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Phenotypes of *dnaA* mutants of *Escherichia coli* sensitive to detergents and organic solvents

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SUMMARY: We examined growth sensitivities of *dnaA* mutants of *Escherichia coli* to detergents (deoxycholic acid, cholic acid) and organic solvents (ethanol, glycerol). The *dnaA602*, *dnaA604*, *dnaA5*, and *dnaA46* mutants with mutations in the putative ATP binding site of DnaA protein showed higher sensitivities to the detergents than did the wild-type strain. The *dnaA508* and *dnaA167* mutants with mutations in the N-terminal region of DnaA protein, however, showed higher sensitivities to organic solvents. The *dnaA204* and *dnaA205* mutants with a mutation in the C-terminal region of DnaA protein showed similar sensitivity as did the wild-type strain to reagents. Complementation analysis with a plasmid carrying the wild-type *dnaA* gene confirmed that *dnaA* mutations are responsible for these phenotypes that are sensitive to the reagents.

Chromosomal DNA replication in *Escherichia coli* initiates at a unique sequence, *oriC*, the origin of chromosomal DNA replication, depending on function of the initiator protein, DnaA protein (1, 2). DnaA protein has a high affinity for ATP and ADP (3); the ATP-binding form is active in the *oriC* replication system *in vitro*, while the ADP-binding form is inactive. The ADP-binding form is activated by acidic phospholipids, cardiolipin and phosphatidylglycerol (4, 5), thereby suggesting that the activity of DnaA protein may be regulated by phospholipids *in vivo*. If such is indeed the case, then reagents that interact with lipids affect growth of *Escherichia coli* cells and this effect is in turn modulated by *dnaA* mutations. Detergents and organic solvents are bactericidal and various mutants sensitive to the reagents have been isolated (6, 7). We report here that some *dnaA* mutants showed higher

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sensitivities than the wild type strain to detergents and organic solvents and that *dnaA* mutations are apparently responsible for the sensitive phenotype of the *dnaA* mutants.

Materials and Methods

Bacterial strains---The following temperature-sensitive mutants of the *dnaA* gene and their parent strain (8-10) were kindly provided by Dr. T. Kogoma (University of New Mexico) through Dr. T. Katayama (Kyushu University).

AQ5425 [*metE46 trp-3 his-4 thi-1 galK2 lacY1 or lacZ4 mtl-1 ara-9 tsx-3 ton-1 rpsL8 or rpsL9 supE44*] λ^- *dnaA*$^+$

AQ4370, AQ5425 *dnaA5*; AQ5448, AQ5425 *dnaA602*; AQ5450, AQ5425 *dnaA604*; AQ5480, AQ5425 *dnaA46*; AQ5481, AQ5425 *dnaA205*; AQ5482, AQ5425 *dnaA204*; AQ5483, AQ5425 *dnaA203*; AQ5484, AQ5425 *dnaA508*; AQ5485, AQ5425 *dnaA167*.

P1 transduction of these AQ strains to W3110 cells was done as described in our previous paper (11). P1 phages were grown in the *dnaA* mutants, transduced into W3110 cells and the transductants were selected on LB agar plates containing 10μg/ml tetracycline. Sensitivity to temperature (42°C) was examined to select *dnaA* mutants with the genetic background of W3110. We named these transductants KS1001-1009 and they were used in all the experiments in this paper.

Plasmid---pHB10 containing the *Hin*dIII-*Xho*I fragment (2535 bp) of the *dnaA* gene inserted into the *Hin*dIII-*Sal*I site of pBR322 (12) was kindly provided by Dr. Y. Sakakibara (National Institute of Health, Japan). As this plasmid contains the *rpmH* gene which locates in the 5'-upstream region of the *dnaA* gene, we deleted the *Cla*I-*Cla*I fragment (659 bp) containing the *rpmH* gene from pHB10 and named the plasmid pHB10S.

Results and Discussion

Sensitivities of *dnaA* mutants to detergents.

Hirota and co-workers reported that a *dnaA46* mutant carries a phenotype sensitive to deoxycholic acid (1). We examined the sensitivities of other temperature-sensitive *dnaA* mutants to detergents. To confirm that the *dnaA* mutations cause the phenotype, a complementation analysis was done with a plasmid carrying the wild type *dnaA* gene. Full growth culture of bacteria cells was diluted and spread on LB agar plates containing various concentrations of deoxycholic acid. The plates were incubated for 20 hours and the numbers of colonies were counted. As shown in Figure 1(A), deoxycholic acid decreased colony forming ability of KS1002 and KS1007 harboring *dnaA5* and *dnaA508* mutations respectively in a concentration-dependent manner, whereas it little affected the colony forming abilities of KS1001, the wild type strain, and KS1006 harboring *dnaA205* mutation. KS1002 and KS1007 showed sensitive-phenotype to deoxycholic acid at 28°C, but differences in sensitivities to deoxycholic acid between the *dnaA* mutants and wild type cells were less at 28°C than those seen at 37°C (data not shown). The time required for generation of the *dnaA*

