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# RecA-mediated SOS response provides a geraniol tolerance in *Escherichia coli*

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ABSTRACT

Geraniol is an important industrial material and a potential candidate of advanced biofuels. One challenge of microbial geraniol production is the toxicity to hosts. However, the poor understanding on geraniol tolerance mechanism is an obstacle for developing geraniol tolerant host. This study genome-widely screened a shot-gun DNA library of *Escherichia coli* and found that *recA* is able to confer geraniol tolerance in *E. coli*. The *recA* knockout mutant was found extremely sensitive to geraniol. Based on our data, it was deciphered that *recA* provided tolerance through SOS response network responding to DNA damage caused by geraniol. RecA-mediated SOS response activates the homologous recombinational repair by RecB and RecN for corrective DNA maintenance. This protection mechanism suggests an effective strategy to combat geraniol toxicity in *E. coli*.



## 1. Introduction

Geraniol (*trans*-3,7-dimethyl-2,6-octadien-1-ol; $C_{10}H_{18}O$) is an acyclic monoterpene alcohol with a rose-like scent. This natural alcohol is widely used as a fragrance ingredient in both cosmetic and household products. It is also known to exhibit antimicrobial, anti-inflammatory and antitumor properties and evaluated as a new class of cancer therapeutic agent (Carnesecchi et al., 2001). The worldwide use of geraniol is greater than 1000 metric tons per annum (Lapczynski et al., 2008). Moreover, geraniol has been considered as a promising biofuel candidate because of its high energy density, low hygroscopicity and low vapor pressure (Dunlop et al., 2011; Fortman et al., 2008). Microbial production of geraniol through metabolic engineering is an eco-friendly and attractive alternative to the commonly used chemical synthesis and plant extraction (Scalcinati et al., 2012). However, a routine challenge in bioprocessing to produce bulk chemicals is that the selected products such as geraniol, are often toxic to microbial hosts (Dunlop et al., 2011). Geraniol has been produced in trace amounts from engineered *Saccharomyces cerevisiae* and *Escherichia coli* (Fischer et al., 2011, 2013). Recent development in metabolic engineering and synthetic biology allows the overproduction of several natural products (Ajikumar et al., 2010; Tsuruta et al., 2009), which would also promote the geraniol titer to a toxic level to hosts in future. Therefore, it will be crucial to develop strategies for increasing geraniol tolerance.

Organic solvents can affect cells by imparting biophysical changes to membranes, unfolding proteins and damaging DNA and RNA (Nicolaou et al., 2010). To combat cytotoxic stress from various solvents, microorganisms employ several strategies including expression of efflux pumps, changes in membrane properties, changes in cell wall composition, and activation of stress response genes (Nicolaou et al., 2010). These associated mechanisms are apparently independent from each other and involve genes widely dispersed on the chromosome (Nicolaou et al., 2010). Engineering strategies for a specific solvent is thus determined by its inherent toxic nature and the associated mechanisms of toxicity.

Unfortunately, little work has been done to elucidate how microorganisms respond to geraniol, which severely limits the development of geraniol tolerance strains to facilitate the improved production. To identify the candidate genes conferring geraniol tolerance in *E. coli*, a genomic shot-gun library was subjected to growth based screening under geraniol stress (Fig. 1A). As discussed later,

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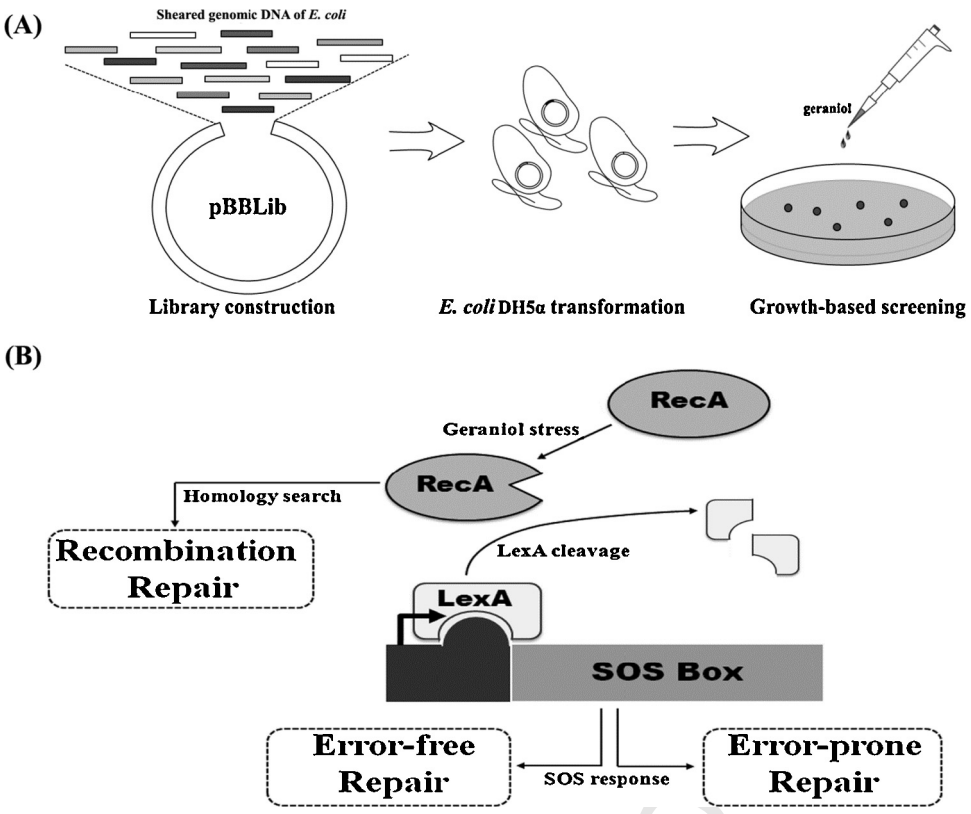


**Fig. 1.** Schematic diagrams of the shot-gun DNA library screening strategy and the role of RecA in *E. coli*. (A) After constructing a shotgun library from genomic DNA of *E. coli* MG1655 on pBBR1MCS-2 plasmid, the library was transformed into the *E. coli* DH5α and spread on LBGMg agar plates. The plates were overlaid with 2% (v/v) concentrations of geraniol solution. Subsequently, geraniol tolerant large colonies were selected from the plates containing the 4% (v/v) geraniol concentration, and the shot-gun plasmids in the selected colonies were isolated to identify the genes conferring geraniol tolerant phenotype. (B) Activated form of RecA functions in homologous recombination by catalyzing DNA strand exchange reactions. Activated RecA also act as a co-protease for auto-cleavage of LexA. LexA is a negative transcriptional repressor of multiple SOS box genes involved in DNA repair mechanisms including error-prone and error-free repair pathways.

