The Journal of Antibiotics (2019) 72:566–573
https://doi.org/10.1038/s41429-019-0160-5

JARA Japan Antibiotics Research Association
The Society for Actinomycetes Japan

ARTICLE

High-throughput identification of the sensitivities of an *Escherichia coli* Δ*recA* mutant strain to various chemical compounds

Tomoya Maeda[1] · Takaaki Horinouchi[1] · Natsue Sakata[1] · Aki Sakai[1] · Chikara Furusawa[1,2]

Received: 24 October 2018 / Revised: 1 February 2019 / Accepted: 4 February 2019 / Published online: 21 February 2019

Abstract

Antibiotic resistance is considered a global threat to public health. Adaptive resistance mutations and the acquisition of resistance genes by horizontal gene transfer are known to be facilitated by the RecA-dependent SOS response during antibiotic treatment, making RecA inhibitors promising agents for the prevention of antibiotic resistance. However, the impact of RecA inactivation on antibiotic sensitivities remains unclear. Therefore, in this study, we performed high-throughput screening to determine the minimum inhibitory concentrations (MICs) of 217 chemicals, including both antibiotics and toxic chemicals of unknown drug action, in the wild-type MDS42 and the Δ*recA* mutant strains of *Escherichia coli*. The Δ*recA* mutant showed increased sensitivity to DNA-damaging agents, DNA replication inhibitors, and chromate stress, as well as to other chemicals, such as *S*-(2-aminoethyl)-L-cysteine, L-histidine, ruthenium red, D-penicillamine, carbonyl cyanide 3-chlorophenylhydrazone (CCCP), cerulenin, and L-cysteine. Microarray analysis showed further that the Δ*recA* mutant had lower expressions of *glnK*, *nac*, and *glnLG*, which encode nitrogen assimilation regulators, as well as *amtB*, which encodes an ammonium transporter, compared with the wild type. These findings suggest that the Δ*recA* mutation affects not only the SOS response but also amino acid metabolism.

Introduction

Emerging antibiotic resistance in bacteria is considered a global threat to public health since it can rapidly evolve in response to clinical doses. It has been suggested previously that antibiotic resistance results from SOS response-mediated mutagenesis and horizontal gene transfer in response to antibiotic treatment [1]. When double-stranded DNA is damaged by ultraviolet (UV) irradiation or DNA-damaging agents, it breaks up into single-stranded DNA (ssDNA) [2], the accumulation of which triggers the SOS response through the activation of RecA coprotease, which promotes the autocatalytic cleavage of the LexA repressor and induction of the SOS response genes [3]. In *Escherichia coli*, more than 40 genes are directly regulated by LexA, including the recombination and repair genes *recA*, *recN*, and *ruvAB*, the nucleotide excision repair genes *uvrAB* and *uvrD*, the error-prone DNA polymerase genes *dinB* encoding Pol IV and *umuDC* encoding Pol V, and *polB* encoding DNA polymerase II [4]. The error-prone DNA polymerases induce a hypermutable state that generates genetic diversity to promote the acquisition of antibiotic resistance [5, 6].

RecA is the key regulatory protein involved in the SOS response and DNA repair; therefore, its inactivation leads to increased sensitivity to some antibiotics. Treatment with the fluoroquinolone antibiotic ciprofloxacin has been shown to lead to an increase in *recA* expression levels as well as activation of the SOS response, as indicated by the induction of error-prone DNA polymerase Pol V [7]. By contrast, deletion of the *recA* gene from a variety of bacteria causes a two- to eight-fold decrease in the minimum inhibitory concentration (MIC) of levofloxacin, another type of fluoroquinolone [8]. Similarly, the inactivation of RecA reduces the antibiotic-mediated mutagenic effect and increases sensitivity to some antibiotics in *E. coli* when

Supplementary information The online version of this article (https://doi.org/10.1038/s41429-019-0160-5) contains supplementary material, which is available to authorized users.

✉ Tomoya Maeda
tomoya.maeda@riken.jp

[1] RIKEN Center for Biosystems Dynamics Research, 6-2-3 Furuedai, Suita, Osaka 565-0874, Japan

[2] Universal Biology Institute, The University of Tokyo, 7-3-1 Hongo, Tokyo 113-0033, Japan

exposed to sublethal concentrations of antibiotics [9], and strains that lack RecA show significantly increased rates of cell death when treated with quinolones, β-lactams, or aminoglycosides [6]. These bactericidal antibiotics generally induce reactive oxygen species (ROS) formation, resulting in DNA damage and induction of the SOS response [6]. However, Ezraty et al. found that antibiotic exposure did not induce ROS production, with lethality probably resulting from the direct inhibition of cell-wall assembly, protein synthesis, and DNA replication [10], and Ezraty et al. also showed that ROS defense mechanisms are dispensable during treatment with bactericidal antibiotics [10]. Therefore, given the involvement of RecA in DNA repair, the SOS response, horizontal gene transfer [11], the control of swarming ability [12], and biofilm formation [13], it is considered a promising target for the development of a new strategy for preventing antibiotic resistance [14]. However, the impact of RecA inactivation on various kinds of antibiotic sensitivities is not fully understood yet. Therefore, in this study, we attempted to investigate the impact of *recA* deletion on sensitivities to antibiotics and chemicals with varying drug actions in the wild-type MDS42 strain and the Δ*recA* mutant strain of *E. coli*. To do this, we determined the MIC of each chemical using a previously developed automated culture system for laboratory evolution that allows us to maintain more than 100-independent culture series under various culture conditions [15].