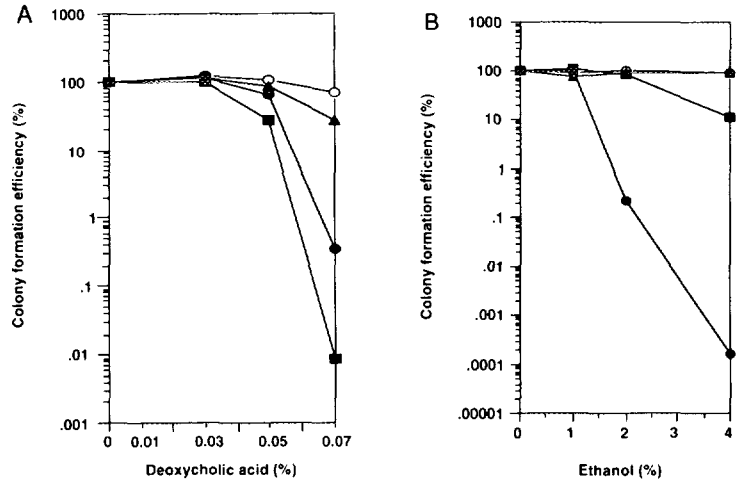

Figure 1.

Colony forming abilities of *dnaA* mutants on agar plates containing deoxycholic acid (A) or ethanol (B). Full growth cultures of bacteria cells were diluted and spread on LB agar plates containing deoxycholic acid. Plates were incubated at 37°C for 20hrs, and numbers of colonies were counted. The number of viable cells in the absence of the reagent is shown as 100% value. ○, KS1001 (*dnaA*$^+$); ▲, KS1006 (*dnaA205*); ●, KS1007 (*dnaA508*); ■, KS1002 (*dnaA5*).

mutants in liquid medium at 37°C, determined by measuring the number of viable cells which formed colonies on agar plates, was approximately 40 min, a time indistinguishable for that of the wild type strain, and size of colonies at 37°C was indistinguishable from that of the wild type strain (data not shown). Thus, the *dnaA* mutants showed no apparent growth phenotype at 37°C.

To examine the relationship between the deoxycholic acid-sensitivity and the position of mutation of the *dnaA* gene, we examined the colony forming abilities of other *dnaA* mutants on LB plates containing 0.07% deoxycholic acid (Table 1). Among the eight *dnaA* mutants tested, colony forming abilities of four strains, KS1002, KS1003, KS1008 and KS1009 decreased to less than 0.1% and colony formation abilities of KS1004 and KS1007 decreased to 1-2% The colony forming abilities of KS1001, KS1005 and KS1006 were little affected by 0.07% deoxycholic acid. Approximately half of the wild type cells (KS1001) could form colonies on agar plates containing the detergent, but the other half could not. This is presumably caused by fluctuation of physiological conditions between cells. The positions of mutations in these *dnaA* mutants have been identified (8-10). As KS1002, KS1003, KS1008 and KS1009 have a mutation in the putative ATP binding site of DnaA protein (8-10), our observations suggest the importance of the mutation for the sensitive phenotype to the detergent in the *dnaA* mutants. The reason why eight *dnaA* mutations showed different sensitivities to the detergent is probably due to structural diversities of mutated DnaA proteins.

Table 1

Influence of detergents on the growth of various *dnaA* mutants with and without pHB10S carrying the wild type *dnaA* gene. Full growth cultures of bacteria cells were diluted and spread on LB agar plates containing 0.07% deoxycholic acid or 0.5% cholic acid. Plates were incubated at 37°C for 20 hrs, and numbers of colonies were counted. Relative colony formation efficiency (%) in the presence of the detergents to the control, without detergents, was calculated.

Strains	0.07% Deoxycholic acid		0.5% Cholic acid	
pHB10S	-	+	-	+
KS1001(*dnaA*$^+$)	66	36	37	68
KS1002(*dnaA5*)	8.8×10^{-3}	42	9.2×10^{-4}	38
KS1003(*dnaA46*)	1.2×10^{-2}	67	5.1×10^{-5}	73
KS1004(*dnaA167*)	1.8	65	1.7×10^{-4}	71
KS1005(*dnaA204*)	20	73	16	35
KS1006(*dnaA205*)	36	30	5.7×10^{-1}	36
KS1007(*dnaA508*)	0.80	58	7.9×10^{-3}	84
KS1008(*dnaA602*)	3.9×10^{-2}	41	5.6×10^{-3}	55
KS1009(*dnaA604*)	2.2×10^{-2}	36	6.4×10^{-4}	56

To confirm that the deoxycholic acid sensitivity was caused by *dnaA* mutations, complementation studies were done. The plasmid, pHB10S, containing the wild type *dnaA* gene, restored both temperature-sensitive and the deoxycholic acid-sensitive phenotypes of the *dnaA* mutants (Table 1), whereas the vector pBR322 did not complement the deoxycholic acid-sensitive phenotype of the *dnaA* mutants (data not shown). Thus, deoxycholic acid-sensitivity of the *dnaA* mutants was caused by the *dnaA* mutations.