RecA was discovered to be critical for providing geraniol tolerance. RecA is DNA-dependent ATPase as well as ATP-dependent DNA binding protein (Lusetti and Cox, 2002). It functions in homologous recombination by catalyzing DNA strand exchange reactions. In the presence of ATP, RecA polymerizes upon DNA to form nucleoprotein filaments and performs a homology search of the duplex DNA until a homologous strand is located (Kuzminov, 1999). Homologous recombination plays a primary role to repair lesions associated with DNA replication of damaged template DNA, specially, double strand breaks (DSBs) (Kuzminov, 1999). The RecA-mediated filaments can also act as a co-protease for auto-proteolysis of LexA (Fig. 1B). LexA is a transcriptional repressor of multiple SOS box genes involved in general DNA repair mechanisms such as nucleotide excision repair, recombination repair and mutagenic bypass repair (Long et al., 2008). SOS box genes such as DNA polymerases *polB* (Pol II) and *dinB* (Pol IV) usually have strong promoters for the factor-independent transcription (Volkert and Landini, 2001). The transcriptional de-repression of SOS genes provides a variety of protective responses via error-free/prone repairs to DNA damage (Volkert and Landini, 2001). Also, the mutations introduced by the low-fidelity DNA polymerases (*polB* and *dinB*) from SOS box are required for the efficient adaptation to stresses during evolution (Galhardo et al., 2009). In order to disclose the role of RecA in geraniol tolerance, the present study investigated the responses to geraniol in various *E. coli* strains with different genotypes for SOS response and DNA repair. It suggested that RecA-mediated SOS response initiates error-free DNA repair, particularly homologous recombination repair to counter geraniol toxicity. This study provides a new finding for engineering geraniol tolerance in *E. coli* host.

## 2. Materials and methods

### 2.1. Bacterial strains and culture conditions

*E. coli* strains used in this study are listed in Table 1. *E. coli* DH5α was used for gene cloning and initial geraniol tolerance screening. *E. coli* MG1655 was used as a parental strain for investigating the role of RecA in geraniol tolerance. *E. coli* BW25113 and its mutants, *E. coli* JW 2669 (Δ*recA*), JW 2788 (Δ*recB*), JW 3784 (Δ*xerC*) and JW 5416 (Δ*recN*) were obtained from Keio collection (Baba et al., 2006). Different alleles of *E. coli* BW25113 were moved into the parental background of MG1655 by P1 transduction (Miller, 2009). The kanamycin resistance cassette from the alleles originated from Keio Collection (National Insitute of Genetics, Shizuoka, Japan) were cured by expressing the FLP recombinase from the helper plasmid pCP20 (Baba et al., 2006). Temperature-sensitive plasmids (pCP20 and pMAKrecA) were cured by growing the cells at 42 °C. Cured colonies were confirmed by growing the individual cells on antibiotic-selective LB agar plates. The curing was also confirmed by standard plasmid extraction and gel analysis. Mutant strains of *E. coli* K996 (*lexA*3(ind⁻)) and GY3448 (*recA*430) were obtained from *E. coli* Genetic Stock Center (Yale University, New Haven, CT) and *E. coli lexA*300(Def) was kindly provided by Prof. Graham C. Walker (Massachusetts Institute of Technology, Cambridge, MA). The geraniol tolerance assays were carried out in a modified Luria-Bertani medium (LBGMg) which contains 1% (w/v) tryptone, 0.5% (w/v) yeast extract, 1% (w/v) NaCl, 0.1% (w/v) glucose and 10 mM $MgSO_4$. Kanamycin (50 μg/mL) and chloramphenicol (50 μg/mL) were added as required.



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**Table 1**
Strains and plasmids used in this work.

| Names | Descriptions | References |
|---|---|---|
| Strains | | |
| *E. coli* DH5α | F⁻, λ⁻, *end*A1 *glnV*44 *thi*-1 *recA*1 *relA*1 *gyr*A96 *deo*R, Φ80, d*lacZ*ΔM15, Δ(*lacZYA-argF*), U169, *hsd*R17($r_K^-$ $m_K^+$), *sup*E44 | ATCC 53868 |
| *E. coli* MG1655 | F⁻, λ⁻, *ilvG*⁻, *rfb*-50, *rph*-1 | ATCC 700926 |
| *E. coli* BW25113 | *lacI*$^q$, Δ*lacZ*$_{WJ16}$, Δ*ara*BAD$_{AH33}$, Δ*rha*BAD$_{LD78}$, *hsd*R514, and r*rnB*$_{T14}$ | Baba et al., 2006 |
| *E. coli* BL21 | F⁻, *dcm*, *ompT*, *hsdS*($r_B$- $m_B$-), gal, [malB⁺]$_{K\text{-}12}$($\lambda^S$) | Stratagene |
| *E. coli* DH10B | F⁻, λ⁻, *endA*1, *recA*1, *galE*15, *galK*16, *rpsL*, Δ*lacX*74, Φ80, *lacZ*ΔM15, *araD*139, Δ(ara,leu)7697, *mcrA*, Δ(mrr-hsdRMS-mcrBC) | Invitrogen |
| *E. coli* JW2669 | *E. coli* BW25113 Δ*recA* | Baba et al., 2006 |
| *E. coli* JW2788 | *E. coli* BW25113 Δ*recB* | Baba et al., 2006 |
| *E. coli* JW3784 | *E. coli* BW25113 Δ*xerC* | Baba et al., 2006 |
| *E. coli* JW5416 | *E. coli* BW25113 Δ*recN* | Baba et al., 2006 |
| *E. coli* MG1655 Δ*recA* | MG1655 with Δ*recA* allele originated from JW2669 by P1 transduction | This study |
| *E. coli* MG1655 Δ*recB* | MG1655 with Δ*recB* allele originated from JW2788 by P1 transduction | This study |
| *E. coli* MG1655 Δ*xerC* | MG1655 with Δ*xerC* allele originated from JW3784 by P1 transduction | This study |
| *E. coli* MG1655 Δ*recN* | MG1655 with Δ*recN* allele originated from JW5416 by P1 transduction | This study |
| *E. coli* K996 | *lexA*3(ind⁻), *malE*300::Tn10 | *E. coli* Genetic Stock Center |
| *E. coli* AB1157 | *lexA300*(Def)::*spec*, Δ*sulA* | Walker Lab, MIT, Cambridge |
| *E. coli* GY3448 | F⁻, λ⁻, *glnV*44(AS), *recA*430, *cysJ*43, *relA*1 | *E. coli* Genetic Stock Center |
| *E. coli lexA*3(ind⁻) | MG1655 with *lexA*3(ind⁻) allele originated from *E. coli* K996 by P1 transduction | This study |
| *E. coli lexA*300(Def) | MG1655 with *lexA*300(Def) allele originated from *E. coli* lexA300(Def) by P1 transduction | This study |
| Plasmids | | |
| pBBR1MCS-2 | $P_{lac}$ and $Km^r$ | Kovach et al., 1995 |
| pBBLib-18 | pBBR1MCS-2 containing positive genome insert | This study |
| pBBmltB | pBBR1MCS-2 containing *mltB* gene from MG1655 | This study |
| pBBygaD | pBBR1MCS-2 containing *ygaD* gene from MG1655 | This study |
| pBBrecA | pBBR1MCS-2 containing *recA* gene from MG1655 | This study |
| pBBrecX | pBBR1MCS-2 containing *recX* gene from MG1655 | This study |
| pBBalaS | pBBR1MCS-2 containing *alaS* gene from MG1655 | This study |
| pBBrecA430 | pBBR1MCS-2 containing *recA*430 gene from GY3448 | This study |
| pBAD-mRFP | $P_{bad}$, *rfp* and $Amp^r$ | Seung Goo Lee Lab, KRIBB |
| pEGFP | $P_{lac}$, *gfp* and $Amp^r$ | Clontech |
| pEmRFPnp | No promoter, *rfp* and $Amp^r$ | This study |
| pEmRFPsulAp | $P_{sulA}$, *rfp* and $Amp^r$ | This study |
| pMAK705 | $P_{lac}$, pSC101(ts) and $Cm^r$ | Hamilton et al., 1989 |
| pMAKrecA | pMAK705 containing *recA* gene from MG1655 | This study |