We found that the Δ*recA* mutation increased the sensitivities to not only SOS response-inducing agents but also *S*-(2-aminoethyl)-L-cysteine, L-histidine, ruthenium red, D-penicillamine, carbonyl cyanide 3-chlorophenylhydrazone (CCCP), cerulenin, and L-cysteine. Furthermore, microarray analysis showed that the Δ*recA* mutation resulted in decreased expression of nitrogen assimilation regulators as well as *amtB*, which encodes an ammonium transporter. Together, these results suggest that the inactivation of RecA affects not only the SOS response but also some metabolic stresses, thereby potentiating several antibiotic activities.

Materials and methods

Bacterial strains and growth media

The insertion sequence (IS)-free *E. coli* strain MDS42 [16] was purchased from Scarab Genomics (Scarab Genomics, Madison, Wisconsin, USA) and used as the wild-type strain. The MDS42 Δ*recA* mutant strain was constructed using the Quick and Easy *E. coli* Gene Deletion Kit (Gene Bridges, Heidelberg, Germany). Deletion of the open reading frame region of *recA* was performed in accordance with the manufacturer's instructions. Briefly, the FRT-cm^r-FRT cassette (A006; Gene Bridges) was amplified by polymerase chain reaction (PCR) using the primer pairs Primer1/Primer2 and Primer3/Primer4 (see Supplementary Table S1) to add 50-bp-long homology arms that corresponded to the sequences flanking the insertion site on the chromosome. The constructed functional cassette was electroporated into the parental strain and inserted into the target locus by λ-Red recombinase using the expression plasmid pRedET. Then, the selection marker was removed from the chromosome by FLPe recombinase using the 709-FLPe expression plasmid (A106; Gene Bridges). The genome modifications and removal of the selection marker cassette were verified by colony PCR using the primer pair Primer5/Primer6 (Table S1) and were then confirmed by direct Sanger sequencing of the PCR products.

The *E. coli* strains were cultured in modified M9 minimal medium containing 17.1 g l^{-1} $Na_2HPO_4{\cdot}12H_2O$, 3.0 g l^{-1} KH_2PO_4, 5.0 g l^{-1} NaCl, 2.0 g l^{-1} NH_4Cl, 5.0 g l^{-1} glucose, 14.7 mg l^{-1} $CaCl_2{\cdot}2H_2O$, 123.0 mg l^{-1} $MgSO_4{\cdot}7H_2O$, 2.8 mg l^{-1} $FeSO_4{\cdot}7H_2O$, and 10.0 mg l^{-1} thiamine hydrochloride (pH 7.0) [17].

Determination of minimum inhibitory concentrations (MICs)

To comprehensively investigate the effect of the Δ*recA* mutation on the susceptibility to different chemicals, we determined the MICs of 217 chemicals with differing mechanisms of action in the wild-type MDS42 strain and the Δ*recA* mutant strain. The selected chemicals included a wide range of antibiotics, such as β-lactams, penicillins, cephalosporins, aminoglycoside, tetracyclines, macrolides, amphenicols, rifamycins, and quinolones. In addition, a number of toxic chemicals other than antibiotics were tested, such as sulfonamides, organic acids, amino acid analogs, metals, chelators, cationic surfactants, and receptor inhibitors. The chemicals we tested have a wide range of biological targets, such as peptidoglycan synthesis, the cell membrane, polysaccharides, 30S ribosomes, 50S ribosomes, protein translation, RNA polymerase, DNA, DNA gyrase, folic acid biosynthesis, metabolic enzymes, oxidative phosphorylation, fatty acid biosynthesis, oxidative stress, metal chelators, acid stress, and osmotic balance. Figure 1 shows the number of chemicals for each biological target.

Supplementary Table S2 lists all of the chemicals that were used in this study and the solvents in which they were dissolved to prepare stock solutions. Chemicals that were not dissolved in the modified M9 medium were added to it at a >20-fold dilution. Cell cultivation, optical density (OD) measurements, and serial dilutions were performed for each chemical using an automated culture system [15] consisting of a Biomek® NX span-8 laboratory automation workstation

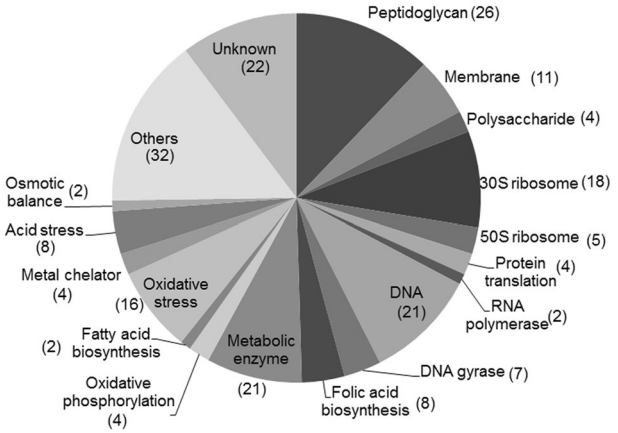

Fig. 1 Biological targets of the chemical compounds used in this study. The proportion (and number) of the 217 chemical compounds tested that fell into each biological target category is shown

(Beckman Coulter, Brea, California, USA) in a clean booth connected to a microplate reader (FilterMax F5; Molecular Devices, San Jose, California, USA), a shaker incubator (STX44; Liconic, Mauren, Liechtenstein), and a microplate hotel (LPX220, Liconic). Serial dilutions of each chemical were prepared in 384-well microplates using the modified M9 medium with doubling dilution steps to determine MICs.

