, Venkateswaran A, Zhai M, Kostandarithes HM, Brim H, Makarova KS *et al*: How radiation kills cells: survival of *Deinococcus radiodurans* and *Shewanella oneidensis* under oxidative stress. *FEMS Microbiol Rev* 2005, 29(2):361–375.
- [59] Messner KR, Imlay JA: Mechanism of superoxide and hydrogen peroxide formation by fumarate reductase, succinate dehydrogenase, and aspartate oxidase. *J Biol Chem* 2002, 277(45):42563–42571.
- [60] Daly MJ: A new perspective on radiation resistance based on *Deinococcus radiodurans*. *Nat Rev Microbiol* 2009, 7(3):237–245.
- [61] Daly MJ, Gaidamakova EK, Matrosova VY, Kiang JG, Fukumoto R, Lee DY, Wehr NB, Viteri GA, Berlett BS, Levine RL: Small-molecule antioxidant proteome-shields in *Deinococcus radiodurans*. *PLoS One* 2010, 5(9):e12570.
- [62] Webb KM, DiRuggiero J: Role of Mn²⁺ and compatible solutes in the radiation resistance of thermophilic Bacteria and Archaea. *Archaea* 2012, 2012:11.
- [63] Robinson CK, Webb K, Kaur A, Jaruga P, Dizdaroglu M, Baliga NS, Place A, Diruggiero J: A major role for nonenzymatic antioxidant processes in the radioresistance of *Halobacterium salinarum*. *J Bacteriol* 2011, 193(7):1653–1662.
- [64] Narumi I, Satoh K, Cui S, Funayama T, Kitayama S, Watanabe H: PprA: a novel protein from *Deinococcus radiodurans* that stimulates DNA ligation. *Mol Microbiol* 2004, 54(1):278–285.
- [65] Tanaka M, Earl AM, Howell HA, Park MJ, Eisen JA, Peterson SN, Battista JR: Analysis of *Deinococcus radiodurans*'s transcriptional response to ionizing radiation and desiccation reveals novel proteins that contribute to extreme radioresistance. *Genetics* 2004, 168(1):21–33.
- [66] Gutman PD, Fuchs P, Minton KW: Restoration of the DNA damage resistance of *Deinococcus radiodurans* DNA polymerase mutants by *Escherichia coli* DNA polymerase I and Klenow fragment. *Mutat Res* 1994, 314(1):87–97.
- [67] Huang L, Hua X, Lu H, Gao G, Tian B, Shen B, Hua Y: Three tandem HRDC domains have synergistic effect on the RecQ functions in *Deinococcus radiodurans*. *DNA Repair (Amst)* 2007, 6(2):167–176.

- [68] Servinsky MD, Julin DA: Effect of a *recD* mutation on DNA damage resistance and transformation in *Deinococcus radiodurans*. *J Bacteriol* 2007, 189(14):5101–5107.
- [69] Zhang L, Yang Q, Luo X, Fang C, Zhang Q, Tang Y: Knockout of *crtB* or *crtI* gene blocks the carotenoid biosynthetic pathway in *Deinococcus radiodurans* R₁ and influences its resistance to oxidative DNA-damaging agents due to change of free radicals scavenging ability. *Arch Microbiol* 2007, 188(4):411–419.
- [70] Bentchikou E, Servant P, Coste G, Sommer S: Additive effects of SbcCD and PolX deficiencies in the in vivo repair of DNA double-strand breaks in *Deinococcus radiodurans*. *J Bacteriol* 2007, 189(13):4784–4790.
- [71] Lecointe F, Shevelev IV, Bailone A, Sommer S, Hubscher U: Involvement of an X family DNA polymerase in double-stranded break repair in the radioresistant organism *Deinococcus radiodurans*. *Mol Microbiol* 2004, 53(6):1721–1730.
- [72] Singh OV, Gabani P: Extremophiles: radiation resistance microbial reserves and therapeutic implications. *J Appl Microbiol* 2011, 110(4):851–861.
- [73] Gabani P, Singh OV: Radiation-resistant extremophiles and their potential in biotechnology and therapeutics. *Appl Microbiol Biotechnol* 2013, 97(3):993–1004.