### 2.2. Chemicals and reagents

Restriction enzymes and T4 DNA ligase were purchased from New England Biolabs (Beverly, MA). Gel extraction and plasmid preparation kits were purchased from Qiagen (Valencia, CA). The geraniol and dodecane were obtained from Sigma–Aldrich (St. Louis, MO). Primers and *Pfu* DNA polymerase were purchased from Bioneer (Daejoen, Korea) and SolGent (Daejoen, Korea), respectively.

### 2.3. Construction of E. coli whole genome shotgun library

Wild type *E. coli* MG1655 was grown in 1 L of LB medium at 37 °C for overnight and harvested for genomic DNA extraction. The resulting genomic DNA was partially digested with *Sau*3AI for 20 min to obtain 3–6 kb DNA fragments. The purified 3–6 kb DNA (2.7 pg) was ligated with 1.4 pg of *Bam*HI-digested and dephosphorylated pBBR1MCS-2 plasmid and transformed into *E. coli* DH5α (Kang et al., 2005; Kovach et al., 1995). All the transformants were harvested from agar plates for shotgun library DNA preparation. The library DNA was stored at −70 °C for further use.

### 2.4. Shotgun library screening for geraniol tolerant phenotype

*E. coli* DH5α was retransformed with 10 ng of the shotgun library DNA and spread on LBGMg plates containing kanamycin. The plates were initially overlaid by 2% (v/v) of geraniol dissolved in dodecane (up to 3 mm thickness) and incubated at 30 °C for 72 h. Dodecane is often used to harvest hydrophobic volatile products in two-phase culture due to its hydrophobic, less-volatile and non-toxic properties (Newman et al., 2006). A subsequent control experiment showed that dodecane had no effect on *E. coli* at the concentration used here. The survival colonies (first round) were picked up and screened again at 4% (v/v) geraniol overlaid plates. Plasmids were isolated from the geraniol tolerant colonies (second round) and retransformed into a clean background *E. coli* DH5α to verify the geraniol tolerant phenotype. The plasmids conferring high tolerance against geraniol were sequenced by SolGent (Daejeon, Korea).

### 2.5. Construction of plasmids

The genomic DNA and plasmids preparation, restriction digestions, transformations and other molecular biological techniques were carried out as described in the literature (Miller, 2009). All PCR amplifications were carried out with *Pfu* DNA polymerase using the primers listed in Table S1. The *mltB*, *ygaD* and *recX* genes were amplified from pBBLib-18 and cloned into the *Kpn*I-*Hin*dIII restriction sites of pBBR1MCS-2 to generate pBBmltB, pBBygaD and pBBrecX. The *recA* and *alaS* genes were amplified from pBBLib-18 and cloned in to the *Sma*I-*Xba*I restriction sites of pBBR1MCS-2 to generate pBBrecA and pBBalaS. The mutated gene *recA*430 was amplified from *E. coli* GY3448 genomic DNA to construct pBBrecA430. The PCR product of *recA* gene was also cloned into corresponding site of pMAK705 (Hamilton et al., 1989) to construct pMAKrecA. To construct the RFP (red florescent protein) reporter plasmid, *rfp* gene was amplified from pBADmRFP plasmid (kindly provided by Dr. Seung Goo Lee, KRIBB) and cloned in to *Nco*I-*Not*I restrictions sites of pEGFP plasmid to replace the green flourescent gene, resulting in pEmRFP plasmid. The *lac* promoter of pEmRFP was removed by PCR amplification using primers pE-F and pE-R to construct promoterless pEmRFPnp plasmid. The *sulA* promoter was



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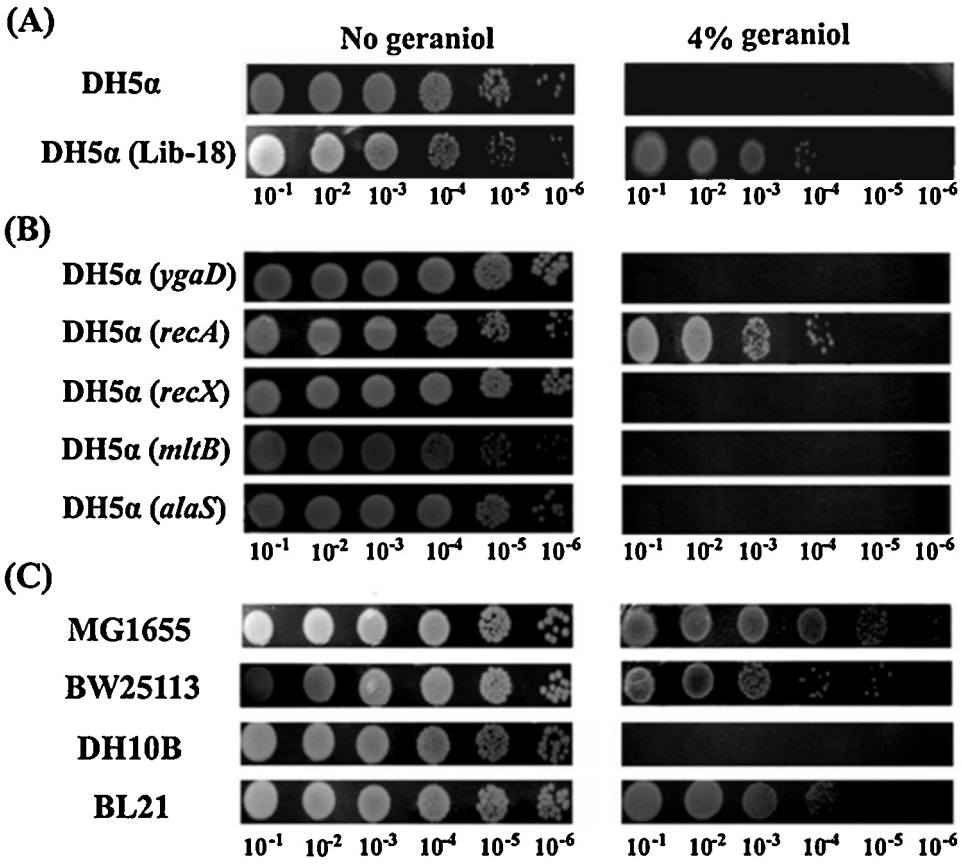