MDS42 and the Δ*recA* mutant cells were inoculated from the frozen glycerol stock into the modified M9 medium and cultivated overnight at 34 °C and 150 rpm. The OD_{620} values of the precultures were measured using the automated culture system, and those precultured cells that were calculated to have initial OD_{620} values of 0.0003 were inoculated into each well (5 µl of diluted overnight culture into 45 µl of medium per well) of 384-well microplates containing serially diluted chemicals to a final volume of 50 µl. After 24 h incubation at 34 °C with agitation at 300 rotations/min, the OD_{620} of the precultures was measured again. The MIC was defined as the lowest concentration of a chemical that reduced the growth to $OD_{620} < 0.09$.

Total RNA purification

Total cellular RNA was isolated from the MDS42 and Δ*recA* mutant cells as follows. Cells were inoculated from the frozen glycerol stock into 5 ml of modified M9 medium in test tubes and cultivated overnight at 34 °C and 150 rpm. Then, the overnight cultures were diluted to an OD_{600} of 0.1 in fresh modified M9 medium in test tubes and cultured. Two volumes of RNAprotect Bacteria Reagent (Qiagen, Hilden, Germany) were added directly to one volume of exponentially growing cultures (OD_{600} of ~1; mid-exponential growth phase) to stabilize the cellular RNA. Then, the cells were harvested by centrifugation at 5000 × *g* for 10 min at 25 °C and total cellular RNA was isolated using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. Total RNA was treated with DNase I at room temperature for 15 min and the purified RNA samples were stored at −80 °C until the microarray experiments.

Transcriptome analysis using microarrays

Microarray experiments were performed as described previously [18] using a custom-designed Agilent 8 × 60 K array for *E. coli* W3110 that included 12 probes for each gene. Briefly, 100 ng of each purified total RNA sample was labeled using the Low Input Quick Amp WT Labeling Kit (Agilent Technologies, Santa Clara, California, USA) with Cyanine3 (Cy3) according to the manufacturer's instructions. Cy3-labeled cRNAs were fragmented and hybridized to the microarray for 17 h at 65 °C in a hybridization oven (Agilent Technologies), following which the microarray was washed and scanned according to the manufacturer's instructions. Microarray image analysis was performed using Feature Extraction version 10.7.3.1 (Agilent Technologies) and expression levels were normalized using the quantile normalization method [19]. To exclude quantitatively unreliable data, genes with low expression levels [<1.5 AU (log base 10)] were excluded from the subsequent analysis (representing ~35% of genes). Each experiment was performed in triplicate, starting from independent cultures. The microarray data have been submitted to the National Center for Biotechnology Information's Gene Expression Omnibus functional genomics data repository under accession number GSE121218.

Results

Effect of the Δ*recA* mutation on chemical susceptibility

To reveal the impact of RecA inactivation on antibiotic sensitivities, we attempted to identify chemicals that strongly inhibit the growth of bacteria lacking the *recA* gene. First, the effect of Δ*recA* mutation on cell growth was examined (Fig. S1). The Δ*recA* mutant strain only showed a slight decrease in growth compared to the wild-type strain. The growth rates at 34 °C in the modified M9 medium were $0.52 \pm 0.01\ h^{-1}$ in the wild-type strain and $0.50 \pm 0.02\ h^{-1}$ in the Δ*recA* mutant strain. Table S2 shows the calculated MIC values for each of the 217 chemicals tested. The Δ*recA* mutant strain showed at least twofold decreases in the MICs for 28 of the 217 chemicals compared with the wild-type strain (Table S2). Table 1 shows the MICs of these 28 chemicals for both strains.

Table 1 Minimum inhibitory concentration (MIC; μg ml^{-1}) of each chemical compound tested for which the Δ*recA* mutant strain was more sensitive than the MDS42 wild-type strain