- [74] Gaidamakova EK, Myles IA, McDaniel DP, Fowler CJ, Valdez PA, Naik S, Gayen M, Gupta P, Sharma A, Glass PJ *et al*: Preserving immunogenicity of lethally irradiated viral and bacterial vaccine epitopes using a radio-protective Mn²⁺-Peptide complex from *Deinococcus*. *Cell Host Microbe* 2012, 12(1):117–124.
- [75] Daly MJ: Engineering radiation-resistant bacteria for environmental biotechnology. *Curr Opin Biotechnol* 2000, 11(3):280–285.
- [76] Appukuttan D, Rao AS, Apte SK: Engineering of *Deinococcus radiodurans* R1 for bioprecipitation of uranium from dilute nuclear waste. *Appl Environ Microbiol* 2006, 72(12):7873–7878.
- [77] Appukuttan D, Seetharam C, Padma N, Rao AS, Apte SK: PhoN-expressing, lyophilized, recombinant *Deinococcus radiodurans* cells for uranium bioprecipitation. *J Biotechnol* 2011, 154(4):285–290.
- [78] Kulkarni S, Ballal A, Apte SK: Bioprecipitation of uranium from alkaline waste solutions using recombinant *Deinococcus radiodurans*. *J Hazard Mater* 2013, 262:853–861.
- [79] Misra CS, Appukuttan D, Kantamreddi VS, Rao AS, Apte SK: Recombinant *D. radiodurans* cells for bioremediation of heavy metals from acidic/neutral aqueous wastes. *Bioeng Bugs* 2012, 3(1):44–48.
- [80] Misra CS, Mukhopadhyaya R, Apte SK: Harnessing a radiation inducible promoter of *Deinococcus radiodurans* for enhanced precipitation of uranium. *J Biotechnol* 2014, 189:88–93.

- [81] Brim H, Venkateswaran A, Kostandarithes HM, Fredrickson JK, Daly MJ: Engineering *Deinococcus geothermalis* for bioremediation of high-temperature radioactive waste environments. *Appl Environ Microbiol* 2003, 69(8):4575–4582.
- [82] Lea D, Catcheside D: The mechanism of the induction by radiation of chromosome aberrations in tradesoantia. *J Genet* 1942, 44:216–245.
- [83] Kellerer AM, Rossi HH: A generalized formulation of dual radiation action. *Radiat Res* 1978, 75(3):471–488.
- [84] Chadwick KH, Leenhouts HP: A molecular theory of cell survival. *Phys Med Biol* 1973, 18(1):78–87.
- [85] Brahme A: Accurate description of the cell survival and biological effect at low and high doses and LET's. *J Radiat Res* 2011, 52(4):389–407.
- [86] Lind BK, Persson LM, Edgren MR, Hedlof I, Brahme A: Repairable-conditionally repairable damage model based on dual Poisson processes. *Radiat Res* 2003, 160(3):366–375.
- [87] Lea D: Actions of radiations on living cells. Cambridge: University Press; 1946.
- [88] Tobias CA: The repair-misrepair model in radiobiology: comparison to other models. *Radiat Res Suppl* 1985, 8:S77–95.
- [89] Curtis SB: Lethal and potentially lethal lesions induced by radiation--a unified repair model. *Radiat Res* 1986, 106(2):252–270.
- [90] Sontag W: A discrete cell survival model including repair after high dose-rate of ionizing radiation. *Int J Radiat Biol* 1997, 71(2):129–144.
- [91] Salata F, D'Orazio A, Fabiani M, D'Alessandro D: Effectiveness of UV radiation for reducing *Aspergillus Niger* and *Actynomices* contamination in air-conditioning systems. In: *Proceedings of Clima 2007 WellBeing Indoors: 10 Jun 2007 – 14 Jun 2007; Helsinki (Finland)*: FINVAC; 2007: 8.
- [92] McCullagh P, Nelder JA: Generalized Linear Models. London: Chapman & Hall; 1989.
- [93] R Development Core Team: R: a language and environment for statistical computing. *R Foundation for Statistical Computing* <http://www.R-project.org> 2007.
- [94] Knezevic SZ, Streibig JC, Ritz C: Utilizing R software package for dose-response studies: the concept and data analysis. *Weed Technol* 2007, 21:840–848.
- [95] Hofner B: Lethal: compute lethal doses (LD) with confidence intervals. *R package*, <https://github.com/hofnerb/lethal> 2014.