**Fig. 2.** RecA leading to geraniol tolerance in *E. coli*. (A) Colony formation efficiencies of *E. coli* DH5α overexpressing recombinant library clone. (B) *E. coli* DH5α strains overexpressing *mltB*, *ygaD*, *recA*, *recX* and *alaS*. (C) *E. coli* MG1655, BW25113 and BL21 have functional *recA* gene whereas DH10B is *recA* defective mutant. Each strain was spotted on LBGMg agar plates in serial ten-fold dilutions, overlaid with 4% (v/v) geraniol solution, and incubated at 30 °C for 24 h.

amplified from the upstream of the RecA-regulated *sulA* gene and cloned into corresponding *Sac*I-*Xho*I restriction sites of pEmRFPnp to produce pEmRFPsulAp.

### 2.6. Plate overlay analysis of geraniol tolerant phenotype

Geraniol tolerances were analyzed by measuring colony forming efficiency (CFE) on the LBGMg agar plate. *E. coli* strains were grown to 1.5 of optical density ($OD_{600}$) at 37 °C in the LBGMg medium. Serial ten-fold dilutions of the culture were prepared with saline solution. Each cell dilutions of 5 μl was spotted onto LBGMg agar plate and then overlaid with 4% (v/v) geraniol solutions. The plates were sealed and incubated at 30 °C for 24 h to measure colony forming efficiency (Okochi et al., 2008). Plates overlaid with dodecane alone (no geraniol) were used as a control for the comparison.

### 2.7. Monitoring SOS induction in individual cells

SOS response reporter plasmid of pEmRFPsulAp was transformed into the wild type *E. coli* MG1655 and MG1655 Δ*recA* mutant. Cells were grown in LBGMg liquid medium (containing 100 μg/mL ampicillin) at 250 rpm and 30 °C to 0.8 $OD_{600}$, and a 4% (v/v) geraniol solution was added in 10% volume of culture broth to induce the RFP expression. After additional cultivation for 2 h, cell broth of 10 μl was loaded onto fresh agarose pads and cover slips were applied. Cells were observed by a Fluoview FV 1000 confocal laser scanning microscope (Olympus Corporation, Tokyo, Japan). Excitation and emission spectra in the RFP channel were at 543 nm and 587–625 nm, respectively. A 100× oil objective lens was used for all photography.

## 3. Results and discussion

### 3.1. Shotgun library screening and isolation of genes conferring improved geraniol tolerance

In order to identify *E. coli* genes conferring geraniol tolerance, *E. coli* MG1655 genomic DNA library (approximately $3 \times 10^4$ colonies) was constructed and screened under the geraniol stress. A total of 5 colonies exhibiting improved growth phenotype (based on colony size) in the presence of 4% (v/v) geraniol were isolated. The plasmids were extracted from the positive clones and re-transformed into native *E. coli* DH5α cells for the further phenotypic confirmation. The result indicates that such a new transformant (pBBLib-18) still maintains the geraniol tolerance phenotype, and exhibits a higher colony forming efficiency (CFE) by ~$10^4$-fold than the control strain harboring an empty vectors (pBBR1MCS-2) (Fig. 2A). The sequencing of the shotgun DNA fragment in pBBLib-18 revealed five intact genes *mltB*, *ygaD*, *recA*, *recX*, and *alaS* with different functions (Table S2). To find out the specific gene corresponding to geraniol tolerance, these genes were sub-cloned individually into pBBR1MCS-2 vector and CFEs of recombinant *E. coli* DH5α overexpressing *mltB*, *ygaD*, *recA*, *recX*, and *alaS* were measured in the presence of 4% (v/v) geraniol (Fig. 2B). Compared to the control strain, the expression of *ygaD*, *recX*, *mltB* and *alaS* did not improve the tolerance of host strain against geraniol. Interestingly, the expression of *recA* resulted in a similar geraniol tolerance with the shot-gun clone containing pBBLib-18 in the presence of 4% (v/v) of geraniol. It should be noted