Compound	Category	Biological target	MDS42 (μg ml^{-1})	Δ*recA* (μg ml^{-1})	Fold change
S-(2-aminoethyl)-L-cysteine	Lysine analog	Aspartate kinase	4.50×10^{2}	7.03	64
Furaltadone	Oxazolidinone	DNA, ribosomes, many macromolecules	2.81	8.80×10^{-2}	32
Mitomycin C	Alkylating agent	DNA	1.76×10^{-1}	1.10×10^{-2}	16
Ornidazole	Nitroimidazole	DNA	4.50×10^{2}	2.81×10	16
Tinidazole	Nitroimidazole	DNA	1.41×10^{3}	8.83×10	16
L-Histidine	L-amino acid	Phosphoribosyl-ATP pyrophosphorylase, glutamine synthetase	4.05×10^{4}	2.53×10^{3}	16
Ofloxacin	Quinolone	DNA gyrase	2.50×10^{-2}	3.13×10^{-3}	8
Ruthenium red	Inorganic dye	Ion channels, Ca^{2+} binding proteins	5.63×10	7.03	8
4-Nitroquinoline 1-oxide	Mutagen	DNA	7.03×10^{-1}	1.76×10^{-1}	4
5-Azacytidine	Antineoplastic	DNA	1.13×10	2.81	4
Enoxacin 1,5-hydrate	Quinolone	DNA gyrase	2.75×10^{-2}	6.87×10^{-3}	4
Lomefloxacin hydrochloride	Quinolone	DNA gyrase	5.50×10^{-2}	1.37×10^{-2}	4
Norfloxacin	Quinolone	DNA gyrase	2.63×10^{-2}	6.57×10^{-3}	4
Pipemidic acid	Quinolone	DNA gyrase	2.08	5.21×10^{-1}	4
Nitrofurantoin	Hydantoin	DNA, ribosomes, many macromolecules	3.54	8.84×10^{-1}	4
D-Penicillamine	Antirheumatic drug	Metal chelator	7.03×10^{2}	1.76×10^{2}	4
Carbonyl cyanide 3-chlorophenylhydrazone (CCCP)	Uncoupling agent	Oxidative phosphorylation	2.81	7.03×10^{-1}	4
Sodium dichromate dihydrate	Metal stress	Oxidative DNA damage	8.79×10^{-1}	2.20×10^{-1}	4
3,5-diamino-1,2,4-triazole (guanazole)	Aromatic amine	Ribonucleoside diphosphate reductase, DNA synthesis	1.13×10^{2}	2.81×10	4
5′-fluoro-5′-deoxyuridine	Antineoplastic	DNA	5.63×10	2.181×10	2
Acriflavine	Acridine	DNA	5.63	2.81	2
Azathioprine	Thiopurine	DNA	1.13×10^{2}	5.63×10	2
Hydroxyurea	Antineoplastic	DNA	1.41	7.03×10^{-1}	2
Phleomycin	Bleomycin	DNA	3.72	1.86	2
Nalidixic acid	Quinolone	DNA gyrase, topoisomerase	3.52	1.76	2
Cerulenin	Antifungal	Fatty acid biosynthesis	1.41×10	7.03	2
L-Cysteine	L-amino acid	Serine *O*-acetyltransferase, cysteine synthase	1.10×10	5.49	2
Cinoxacin	Quinolone	DNA gyrase	1.47	7.37×10^{-1}	2

The MICs are the means from three-independent experiments

We found that the MICs of all seven of the quinolones tested were at least twofold lower for the Δ*recA* mutant than for the wild-type strain (Table 1). The Δ*recA* mutant also showed decreased MICs for many chemicals that cause DNA damage, i.e., acriflavine, mitomycin C, hydroxyurea, phleomycin, 4-nitroquinoline 1-oxide, ornidazole, and tinidazole (Table 1). However, it should be noted that the Δ*recA* mutant did not show decreased MICs for semicarbazide, netropsin, *N*-methyl-*N*-nitrosourea, bleomycin, or 9-aminoacridine, all of which also cause DNA damage (Table S2).

The Δ*recA* mutant also showed decreased MICs for chemicals that inhibit nucleotide biosynthesis, i.e., 5-azacytidine, 5′-fluoro-5′-deoxyuridine, and azathioprine, although the MICs for cytosine β-D -arabinofuranoside, 6-mercaptopurine, and 3,5-diamino-1,2,4-triazole, which inhibit nucleotide biosynthesis, and for the sulfonamides, which inhibit folic acid biosynthesis (which is a precursor of nucleotide biosynthesis), were comparable between the two strains (Table S2). In addition, the MICs for nitrofurantoin and furaltadone, which target many macromolecules, including DNA and ribosomes, were 4- and 32-fold lower, respectively, in the Δ*recA* mutant than in the wild-type strain, and the Δ*recA* mutant showed increased sensitives to sodium dichromate, which causes oxidative DNA damage (Table 1).

We also found that the Δ*recA* mutation caused increased sensitivities to ruthenium red (an ion channel and Ca^{2+} binding protein inhibitor), D-penicillamine (a metal chelator), L-amino acids (L-histidine and L-cysteine), and some metabolic inhibitors, i.e., *S*-(2-aminoethyl)-L-cysteine (a lysine analog), cerulenin (a fatty acid synthesis inhibitor), and carbonyl cyanide m-chlorophenyl hydrazine (CCCP; an oxidative phosphorylation inhibitor) (Table 1). Among the 217 chemicals tested, *S*-(2-aminoethyl)-L-cysteine was most effective in inhibiting the growth of the Δ*recA* mutant, with a 64-fold lower MIC for this strain compared with the wild-type strain (Table 1).

