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

Asad Ali Shah[a,1], Chonglong Wang[a,1], Sang-Hwal Yoon[a], Jae-Yean Kim[a], Eui-Sung Choi[b,**], Seon-Won Kim[a,*]

[a] Division of Applied Life Science (BK21 Program), PMBBRC, Gyeongsang National University, 501 Jinju Dae-ro, Jinju 660-701, Republic of Korea
[b] Industrial Biotechnology Research Center, Korea Research Institute of Bioscience & Biotechnology, 125 Gwahangno, Yuseong-gu, Daejeon 305-806, Republic of Korea

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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,

* Corresponding author. Tel.: +82 55 772 1362; fax: +82 55 759 9363.
** Corresponding author. Tel.: +82 42 860 4453; fax: +82 42 860 4489.
E-mail addresses: choi4162@kribb.re.kr (E.-S. Choi), swkim@gnu.ac.kr (S.-W. Kim).
[1] Both authors contributed equally to this paper.

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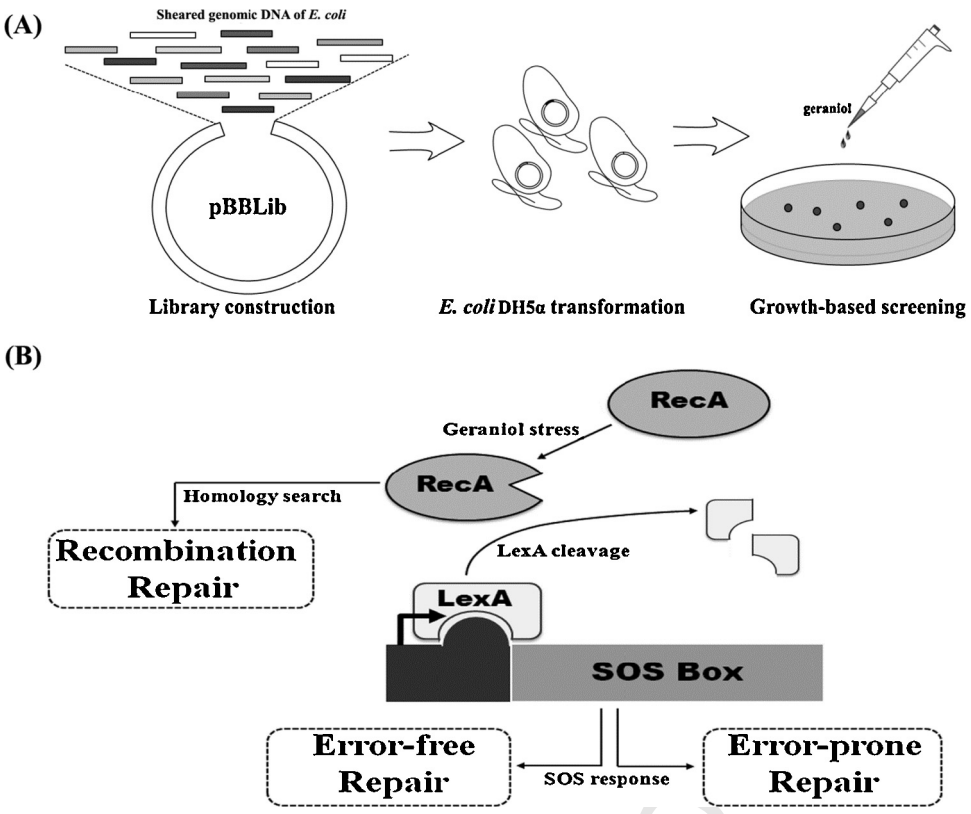

Fig. 1. Schematic diagrams of the shot-gun DNA library screening strategy and the role of RecA in *E. coli*. (A) After constructing a shotgun library from genomic DNA of *E. coli* MG1655 on pBBR1MCS-2 plasmid, the library was transformed into the *E. coli* DH5α and spread on LBGMg agar plates. The plates 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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