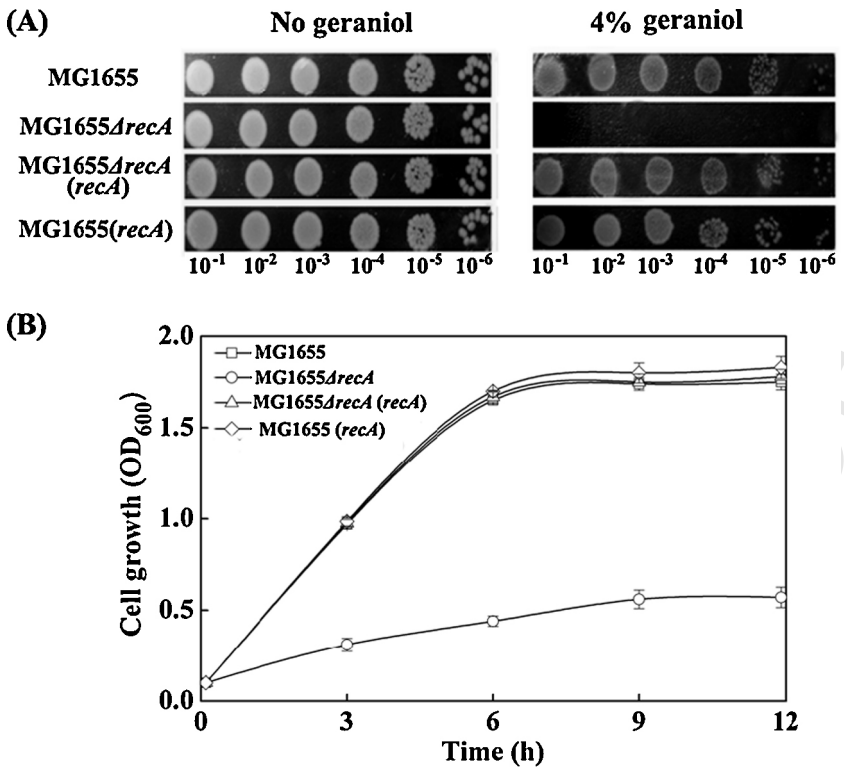


**Fig. 3.** Plate and broth overlay of wild type *E. coli* MG1655 and its ΔrecA mutants. (A) Colony formation efficiencies of wild type *E. coli* MG1655 and MG1655 Δ*recA* overexpressing *recA* on geraniol overlay plates. Each strain was spotted on LBGMg agar plates in serial ten-fold dilutions, overlaid with 4% (v/v) geraniol solution, and incubated at 30 °C for 24 h. (B) Cell growth of *E. coli* MG1655 and MG1655 Δ*recA* mutants in geraniol overlay broth culture. When the cell growth ($OD_{600}$) reached to 0.1, the culture was overlaid with the 4% (v/v) geraniol solutions in 10% volume of culture broth and cultivated at 250 rpm and 30 °C in shaking incubator. Cell growth was measured every 3 h during 12 h of the cultivation. Error bar indicates the standard error of mean.

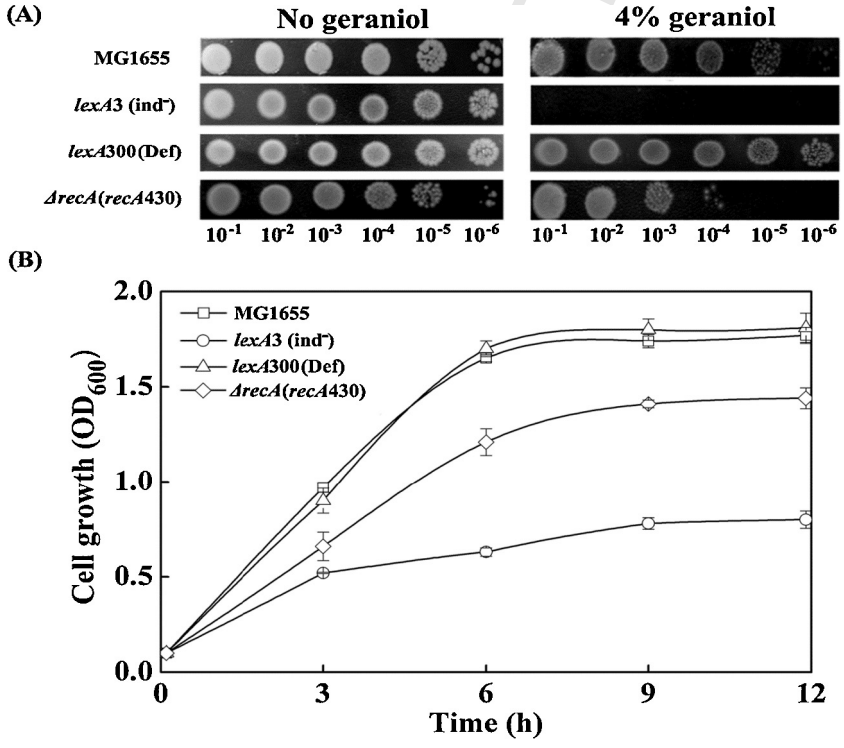


**Fig. 4.** SOS response mediating geraniol tolerance in *E. coli*. (A) Colony formation efficiencies of wild type *E. coli* MG1655 and its mutants with altered capacity for SOS induction after geraniol overlay. Each strain was spotted on LBGMg agar plates in serial ten-fold dilutions, overlaid with 4% (v/v) geraniol solutions, and incubated at 30 °C for 24 h. (B) Cell growth in broth overlay. When the cell growth ($OD_{600}$) reached to 0.1, the culture was overlaid with the 4% (v/v) geraniol solutions in 10% volume of culture broth and cultivated at 250 rpm and 30 °C in shaking incubator. Cell growth was measured every 3 h during 12 h of the cultivation. Error bar indicates the standard error of mean.

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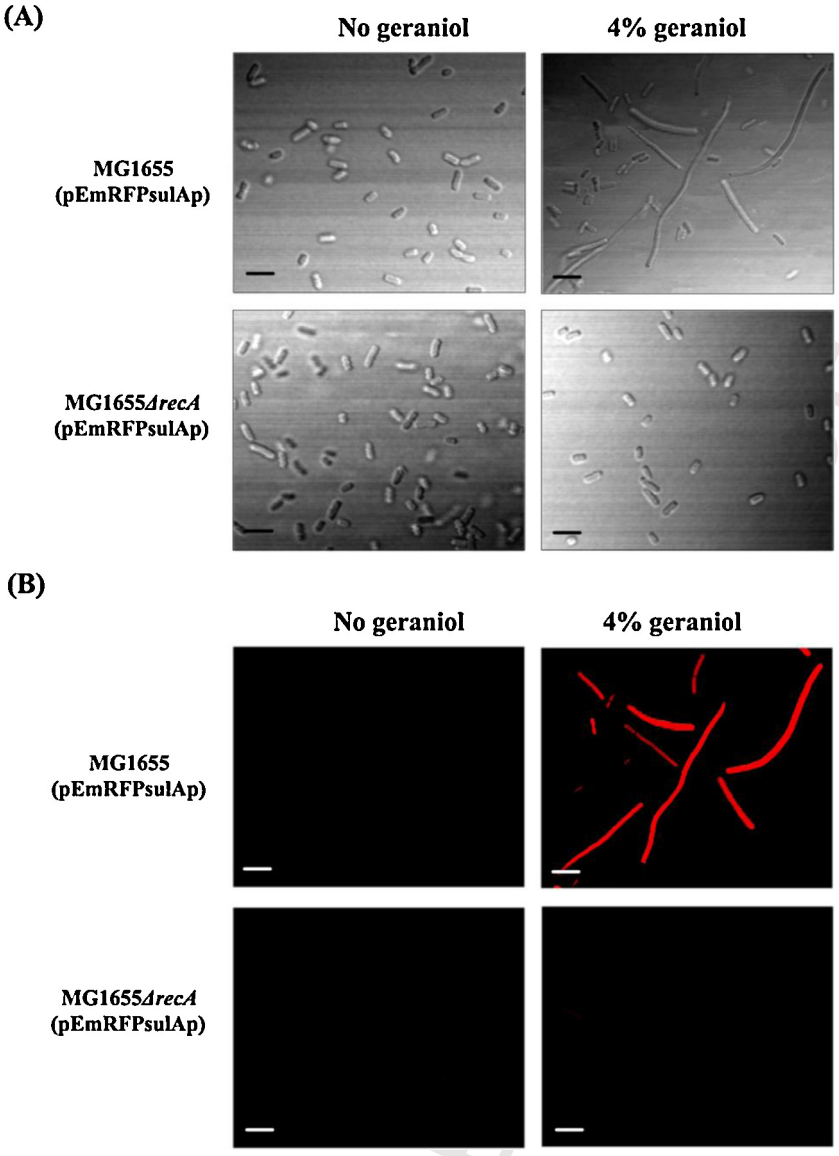