Effect of the Δ*recA* mutation on global gene expression

Since our MIC measurements revealed that the Δ*recA* mutation resulted in increased sensitivities to chemicals in addition to DNA-damaging agents and DNA synthesis inhibitors, we compared the transcriptomes of the Δ*recA* mutant and wild-type strains using DNA microarray analysis. Overall, 19 genes showed ≥2.0-fold increases and 14 genes showed ≥2.0-fold decreases in mRNA levels in the Δ*recA* mutant compared with the wild type. These genes and their annotated functions and mRNA ratios are listed in Table 2. The Δ*recA* mutant showed significantly decreased expression of nitrogen regulator genes, including a 7.8-fold decrease in *glnK*, which encodes a nitrogen assimilation regulator; a 4.3-fold decrease in *nac*, which encodes a nitrogen assimilation control gene; a 3.6-fold decrease in *amtB*, which encodes an ammonium transporter; a 2.7-fold decrease in *glnLG*, which encodes a two-component regulatory system for nitrogen regulation; and a 2.1-fold decrease in *glnA*, which encodes glutamine synthetase. The Δ*recA* mutant also showed decreased expressions of *recN*, which encodes a recombination and repair protein, and *sulA*, which encodes an SOS cell division inhibitor. By contrast, the Δ*recA* mutant had increased expressions of several genes that encode chaperone proteins, including a 3.9-fold increase in *ibpB*, a 2.5-fold increase in *ibpA*, a 2.4-fold increase in *clpB*, and a 2.0-fold increase in *htpG*.

Discussion

In this study, we determined MICs for 217 chemicals with a wide range of biological targets in the wild-type and Δ*recA* mutant strains of *E. coli*. We found that the Δ*recA* mutant showed increased sensitivities to various chemicals, including not only DNA-damaging agents but also some metabolic inhibitors, such as the lysine analog *S*-(2-aminoethyl)-L-cysteine and L-histidine. To understand the effect of *recA* deletion on global gene expression better, we also performed transcriptome analysis, which showed that overall deletion of the *recA* gene did not affect the global transcriptome profile. However, the Δ*recA* mutant strain did show decreased expressions of nitrogen assimilation regulators and their regulons, *amtB*, which encodes an ammonium transporter, and *glnA*, which encodes glutamine synthetase.

Previous studies have shown that deletion of the *recA* gene in a number of bacteria results in increased sensitivities to quinolones, which inhibit DNA gyrase [8, 9]. Similarly, in this study, we found that the *recA* mutant showed two- to eight-fold lower MICs for all of the tested quinolones compared with the wild type (Table 1). In addition, we found that deletion of the *recA* gene resulted in decreased MICs for many DNA-damaging agents and sodium dichromate. Since both double-strand breaks in the DNA caused by DNA-damaging agents, such as mitomycin C and nitroimidazole derivatives, and chromate shock induce the SOS response [20], the observation of decreased MICs for these chemicals in the Δ*recA* mutant is reasonable. A previous study showed that treatment of an *E. coli* Δ*recA* mutant with UV irradiation and 4-nitroquinoline-1-oxide treatment resulted in rapid chromosome degradation and cell death [21], indicating that the SOS response and/or RecA-dependent recombination are required for the prevention of DNA degradation and cell death. It has also been shown that alterations to the deoxyribonucleoside triphosphate (dNTP) pool caused by the deletion of either *ndk*, which encodes nucleoside diphosphate kinase, or *dcd*, which encodes deoxycytidine triphosphate (dCTP) deaminase, result in hypermutability and error catastrophe [22–24]. In the present study, we found that the Δ*recA* mutant showed increased sensitivities to the nucleic acid synthesis inhibitors 5-azacytidine (a cytidine analog) and 5′-fluoro-5′-deoxyuridine (a pyrimidine analog) (Table 1). Therefore, since dNTP pool imbalances lead to an increased production of mispairing errors and reduced exonucleolytic proofreading of any mispairings [24], treatment with these inhibitors may be more deleterious to the Δ*recA* mutant.

Bactericidal drugs such as quinolones, β-lactams, and aminoglycosides have been shown to induce hydroxyl radical formation in a Δ*recA* strain, causing DNA damage and cell death, and thereby potentiating the killing efficiency [6]. Furthermore, Thi et al. [9] found that deletion of the *recA* gene results in increased sensitivities to some bactericidal antibiotics, such as ceftazidime (cephalosporin), fosfomycin (phosphonic antibiotic inhibiting peptidoglycan synthesis), trimethoprim/sulfamethoxazole, and colistin, although there was no evidence of increased sensitivities to other bactericidal drugs, such as ampicillin (β-lactam) and gentamicin (aminoglycoside). However, none of these bactericidal drugs produced ROS, indicating that the ROS defense mechanisms are dispensable during treatment with

Table 2 Effect of the Δ*recA* mutation on the expression of *Escherichia coli* genes

