Mutation Research 793–794 (2016) 1–14

Contents lists available at ScienceDirect

Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis

journal homepage: www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

Screen for genes involved in radiation survival of *Escherichia coli* and construction of a reference database

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ARTICLE INFO

Article history:
Received 17 June 2015
Received in revised form
29 September 2016
Accepted 1 October 2016
Available online 3 October 2016

Keywords:
Escherichia coli
Keio strains
Radiation survival
UV-radiation
X-rays

ABSTRACT

A set of 3907 single-gene knockout (Keio collection) strains of *Escherichia coli* K-12 was examined for strains with increased susceptibility to killing by X- or UV-radiation. After screening with a high-throughput resazurin-based assay and determining radiation survival with triplicate clonogenic assays, we identified 76 strains (and associated deleted genes) showing statistically-significant increased radiation sensitivity compared to a control strain. To determine gene novelty, we constructed a reference database comprised of genes found in nine similar studies including ours. This database contains 455 genes comprised of 103 common genes (found 2–7 times), and 352 uncommon genes (found once). Our 76 genes includes 43 common genes and 33 uncommon (potentially novel) genes, i.e., *appY, atoS, betB, bglJ, clpP, cpxA, cysB, cysE, ddlA, dgkA, dppF, dusB, elfG, eutK, fadD, glnA, groL, guaB, intF, prpR, queA, rplY, seqA, sufC,yadG, yagJ, yahD, yahO, ybaK, ybfA, yfaL, yhjV,* and *yiaL.* Of our 33 uncommon gene mutants, 4 (12%) were sensitive only to UV-radiation, 10 (30%) only to X-radiation, and 19 (58%) to both radiations. Our uncommon mutants vs. our common mutants showed more radiation specificity, i.e., 12% vs. 9% (sensitive only to UV-); 30% vs. 16% (X-) and 58% vs. 74% (both radiations). Considering just our radiation-sensitive mutants, the median UV-radiation survival (75 J m^{-2}) for 23 uncommon mutants was 6.84E-3 compared to 1.85E-3 for 36 common mutants (P = 0.025). Similarly, the average X-radiation survival for 29 uncommon mutants was 1.08E-2, compared to 6.19E-3 for 39 common mutants (P = 0.010). Comparing gene functions using MultiFun terms, uncommon genes tended to show less involvement in DNA repair-relevant categories (information transfer and cell processes), but greater involvement in seven other categories. Our analysis of 455 genes suggests cell survival and DNA repair processes are more complex than previously understood, and may be compromised by deficiencies in other processes.
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1. Introduction

Genomic DNA is subject to damage produced by environmental agents such as radiation and chemicals, and by endogenous agents such as reactive oxygen species [1]. To deal with this DNA damage and ensure cell survival, multiple repair and damage tolerance systems have developed over time. *Escherichia coli* has been well-studied as a target for DNA damaging agents, and for the subsequent multiple repair or damage avoidance mechanisms that come into play to facilitate cell survival [2].

The *E. coli* genome encodes at least 63 proteins involved in DNA repair processing, which have been categorized (in descending frequency) as recombinational repair, damage avoidance (SOS), base excision repair, mismatch repair, damage reversal, and/or nucleotide excision repair [3]. Genome sequencing has identified 4464 genes in *E. coli* [4,5], however, as of 2012, only 10% of these genes were well-understood; 60% were named, but incompletely characterized (lacking biochemical or physiological roles); and 30% were unnamed genes ("y-genes"), which largely have unknown functions (homology and expression data only) [6]. Given the large number of *E. coli* genes that have not been studied in depth, it seems likely that many genes relevant to DNA repair may yet be unknown or poorly understood for this organism. A more complete understanding of the *E. coli* genes involved in DNA repair is likely to further advance this field of study, which has already contributed greatly to the general understanding of mutagenesis and cancer biology in humans. For this reason, we tested the Keio collection [7] of 3907 single-gene knock-out, non-essential mutants of *E. coli* K-12 to identify previously uncharacterized genes involved in survival and potentially in DNA repair in irradiated cells.

The selection of this screening protocol was based on several principles. Broad searches for DNA repair genes in the past often

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http://dx.doi.org/10.1016/j.mrfmmm.2016.10.001
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relied on chemical mutagens that produced a variety of types of mutations (often multiple mutations), and these mutations had to be mapped and purified to complete the study [8,9]. The current study used the Keio collection of well-defined single-gene knockout mutants [7] to greatly facilitate data analysis, but with the limitation that only non-essential genes could be studied. The selection phenotype in this study was sensitivity to X- and/or UV-radiation, because this approach involving DNA damage consisting of strand breaks (X-rays) and replication fork-blocking lesions (UV radiation) would cover a broad range of DNA repair processes. To improve the ability to detect genes involved in cell survival after irradiation, cells were grown in rich bacterial culture media supplemented with glucose, which produces glucose-induced resistance (GIR) in *E. coli* [10], and increases the radiation-sensitivity phenotype differential between DNA repair proficient and deficient *E. coli* strains, especially after X-irradiation [11]. In addition, each mutant in the Keio collection exhibits a null phenotype, whereas the phenotypic range of chemically-induced mutations can also include partial or leaky phenotypes.

2. Materials and methods

2.1. Media and chemicals

Luria-Bertani-Miller (LB) broth [12] is 1% tryptone (Difco), 0.5% yeast extract (Difco) and 1% NaCl. LB agar is LB broth solidified with 1.5% agar (Fisher). LBG [11] is LB broth supplemented with 1% glucose. LB-Kn-50 is LB agar supplemented with 50 μg/ml kanamycin sulfate (Fisher). Phosphate buffer (PB) is 67 mM NaK phosphate buffer at pH 7 [9]. Resazurin (Fisher) was dissolved in sterile distilled water at 0.675% (w/v).