**Fig. 5.** Detecting the SOS response from individual cells. (A) Bright field images and (B) Fluorescence images of the wild type *E. coli* MG1655 and its *recA* knock-out mutant in presence and absence of 4% (v/v) geraniol in dodecane. Geraniol solution was added in culture of each strain at 0.8 of $OD_{600}$ and cells were grown for an additional 2 h before imaging. Scale bar represents 5 μm.

that *E. coli* DH5α is a *recA* defective mutant. The higher geraniol tolerance was also observed in untransformed *E. coli* strains with functional *recA* gene such as *E. coli* MG1655, BW25113 and BL21, but not in the *recA*-defective *E. coli* DH10B (Fig. 2C). Therefore, the *recA* deletion was suspected to impair tolerance of *E. coli* against geraniol. In the presence of geraniol, the cell growth of *E. coli* MG1655 was defective by *recA* deletion and restored by *recA* complementation (Fig. 3). Taken together, it was concluded that *recA* plays a crucial role in *E. coli* for geraniol tolerance.

### 3.2. RecA-mediated SOS response provides geraniol tolerance

RecA performs recombinational DNA repair and induces bacterial SOS response through LexA cleavage (Fig. 1B). To decipher the RecA role on geraniol tolerance, various *E. coli* strains with altered capacity of DNA repair and SOS induction was investigated. The *lexA*3(ind-) allele of *E. coli* encodes a non-cleavable mutant LexA protein. RecA cannot proteolyze this mutated LexA to induce SOS response but is still proficient in homologous recombination (Mount et al., 1972). In contrast, the variant in *E. coli* MG1655 LexA300(Def) constitutively induce the expression of SOS response genes regardless of RecA (Dorr et al., 2009). Both LexA mutations did not have any significant effect on CFEs in the absence of geraniol (Fig. 4A). However, *E. coli lexA*3(ind$^-$) was as hypersensitive to geraniol as the *E. coli* MG1655 Δ*recA* mutant whereas *E. coli lexA*300(Def) showed equal tolerance to geraniol as *E. coli* MG1655. *E. coli* MG1655Δ*recA* harboring a mutated RecA430, which exhibits a low LexA cleavage efficiency (Menetski and Kowalczykowski, 1990), had an intermediate geraniol tolerance (Fig. 4A).

To further examine the effect of these mutants toward geraniol, susceptibility test in liquid culture was also conducted. The outcome of cell growth pattern was similar to the results of plate overlay (Fig. 4B).

The exposure of *E. coli* to geraniol was thought to induce SOS response resulting in geraniol tolerance. To confirm the induction of SOS response by geraniol, a *sulAp*-RFP reporter system was constructed by fusion of the LexA-regulated *sulA* promoter with RFP in a low-copy plasmid. SulA is tightly repressed by LexA under the normal growth condition but inhibits cell division during the SOS response until DNA damage is repaired (McCool et al., 2004). As

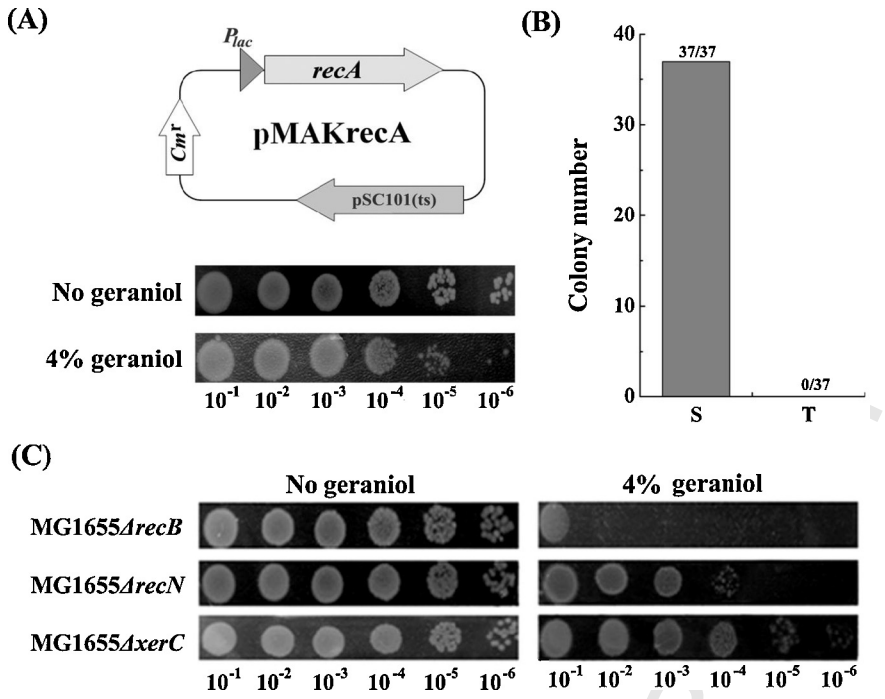


**Fig. 6.** SOS response mediated homologous recombination repair conferring geraniol tolerance in *E. coli*. (A) Plate overlay results of *E. coli* MG1655 Δ*recA* mutant overexpressing *recA* on the pMAKrecA plasmid. It contains the heat-sensitive origin of replication (pSc101), chloramphenicol resistance marker, and *recA* gene under the control of *lac* promoter. (B) Number of sensitive (S) and tolerant (T) colonies after plasmid curing. LBGMg agar plates were overlaid with 4% (v/v) geraniol solution. (C) Colony formation efficiencies of *E. coli* MG1655 mutants deficient in different recombination repair systems after geraniol overlay. Each strain was spotted on LBGMg agar plates in serial ten-fold dilutions, overlaid with 4% (v/v) geraniol solutions, and incubated at 30 °C for 24 h.

expected, the addition of geraniol (4%, v/v) resulted in cell filamentation of the wild type *E. coli* MG1655 and a large population of cells also exhibited a significant red fluorescence as compared to those of no gerniol treatment (Fig. 5A and B).