Gene	Fold change	*P*-value	Category	Function
glnK	0.13	−4.00	Nitrogen assimilation	Nitrogen assimilation regulatory protein for GlnL, GlnE, and AmtB
nac	0.23	−4.42	Nitrogen assimilation	DNA-binding transcriptional dual regulator
amtB	0.28	−3.87	Nitrogen assimilation	Ammonium transporter
glnG	0.37	−4.77	Nitrogen assimilation	Fused DNA-binding response regulator in two-component regulatory system with GlnL, nitrogen regulator I (NRI)
glnL	0.37	−4.67	Nitrogen assimilation	Sensory kinase in two-component regulatory system with GlnG
glnA	0.47	−3.57	Nitrogen assimilation	Glutamine synthetase
recN	0.19	−4.91	SOS response	Recombination and repair protein
sulA	0.38	−4.62	SOS response	SOS cell division inhibitor
yebG	0.47	−4.37	SOS response	Conserved protein regulated by LexA
cspG	0.38	−3.29	Cold shock	DNA-binding transcriptional regulator
ddg	0.46	−2.92	Cold shock	Palmitoleoyl-acyl carrier protein (ACP)-dependent acyltransferase
deaD	0.47	−4.01	Cold shock	ATP-dependent RNA helicase
cspA	0.48	−2.86	Cold shock	Major cold shock protein
htpG	2.00	−3.60	Heat shock	Molecular chaperone HSP90 family
clpB	2.45	−3.43	Heat shock	Protein disaggregation chaperone
ibpA	2.51	−3.89	Heat shock	Heat shock chaperone
ibpB	3.89	−4.49	Heat shock	Heat shock chaperone
hdeB	1.95	−3.49	Acid resistance	Acid-resistance protein
gadB	2.00	−2.49	Acid resistance	Glutamate decarboxylase B, pyridoxal 5′-phosphate (PLP)-dependent
hdeA	2.09	−3.16	Acid resistance	Stress response protein acid-resistance protein
hdeD	2.51	−3.06	Acid resistance	Acid-resistance membrane protein
csgE	2.00	−2.14	Biofilm formation	Predicted transport protein
csgF	2.40	−3.14	Biofilm formation	Predicted transport protein
nrdB	1.95	−3.95	Deoxyribonucleotide biosynthesis	Ribonucleoside diphosphate reductase 1, beta subunit, ferritin-like
nrdA	1.95	−3.50	Deoxyribonucleotide biosynthesis	Ribonucleoside diphosphate reductase 1, alpha subunit
dps	2.00	−2.57	DNA protection	Fe-binding and storage protein
wrbA	1.95	−3.41	Oxidative stress	Predicted flavoprotein in Trp regulation
yhdW	0.47	−3.49	Pseudogene	Predicted amino acid transporter subunit
pstS	2.09	−3.61	Sugar metabolism	Phosphate transporter subunit
sbp	2.34	−3.10	Sulfur metabolism	Sulfate transporter subunit
rbbA	2.45	−3.25	Translation	Fused ribosome-associated ATPase
ycaC	2.09	−4.45	Unknown	Predicted hydrolase
yiaG	2.63	−4.93	Unknown	Predicted transcriptional regulator

Relative ratios of the mRNA levels in the Δ*recA* mutant strain to the MDS42 wild-type strain are shown. The indicated genes had ≥twofold increases and decreases in mRNA levels in the Δ*recA* mutant. The values represent the means from three-independent experiments. *P*-values of two-tailed paired Student's *t*-tests were calculated using log10 values

these drugs [10, 25]. Therefore, the effect of *recA* inactivation on susceptibilities to bactericidal drugs remains unclear. In the present study, we did not observe any potentiating effects of β-lactams or aminoglycosides on growth inhibition of the Δ*recA* strain (Table S2). These differences may have resulted from differences in the media used for the determination of MICs. Previous studies showed that the growth arrest of *E. coli* cells caused by

nutrient limitation triggers the production of oxidative stress proteins e.g. catalases which result in the resistance to hydrogen peroxide [26, 27]. We used the modified M9 minimal medium while Kohanski et al. [6] and Thi et al. [9] used nutrient-rich medium (LB medium). Therefore, the effect of Δ*recA* mutation on susceptibilities to β-lactams and aminoglycosides may be suppressed in this study.

We found that the Δ*recA* mutant also showed increased sensitivities to chemicals other than DNA-damaging agents, such as a lysine analog [*S*-(2-aminoethyl)-L-cysteine], L-amino acids (L-histidine and L-cysteine), an ion channel and Ca^{2+} binding protein inhibitor (ruthenium red), a metal chelator (D-penicillamine), an uncoupling agent that inhibits oxidative phosphorylation (CCCP), and a fatty acid biosynthesis inhibitor (cerulenin). To understand how the absence of *recA* increases the sensitivities to these chemicals better, we performed microarray analysis. This showed that the Δ*recA* mutant had significantly lower expressions of the *glnALG* operon, which encodes glutamine synthetase (GS), the nitrogen regulatory sensor kinase and response regulator *nac*, which encodes nitrogen assimilation control, and the *glnK-amtB* operon, which encodes a nitrogen regulator and ammonium transporter (Table 2). In *E. coli*, the master regulator of the nitrogen-limited stress response is the NtrBC two-component system, which is encoded by the *glnGL* genes [28]. Under nitrogen-limited conditions, the nitrogen regulator GlnK and the nitrogen assimilation control protein Nac also activate genes that are involved in nitrogen assimilation [29–32]. However, the Δ*recA* mutant showed decreased expressions of genes that are important for nitrogen assimilation (i.e., *glnALG*, *nac*, *glnK*, and *amtB*), meaning that the biosynthesis of glutamine and other amino acids may be limited. Interestingly, the Δ*recA* mutant also showed increased sensitivity to L-histidine, which is known to inhibit GS encoded by the *glnA* gene [33]. However, the Δ*recA* mutant did not show decreased MICs for L-methionine sulfoximine, bialaphos, or glufosinate, all of which are known as GS inhibitors [34] (Table S2). It was shown that NtrC couples the stringent response and NtrC regulates not only nitrogen assimilation related genes but also *argT* encoding lysine/arginine/ornithine ABC transporter and *hisJQMP* encoding histidine ABC transporter in *E. coli* [35]. Although these gene expressions were not significantly changed in the Δ*recA* mutant, decreased expression of *glnG* in the Δ*recA* mutant may also affect lysine and histidine metabolism. Since, we used the minimal medium for MIC measurements, such a deficiency in amino acid biosynthesis may result in an increased sensitivity to the lysine analog, L-histidine, and L-cysteine. These results suggest that deletion of the *recA* gene results in a limitation of amino acid biosynthesis.

