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Short communication

Plasmid expression of *mutS*, *-L* and/or *-H* gene in *Escherichia coli dam* cells results in strains that display reduced mutation frequency

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Abstract

Escherichia coli dam cells have an active but non-directed mismatch repair system; therefore, assembly of MutSLH complex at a mismatched base pair can result in MutH-mediated cleavage of GATC sites in both DNA strands. Unpaired double-strand breaks on a fraction of the replication errors occurring in *dam* cells presumably cause cell death, selectively eliminating these putative mutants from the population. We show that *E. coli dam* cells transformed with plasmids containing either the *mutS*, *mutL* or *mutH* gene display a mutation frequency three to eight times lower than that of the parental *dam* strain, due to increased mismatch-stimulated cell killing. Transformed strains are also more susceptible to killing by the base analogue 2-aminopurine. However, *dam* and *dam* transformed cells have similar duplication time, proportion of live/dead cells and morphology.

Keywords: MutS; MutL; MutH; Dam; Mismatch; Mutation frequency

1. Introduction

Cells lacking the mismatch repair system (MMRS) are mutators [1]. *Escherichia coli dam* cells, deficient in adenine methylation, have an undirected MMRS. Although undirected mismatch repair should have the same effect on mutation frequencies as no mismatch repair, the spontaneous mutation frequency of *dam* cells is lower than that of *mutS*, *mutL* or *mutH* single mutants or *dam mut* double mutants, all of which are deficient in mismatch repair [2,3]. This is because undirected mismatch repair can cause cell death by creating lethal double-strand breaks (DSBs) in DNA [3]. This hypothesis is supported by the findings that double mutants deficient in both Dam and recombinational repair (*dam recA*, *-B* and *-C* and *dam ruvA*, *-B* and *-C*) are inviable [4,5], but *dam recA mut* triple mutants are viable [2]. Such mismatch-stimulated cell killing would also account for the findings that *dam* mutants are more sensitive than wild type bacteria to base analogs, and UV irradiation, and that these sensitivities can be alleviated by addition of a *mut* mutation, which renders the *dam* cells deficient in mismatch repair [3]. Recent studies using single-cell electrophoresis showed that *E. coli dam* cells have a high level of DSBs, whose formation is dependent on functional mismatch repair [6].

Abbreviations: 2-AP, 2-aminopurine; Dam, DNA-adenine methylase; DSB, double-strand break; LB, Luria–Bertani medium; MMRS, mismatch repair system; Rif^r, rifampicin-resistant.

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Overproduction of Dam interferes with the normal action of SeqA following the replication fork resulting in a fully methylated DNA [7]. This modified DNA is resistant to MutH incision also resulting in a mutator phenotype [8–11]. On the contrary, in particular cases, overproduction of MutS, MutL or MutH reduces the rates of particular mutations in exponentially growing liquid culture or in stationary phase [12,13]. It also restores mismatch repair of *E. coli mutD5* mutant, which has mutated the *dnaQ* gene encoding the proofreading exonuclease of DNA polymerase III holoenzyme, even in log phase [14]. Similarly, saturation of DNA mismatch repair by a base analogue can be alleviated by introduction of a plasmid containing *mutL* [15]. These results show that plasmid expression of *mutS*, *-L* or *-H* gene improves functioning of the MMRS. Therefore, introduction of plasmids containing *mutS*, *mutL* or *mutH* gene into *E. coli dam* cells is expected to improve the MMRS, and consequently increase mismatch-stimulated cell killing. This hypothesis was tested in the present study. Our results show that introduction of plasmids containing any of the *mut* (*S*/*L*/*H*) genes into *E. coli dam* cells increases mismatch-stimulated cell killing, resulting in strains that display a mutation frequency three to eight times lower than that of the parental *dam* strain.