Cellular filamentation in wild type *E. coli* is specific indication of DNA damage and induction of the SOS response (Justice et al., 2006; Kamensek et al., 2010). However, *E. coli* MG1655 Δ*recA* failed to induce SOS response, and filamentations and red fluorescence were not observed for this strain. Together, these results suggest that the geraniol tolerance is indeed dependent on a functional RecA-mediated SOS response. As RecA is an inducer of the SOS response and not a direct participant in geraniol tolerance, the additional RecA overexpression would not further improve the tolerance of wild type *E. coli* MG1655 (Fig. 3). SOS response is usually triggered by problematic DNA replication or DNA damage which is caused from a variety of toxic heavy metal ions, chemicals and antibiotics (Dorr et al., 2009; Kozubek et al., 1989; van der Veen et al., 2010). In a similar manner, exposure of *E. coli* to geraniol may also lead to DNA lesions directly or indirectly.

### 3.3. SOS-mediated recombinational DNA repair contributes to geraniol tolerance

SOS response can activate genes required for error-free DNA repair such as nucleotide excision repair and homologous recombination repair (Friedman et al., 2005). It also can stimulate some DNA polymerases such as *dinB* involved in mutagenic bypass replication of lesions, which is termed as error-prone DNA repair (Hori et al., 2010). The occurrence of SOS-induced mutations accelerates the adaptive evolution of resistant mutants (McKenzie et al., 2000). To further dissect the possible mechanisms for geraniol tolerance between error-free and error-prone repair, RecA was expressed from a temperature-sensitive plasmid (pMAKrecA) in *E. coli* MG1655 Δ*recA* for the geraniol induction of SOS response (Fig. 6A). If the SOS-induced adaptive mutation results in the geraniol tolerance in *E. coli* MG1655 Δ*recA*, the adapted resistant mutants (survived colonies in the presence of 4% (v/v) geraniol) would maintain their tolerant phenotypes even after removal of pMAKrecA and no more expression of RecA. However, such an outcome was not obtained. All of 37 "tolerant" strains became sensitive to geraniol like the parental *E. coli* MG1655 Δ*recA* after curing of the temperature-sensitive pMAKrecA plasmid (Fig. 6B and Fig. S1). This result suggests that mechanism of SOS-induced adaptive mutation is not involved in conferring geraniol tolerance in *E. coli*.

The error-free DNA repair activated by RecA could be a responsible route for geraniol tolerance in *E. coli*. Therefore, some representative DNA recombinational repair pathways were investigated to probe the possible role in geraniol tolerance. Deletions of *recB* and *recN* genes resulted in *E. coli* MG1655 Δ*recB* and Δ*recN* sensitive to geraniol, although RecA was present in the mutant strains (Fig. 6C). RecB is the key protein of homologous recombinational pathway and hallmark for double strand breaks (DSBs) repair in *E. coli* (Dorr et al., 2009). RecN protein belongs to an alternative pathway of recombination repair. The RecN deficient strain is unable to repair single strand breaks (SSBs), but partially active in DSB repair (Dorr et al., 2009). XerC is a recombinase for Xer site-specific recombination which resolves chromosome dimers at *dif* site in the abnormal recombination events (Blakely et al., 1991). The deletion of *xerC* does not affect the proficiency for induction of SOS response (Dorr et al., 2009). As a result, *xerC* deletion did not impair the geraniol tolerance in *E. coli* (Fig. 6C). It has been reported that geraniol is a potent inducer of DNA breaks in the cultured plant shoot primordia of *Matricaria chamomilla* (Izumi et al., 1999). Therefore, we suspect that geraniol likely leads to DSB lesions and induces SOS response in *E. coli*. The functional RecA-mediated SOS response most likely initiates homologous recombinational repair to combat DNA damage and promotes survival of *E. coli* against geraniol toxicity.



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## 4. Conclusion

In this study, we found an unexpected role of RecA in providing geraniol tolerance based on SOS response. SOS response has been known to contribute to the survival of many bacteria against a variety of stresses (van der Veen et al., 2010). Our data implicated that geraniol putatively causes DNA damge that leads to SOS response induction. The SOS-induced recombinational repair plays a major role to combat geraniol toxicity through the corrective repair of damaged DNA. In metabolic engineering *E. coli* strains are required to be RecA deficient to ensure a stability of engineered exogenous plasmids in the hosts (Ajikumar et al., 2010; Tsuruta et al., 2009). The RecA deficient strains cannot serve as an ideal host for overproduction of geraniol. A specific strain, in which RecA is deficient but SOS response is constitutively activated may be a good host for geraniol production. It is expected that our results provide a mechanistic understanding of geraniol tolerance, which will benefit the development of resistant hosts for its production.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jbiotec.2013.07.023.

## References

Ajikumar, P.K., Xiao, W.H., Tyo, K.E., Wang, Y., Simeon, F., Leonard, E., Mucha, O., Phon, T.H., Pfeifer, B., Stephanopoulos, G., 2010. Isoprenoid pathway optimization for taxol precursor overproduction in *Escherichia coli*. Science 330, 70–74.

Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., Baba, M., Datsenko, K.A., Tomita, M., Wanner, B.L., Mori, H., 2006. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the keio collection. Molecular Systems Biology 2, 0008.

Blakely, G., Colloms, S., May, G., Burke, M., Sherratt, D., 1991. *Escherichia coli* XerC recombinase is required for chromosomal segregation at cell division. New Biologist 3, 789–798.

Carnesecchi, S., Schneider, Y., Ceraline, J., Duranton, B., Gosse, F., Seiler, N., Raul, F., 2001. Geraniol, a component of plant essential oils, inhibits growth and polyamine biosynthesis in human colon cancer cells. Journal of Pharmacology and Experimental Therapeutics 298, 197–200.

Dorr, T., Lewis, K., Vulic, M., 2009. SOS response induces persistence to fluoroquinolones in *Escherichia coli*. PLoS Genetics 5, e1000760.

Dunlop, M.J., Dossani, Z.Y., Szmidt, H.L., Chu, H.C., Lee, T.S., Keasling, J.D., Hadi, M.Z., Mukhopadhyay, A., 2011. Engineering microbial biofuel tolerance and export using efflux pumps. Molecular Systems Biology 7, 487.

Fischer, M.J., Meyer, S., Claudel, P., Bergdoll, M., Karst, F., 2011. Metabolic engineering of monoterpene synthesis in yeast. Biotechnology and Bioengineering 108, 1883–1892.

Fischer, M.J., Meyer, S., Claudel, P., Perrin, M., Ginglinger, J.F., Gertz, C., Masson, J.E., Werck-Reinhardt, D., Hugueney, P., Karst, F., 2013. Specificity of *Ocimum basilicum* geraniol synthase modified by its expression in different heterologous systems. Journal of Biotechnology 163, 24–29.

Fortman, J.L., Chhabra, S., Mukhopadhyay, A., Chou, H., Lee, T.S., Steen, E., Keasling, J.D., 2008. Biofuel alternatives to ethanol: pumping the microbial well. Trends in Biotechnology 26, 375–381.