Since bacteria easily evolve antibiotic resistance in response to clinical doses, new strategies for preventing antibiotic resistance are required for public health. One such strategy is drug combination therapy [36]. In some cases, combinations of drugs act synergistically, causing them to kill pathogens more efficiently and, thus, suppressing antibiotic resistance [37, 38]. RecA inhibitors are considered promising for the prevention of antibiotic resistance [14]. Suramin (polysulphonated naphthylurea) is considered a potent and selective inhibitor of RecA and the SOS response in *Mycobacterium tuberculosis* [39]. In addition, phthalocyanine tetrasulfonic acid (PcTs)-based RecA inhibitors are known to block ATPase, DNA binding, DNA strand exchange, and LexA proteolysis activities of RecA, potentiating the activity of quinolone, β-lactam, and aminoglycoside family antibiotics and reducing the ability of bacteria to acquire antibiotic resistance [14]. Therefore, an additional dosage of a drug that shows synergistic activity to the RecA inhibitor could potentiate the inhibition of antibiotic resistance. In this study, we identified a number of chemical compounds that effectively inhibited the growth of the *recA* deletion mutant, which may contribute to the development of synergistic combination therapy using RecA inhibitors.

Acknowledgements This work was supported in part by Grant-in-Aid for Scientific Research (B) [15KT0085, 17H03622] and Grant-in-Aid for Scientific Research (S) [15H05746] from Japan Society for the Promotion of Science (JSPS).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Baharoglu Z, Bikard D, Mazel D. Conjugative DNA transfer induces the bacterial SOS response and promotes antibiotic resistance development through integron activation. PLoS Genet. 2010;6:1–10.
2. Baharoglu Z, Mazel D. SOS, the formidable strategy of bacteria against aggressions. FEMS Microbiol Rev. 2014;38:1126–45.
3. Butala M, Žgur-Bertok D, Busby SJW. The bacterial LexA transcriptional repressor. Cell Mol Life Sci. 2009;66:82–93.
4. Courcelle J, Hanawalt PC. Participation of recombination proteins in rescue of arrested replication forks in UV-irradiated *Escherichia coli* need not involve recombination. Proc Natl Acad Sci USA. 2001;98:8196–202.
5. Cirz RT, Chin JK, Andes DR, De Crécy-Lagard V, Craig WA, Romesberg FE. Inhibition of mutation and combating the evolution of antibiotic resistance. PLoS Biol. 2005;3:1024–33.
6. Kohanski MA, Dwyer DJ, Hayete B, Lawrence CA, Collins JJ. A Common mechanism of cellular death induced by bactericidal antibiotics. Cell. 2007;130:797–810.
7. Schröder W, Goerke C, Wolz C. Opposing effects of aminocoumarins and fluoroquinolones on the SOS response and adaptability in *Staphylococcus aureus*. J Antimicrob Chemother. 2013;68:529–38.