2.2. Bacterial strains

The Keio collection, provided for our use, was comprised of 3909 unique *E. coli* mutant strains created from host strain K-12 W3110, BW25113 [7], and purchased from the National BioResource Project-*E. coli* at the National Institute of Genetics in Mishima, Japan (NBRP-E.coli at NIG). Two strains, *ompC* and *yfdR,* were dropped from our studies, because of slow-growth issues, so only 3907 strains were used in this study. The Keio collection strains were derived from strain BW25113 by gene replacement with a kanamycin cassette [7]. Strain BW25113 has a genotype of F-Δ *(araD-araB)567* Δ*lacZ4787*(:rrnB-3) *rph-1* Δ*(rhaD-rhaB)568 hsdR514* λ; strain JW5807 (used as the putative repair-proficient control strain in our study) has the same genotype, except for an additional Δ*leuB*:*kan* mutation. All other Keio strains used in this study resemble strain JW5807, except that their single knockout mutation involves a gene other than *leuB.*

2.3. Irradiation

X-radiation was generated by a Polaris Model XR160 cabinet irradiator (Kimtron) with a 3000 W, Varian NDI-161 tube running at 160 kV and 15 mA. For irradiating multiple strains simultaneously, cell suspensions in 96-well microtiter plates were irradiated 14 cm below the radiation port with a dose-rate of 18 Gy min^{-1}. For multi-dose irradiation, a conical, 50-ml centrifuge tube containing a 10-ml cell suspension was serially irradiated at a position closer to the radiation port with a dose-rate of 56.6 Gy min^{-1}, while being bubbled with air. UV-radiation was supplied by an 8-W germicidal lamp (GE, G8T5) emitting primarily at 254 nm. For UV-irradiating multiple strains simultaneously, cell suspensions in 96-well microtiter plates were irradiated at 47 cm below the lamp at a dose-rate of 1.42 J m^{-2} s^{-1}, and under gold ambient light (GEF40GO Gold 40 W lamps) to prevent photoreactivation. For multi-dose irradiation, a 10-ml cell suspension was serially irradiated in a Petri dish on a mixing platform. Details on dosimetry and procedures for both types of irradiation have been reported earlier [13].

2.4. Resazurin-based screen for radiation-sensitive mutants

Resazurin is a purple, non-toxic, oxidation-reduction indicator that becomes pink when reduced to resorufin by cellular oxidoreductases [14]. The concentration of viable cells in a suspension containing resazurin directly determines the time-point for a visible conversion from purple to a pink color. To test for radiation sensitivity, cells were inoculated into 5-ml volumes of LBG broth and tube cultures were incubated at 37 °C for 17 h in a tube roller. Culture OD_{600} values (NanoDrop, Fisher) were determined for each culture (typically 95 test strains and a control strain), and cells were diluted to ∼150 × 10^8 colony-forming units per ml (CFU/ml) with PB. A 50-μl diluted sample of each culture was placed in wells in duplicate 96-well microtiter plates; one for UV-irradiation at 75 J m^{-2} and one for X-irradiation at 88.5 Gy. Doses were selected to give about 10% survival with UV-radiation and about 90% survival after X-radiation, thinking this would yield could numbers of both slightly sensitive and very sensitive strains. After irradiation, 150 μl of LB broth and 10 μl of 0.675% resazurin solution were added to each well and plates were incubated at 37 °C for at least 3.25 h (X-ray) or 5.5 h (UV-radiation). Incubation was terminated when the culture color changed from purple to pink for the control strain, JW5807, signifying the endpoint for normal post-irradiation growth. At this time-point, cultures that lagged behind the control culture (i.e., they were less pink in color intensity or were still purple) were judged by visual inspection to be potentially more radiation sensitive than the control strain, and these strains were scheduled for clonogenic testing for radiation sensitivity. All "hard-to-call" results were repeated. Details on the resazurin-based assay have been reported earlier [13].

2.5. Clonogenic-plating screen for radiation-sensitive mutants

Each mutant strain judged to be radiation sensitive by the resazurin screen was grown for 17 h in a 5-ml LBG broth tube culture at 37 °C. Cells were plated for CFU/ml, or diluted 10^4-fold with PB before placing 300-μl volumes in single wells in duplicate microtiter plates; one for X-irradiation (400 Gy), and one for UV-irradiation (75 J m^{-2}). Unlike our screening protocol, we decided that both doses should give about 10% survival in the control strain. Wells in the perimeter two rows of the plate were avoided to improve the uniformity of radiation doses between the wells used. Cell suspensions for the control strain were placed at 3 positions on each plate (center, upper right corner, and lower left) to provide triplicate control strain data. Following irradiation, cells were diluted further in PB and 100 μl-samples were plated on duplicate LB agar plates to determine CFU/ml. These assays were performed in triplicate.

3. Results

3.1. Control strain for radiation studies

All of our Keio mutants are kanamycin-resistant, because of the *kan* cassette used to derive the Keio mutants [7]. Since the Keio parental strain, BW25113, is kanamycin-sensitive, we chose to use the kanamycin-resistant Keio strain, JW5807 (Δ*leuB*:*kan*), as the control strain for our studies after confirming it showed the same radiation survival phenotype as strain BW25113 for X- or UV-radiation. The UV- and X-radiation survival curves for mean data from triplicate experiments were congruent (data not shown). For example, at 80 J m^{-2}, the UV-radiation surviving fractions were the

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