Friedman, N., Vardi, S., Ronen, M., Alon, U., Stavans, J., 2005. Precise temporal modulation in the response of the SOS DNA repair network in individual bacteria. PLoS Biology 3, e238.

Galhardo, R.S., Do, R., Yamada, M., Friedberg, E.C., Hastings, P.J., Nohmi, T., Rosenberg, S.M., 2009. DinB upregulation is the sole role of the SOS response in stress-induced mutagenesis in *Escherichia coli*. Genetics 182, 55–68.

Hamilton, C.M., Aldea, M., Washburn, B.K., Babitzke, P., Kushner, S.R., 1989. New method for generating deletions and gene replacements in *Escherichia coli*. Journal of Bacteriology 171, 4617–4622.

Hori, M., Yonekura, S., Nohmi, T., Gruz, P., Sugiyama, H., Yonei, S., Zhang-Akiyama, Q.M., 2010. Error-prone translesion DNA synthesis by *Escherichia coli* DNA polymerase IV (DinB) on templates containing 1,2-dihydro-2-oxoadenine. Journal of Nucleic Acids 2010, 807579.

Izumi, S., Takashima, O., Hirata, T., 1999. Geraniol is a potent inducer of apoptosis-like cell death in the cultured shoot primordia of *Matricaria chamomilla*. Biochemical and Biophysical Research Communications 259, 519–522.

Justice, S.S., Hunstad, D.A., Seed, P.C., Hultgren, S.J., 2006. Filamentation by *Escherichia coli* subverts innate defenses during urinary tract infection. Proceedings of the National Academy of Sciences of the United States of America 103, 19884–19889.

Kamensek, S., Podlesek, Z., Gillor, O., Zgur-Bertok, D., 2010. Genes regulated by the *Escherichia coli* SOS repressor LexA exhibit heterogeneous expression. BMC Microbiology 10, 283.

Kang, M.J., Lee, Y.M., Yoon, S.H., Kim, J.H., Ock, S.W., Jung, K.H., Shin, Y.C., Keasling, J.D., Kim, S.W., 2005. Identification of genes affecting lycopene accumulation in *Escherichia coli* using a shot-gun method. Biotechnology and Bioengineering 91, 636–642.

Kovach, M.E., Elzer, P.H., Hill, D.S., Robertson, G.T., Farris, M.A., Roop 2nd, R.M., Peterson, K.M., 1995. Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. Gene 166, 175–176.

Kozubek, S., Krasavin, E.A., Soska, I., Drasil, V., Amirtayev, K.G., Tokarova, B., Bonev, M., 1989. Induction of the SOS response in *Escherichia coli* by heavy ions. Mutation Research 215, 49–53.

Kuzminov, A., 1999. Recombinational repair of DNA damage in *Escherichia coli* and bacteriophage lambda. Microbiology and Molecular Biology Reviews: MMBR 63, 751–813, table of contents.

Lapczynski, A., Bhatia, S.P., Foxenberg, R.J., Letizia, C.S., Api, A.M., 2008. Fragrance material review on geraniol. Food and chemical toxicology: an International Journal Published for the British Industrial Biological Research Association 46 (Suppl. 11), S160–S170.

Long, J.E., Renzette, N., Centore, R.C., Sandler, S.J., 2008. Differential requirements of two recA mutants for constitutive SOS expression in *Escherichia coli* K-12. PLoS ONE 3, e4100.

Lusetti, S.L., Cox, M.M., 2002. The bacterial RecA protein and the recombinational DNA repair of stalled replication forks. Annual Review of Biochemistry 71, 71–100.

McCool, J.D., Long, E., Petrosino, J.F., Sandler, H.A., Rosenberg, S.M., Sandler, S.J., 2004. Measurement of SOS expression in individual *Escherichia coli* K-12 cells using fluorescence microscopy. Molecular Microbiology 53, 1343–1357.

McKenzie, G.J., Harris, R.S., Lee, P.L., Rosenberg, S.M., 2000. The SOS response regulates adaptive mutation. Proceedings of the National Academy of Sciences of the United States of America 97, 6646–6651.

Menetski, J.P., Kowalczykowski, S.C., 1990. Biochemical properties of the *Escherichia coli* recA430 protein. Analysis of a mutation that affects the interaction of the ATP-recA protein complex with single-stranded DNA. Journal of Molecular Biology 211, 845–855.

Miller, J.H., 2009. A Short Course in Bacterial Genetics: A Laboratory Manual and Handbook for *Escherichia coli* and Related Bacteria. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Mount, D.W., Low, K.B., Edmiston, S.J., 1972. Dominant mutations (lex) in *Escherichia coli* K-12 which affect radiation sensitivity and frequency of ultraviolet light-induced mutations. Journal of Bacteriology 112, 886–893.

Newman, J.D., Marshall, J., Chang, M., Nowroozi, F., Paradise, E., Pitera, D., Newman, K.L., Keasling, J.D., 2006. High-level production of amorpha-4,11-diene in a two-phase partitioning bioreactor of metabolically engineered *Escherichia coli*. Biotechnology and Bioengineering 95, 684–691.

Nicolaou, S.A., Gaida, S.M., Papoutsakis, E.T., 2010. A comparative view of metabolite and substrate stress and tolerance in microbial bioprocessing: from biofuels and chemicals, to biocatalysis and bioremediation. Metabolic Engineering 12, 307–331.

Okochi, M., Kanie, K., Kurimoto, M., Yohda, M., Honda, H., 2008. Overexpression of prefoldin from the hyperthermophilic archaeum *Pyrococcus horikoshii* OT3 endowed *Escherichia coli* with organic solvent tolerance. Applied Microbiology and Biotechnology 79, 443–449.

Scalcinati, G., Knuf, C., Partow, S., Chen, Y., Maury, J., Schalk, M., Daviet, L., Nielsen, J., Siewers, V., 2012. Dynamic control of gene expression in *Saccharomyces cerevisiae* engineered for the production of plant sesquitepene alpha-santalene in a fed-batch mode. Metabolic Engineering 14, 91–103.

Tsuruta, H., Paddon, C.J., Eng, D., Lenihan, J.R., Horning, T., Anthony, L.C., Regentin, R., Keasling, J.D., Renninger, N.S., Newman, J.D., 2009. High-level production of amorpha-4,11-diene, a precursor of the antimalarial agent artemisinin, in *Escherichia coli*. PLoS ONE 4, e4489.

van der Veen, S., van Schalkwijk, S., Molenaar, D., de Vos, W.M., Abee, T., Wells-Bennik, M.H., 2010. The SOS response of *Listeria monocytogenes* is involved in stress resistance and mutagenesis. Microbiology 156, 374–384.

Volkert, M.R., Landini, P., 2001. Transcriptional responses to DNA damage. Current Opinion in Microbiology 4, 178–185.