8. Singh R, Ledesma KR, Chang KT, Tam VH. Impact of r*ecA* on levofloxacin exposure-related resistance development. Antimicrob Agents Chemother. 2010;54:4262–8.
9. Do ThiT, López E, Rodríguez-Rojas A, Rodríguez-Beltrán J, Couce A, Guelfo JR, et al. Effect of r*ecA* inactivation on mutagenesis of E*scherichia coli* exposed to sublethal concentrations of antimicrobials. J Antimicrob Chemother. 2011;66:531–8.
10. Ezraty B, Vergnes A, Banzhaf M, Duverger Y, Huguenot A, Brochado AR, et al. Fe-S cluster biosynthesis controls uptake of aminoglycosides in a ROS-less death pathway. Science. 2013;340:1583–8.
11. Beaber JW, Hochhut B, Waldor MK. SOS response promotes horizontal dissemination of antibiotic resistance genes. Nature. 2004;427:72–4.
12. Gómez-Gómez JM, Manfredi C, Alonso JC, Blázquez J. A novel role for RecA under non-stress: promotion of swarming motility in *Escherichia coli* K-12. BMC Biol. 2007;5:1–15.
13. Boles BR, Thoendel M, Singh PK. Self-generated diversity produces "insurance effects" in biofilm communities. Proc Natl Acad Sci USA. 2004;101:16630–5.
14. Alam MK, Alhhazmi A, Decoteau JF, Luo Y, Geyer CR. RecA inhibitors potentiate antibiotic activity and block evolution of antibiotic resistance. Cell Chem Biol. 2016;23:381–91.
15. Horinouchi T, Minamoto T, Suzuki S, Shimizu H, Furusawa C. Development of an automated culture system for laboratory evolution. J Lab Autom. 2014;19:478–82.
16. Pósfai G, Umenhoffer K, Kolisnychenko V, Stahl B, Sharma SS. Emergent properties of reduced-genome *Escherichia coli*. Science. 2006;312:1044–7.
17. Mori E, Furusawa C, Kajihata S, Shirai T, Shimizu H. Evaluating 13C enrichment data of free amino acids for precise metabolic flux analysis. Biotechnol J. 2011;6:1377–87.
18. Suzuki S, Horinouchi T, Furusawa C. Prediction of antibiotic resistance by gene expression profiles. Nat Commun. 2014;5:5792.
19. Bolstad BM, Irizarry RA, Astrand M, Speed TP. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics. 2003;19:185–93.
20. Qin T, Kang H, Ma P, Li P, Huang L, Gu B. SOS response and its regulation on the fluoroquinolone resistance. Ann Transl Med. 2015;3:358.
21. Williams AB, Hetrick KM, Foster PL. Interplay of DNA repair, homologous recombination, and DNA polymerases in resistance to the DNA damaging agent 4-nitroquinoline-1-oxide in E*scherichia coli*. DNA Repair. 2010;9:1090–7.
22. Ahluwalia D, Bienstock RJ, Schaaper RM. Novel mutator mutants of E. *coli nrdAB r*ibonucleotide reductase: insight into allosteric regulation and control of mutation rates. DNA Repair. 2012;11:480–7.
23. Schaaper RM, Mathews CK. Mutational consequences of dNTP pool imbalances in *E. coli*. DNA Repair. 2013;12:73–9.
24. Ahluwalia D, Schaaper RM. Hypermutability and error catastrophe due to defects in ribonucleotide reductase. Proc Natl Acad Sci USA. 2013;110:18596–601.
25. Liu Y, Imlay JA. Cell death from antibiotics without the involvement of reactive oxygen species. Science. 2013;339:1210–3.
26. Jenkins DE, Schultz JE, Matin A. Starvation-induced cross protection against heat or H_2O_2 challenge in *Escherichia coli*. J Bacteriol. 1988;170:3910–4.
27. Dukan S, Nyström T. Oxidative stress defense and deterioration of growth-arrested *Escherichia coli* cells*. J Biol Chem. 1999;274:26027–32.
28. Jiang P, Ninfa AJ. Regulation of autophosphorylation of *Escherichia coli* nitrogen regulator II by the PII signal transduction protein. J Bacteriol. 1999;181:1906–11.
29. Muse WB, Bender RA. The *nac* (Nitrogen Assimilation Control) gene from *Escherichia coli*. J Bacteriol. 1998;180:1166–73.
30. Zimmer DP, Soupene E, Lee HL, Wendisch VF, Khodursky AB, Peter BJ, et al. Nitrogen regulatory protein C-controlled genes of *Escherichia coli:* scavenging as a defense against nitrogen limitation. Proc Natl Acad Sci USA. 2000;97:14674–9.
31. Blauwkamp TA, Ninfa AJ. Physiological role of the GlnK signal transduction protein of *Escherichia coli:* survival of nitrogen starvation. Mol Microbiol. 2002;46:203–14.
32. Atkinson MR, Blauwkamp T, Ninfa AJ. Context-dependent functions of the PII signal transduction protein of *Escherichia coli*. J Bacteriol. 2002;184:5364–75.
33. Hubbard JS, Stadtman ER. Regulation of synthesis of glutamine synthetase VI. Interactions of inhibitors for *Bacillus licheniformis* glutamine synthetase. J Bacteriol. 1967;94:1016–24.
34. Berlicki L. Inhibitors of glutamine synthetase and their potential application in medicine. Mini Rev Med Chem. 2008;8:869–78.
35. Brown DR, Barton G, Pan Z, Buck M, Wigneshweraraj S. Nitrogen stress response and stringent response are coupled in *Escherichia coli*. Nat Commun. 2014;5:1–8.
36. Wambaugh MA, Shakya VPS, Lewis AJ, Mulvey MA, Brown JCS. High-throughput identification and rational design of synergistic small-molecule pairs for combating and bypassing antibiotic resistance. PLoS Biol. 2017;15:e2001644.
37. Mitchison DA. Prevention of drug resistance by combined drug treatment of tuberculosis. Handb Exp Pharmacol. 2012;212:87–98.
38. Hill JA, Cowen LE. Using combination therapy to thwart drug resistance. Future Microbiol. 2015;10:1719–26.
39. Nautiyal A, Patil KN, Muniyappa K. Suramin is a potent and selective inhibitor of M*ycobacterium tuberculosis R*ecA protein and the SOS response: RecA as a potential target for antibacterial drug discovery. J Antimicrob Chemother. 2014;69:1834–43.