2. Materials and methods

2.1. Bacterial strains and plasmids

E. coli wild type strain (BW25113, *E. coli* K-12 W3110) and *E. coli mutL* (JWK4128-1/2), *mutS* (JWK2703-1/2), *mutH* (JWK2799-1/2) and *dam* (JWK3350-2/3) isogenic mutant strains (all in-frame single-gene knockout mutants) were from the Keio collection (http://www.ecoli.naist.jp) [16]. *E. coli dam mut* double mutant strains were obtained by isolation of 2-aminopurine (2-AP) resistant second-site revertants of *E. coli dam* mutants (JWK3350-2/3) as described previously [2]. Several 2-AP-resistant colonies were isolated and theirs *mutS*, *mutL* and *mutH* gene were PCR amplified and sequenced. Five strains having a nonsense mutation in the *mutS* (*dam mutS1*: codon 202, TGG to TGA; *dam mutS2*: codon 264, CAG to TAG), *mutL* (*dam mutL1*: codon 118, CAG to TAG; *dam mutL2*: codon 207, CAA to TAA) or *mutH* (*dam mutH1*: codon 23, CAA to TAA) gene were selected and further used. Plasmids pTX412 (pETS), pTX417 (pETL) and pTX418 (pETH) (pET-15b derivatives containing the *E. coli mutS*, *mutL* and *mutH* gene, respectively) were from Feng and Winkler [17]. Plasmid p5S corresponding to plasmid PMCS-*his-mutSc* [18], and plasmid p5L corresponding to plasmid p5-Lc [19], are p5 derivatives that carry N-terminal histidine tagged *E. coli* MutS and MutL proteins, respectively. It was previously observed that N-terminal histidine tagged MutS [18], MutL [20] and MutH [21] proteins are functional *in vivo*, and are also active *in vitro*. Plasmid pET15b (Novagen, Madison, WI, USA) carries a gene that confers resistance to ampicillin; pBBRMCS5 (p5) [22] carries a gene that confers resistance to gentamicin. pET and p5 derivatives have *dam* or *mut* genes under the control of bacteriophage T7 promoter. Strains used in this study do not contain the gene encoding T7 RNA polymerase, so expression of *dam* and *mut* genes depend on the adventitious use of an RNA polymerase promoter on the plasmid as it has been described [20]. We showed previously that expression of these plasmids are able to complement *mut* mutant strains [18,19]. A recent study using polyclonal antisera showed that *E. coli* MutL expression from pET plasmid without induction is approximately five-fold higher than normal chromosomal levels [20]. Plasmid JW3350 (pCA24N derivative containing the *E. coli dam* gene) was from the ASKA library (http://www.ecoli.naist.jp). Plasmid p5Dam resulted from integration of the EcoRI–SalI fragment from plasmid JW3350, containing the *E. coli dam* gene, into plasmid p5 (N. Morero *et al*., unpublished).

2.2. Cell transformation and determination of mutation frequency

In order to transform or to complement mutant strains, the corresponding plasmid(s) was introduced into *E. coli* strains by electroporation or by $CaCl_2$ method. Transformed strains were selected onto LB-agar plates containing the corresponding antibiotic (ampicillin 100 μg/ml, gentamicin 10 μg/ml or both). As pET and p5 derivatives have compatible replication origins and confer resistance to different antibiotics, these two set of plasmids were used in order to insert two *mut* genes in the same cell. For determination of mutation frequency, single colonies of transformed strains were grown in LB medium containing 10 μg/ml gentamicin (for p5-derivative transformed strains), 100 μg/ml ampicillin (for pET-derivative transformed strains) or gentamicin (10 μg/ml) and ampicillin (100 μg/ml) (for p5-derivative and pET-derivative transformed strains). Non-transformed controls were grown in LB without antibiotic. To calculate mutation frequencies, appropriate dilutions of overnight cultures were plated onto LB-agar to determine the total number of viable cells; or onto LB-agar containing 100 μg/ml rifampicin to determine number of rifampicin-resistant (Rif^r) cells, following incubation overnight at 37 °C. Mutation frequency was expressed as the number of Rif^r colonies per 10^8 viable cells. Results were grouped when differences between them were not statistically significant.

2.3. Bacterial growth in liquid culture and flow cytometry analysis

E. coli dam strain and transformed *E. coli dam* strains containing the empty plasmid or a plasmid carrying *mutS* or *mutL* gene, were cultured in LB with or without gentamicin (10 μg/ml) or ampicillin (100 μg/ml) with shaking overnight at 37 °C. Resulting strains were subcultured 1:100 or 1:130 into fresh LB with or without corresponding antibiotics, and incu-