We also examined the sensitivities of *dnaA* mutants to cholic acid (Table 2). On the plate containing 0.5% cholic acid, the colony forming abilities of six strains, KS1002, KS1003, KS1004, KS1007, KS1008 and KS1009 decreased to less than 0.01% of that of control. KS1005 and KS1006 were less sensitive to cholic acid. Complementation analysis using pHB10S confirmed that mutations in the *dnaA* gene were responsible for the sensitivities to cholic acid.

Sensitivities of the *dnaA* mutants to organic solvents.

As ethanol as well as detergents affect the dynamics of membranes (13), and ethanol is bactericidal, we examined the sensitivity of the *dnaA* mutants to ethanol. As shown in Figure 1(B), colony forming ability of KS1007 on LB agar plates decreased to 0.25% on LB plates containing 2% ethanol, whereas colony forming abilities of KS1001, KS1002 and KS1006

Table 2

Influence of organic solvents on the growth of various *dnaA* mutants with and without pHB10S carrying the wild type *dnaA* gene. Full growth cultures of bacteria cells were diluted and spread on LB agar plates containing 4% ethanol or 10% glycerol. Plates were incubated at 37°C for 20 hrs, and numbers of colonies were counted. Relative colony formation efficiency (%) in the presence of organic solvents to the control, without organic solvents,was calculated.

Strains	4% Ethanol		10% Glycerol	
pHB10S	-	+	-	+
KS1001(*dnaA*$^+$)	92	80	100	87
KS1002(*dnaA5*)	11	84	75	92
KS1003(*dnaA46*)	88	90	100	95
KS1004(*dnaA167*)	5.2×10^{-5}	72	2.5×10^{-4}	93
KS1005(*dnaA204*)	76	93	110	79
KS1006(*dnaA205*)	92	83	120	120
KS1007(*dnaA508*)	1.6×10^{-4}	78	1.0×10^{-3}	100
KS1008(*dnaA602*)	95	93	100	120
KS1009(*dnaA604*)	81	110	96	94

were little affected by 4% ethanol. To determine the allele specificity of ethanol-sensitivity in *dnaA* mutants in detail, we examined the sensitivities of other *dnaA* mutants to 4% ethanol (Table 2). The colony forming abilities of two strains, KS1004 and KS1007 decreased to less than 0.0002% in the presence of 4% ethanol, whereas the colony forming abilities of other strains were little affected by 4% ethanol. With the introduction of pHB10S, the sensitive phenotypes of the *dnaA* mutants to ethanol were complemented, thereby suggesting that the *dnaA* mutation is responsible for the sensitive phenotype to ethanol (Table 2). As KS1004 and KS1007 carry mutations in the N-terminal region of the *dnaA* gene (8-10), the N-terminal region of DnaA protein may be responsible for the phenotype to be sensitive to ethanol.

When we examined the sensitivities of *dnaA* mutants to glycerol, the results were much the same as seen with ethanol. On the plate containing 10% glycerol, the colony forming abilities of two strains, KS1004 and KS1007 decreased to less than 0.001% of that on the control plate (Table 2). On the other hand, the colony forming abilities of the other strains were unaffected by glycerol. The complementation analysis using pHB10S revealed that the sensitive phenotypes of the these strains to glycerol were caused by *dnaA* mutations (Table 2).

The mechanism of sensitivity of *dnaA* mutants to the reagents remains to be elucidated. As detergents and organic solvents affect the phospholipid bilayer structure in cytoplasmic

membranes, one possible explanation for this sensitivity is that DnaA protein is activated by phospholipids *in vivo* and the reagents tested in this paper inhibit this activation resulting in an inhibition of chromosomal DNA replication. Mutated DnaA protein may interact weakly with phospholipids, hence *dnaA* mutants would show a sensitive phenotype to reagents. But we cannot exclude a possibility that the reagents directly affect the function of DnaA protein. Therefore, screening of drugs which specifically inhibit the interaction of DnaA protein with phospholipids is needed for further studies.

Biochemical analysis of mutated DnaA protein is difficult because mutated proteins from temperature-sensitive mutants are usually too unstable to withstand purification procedures. Mutated DnaA proteins from temperature-resistant *dnaA* mutants with sensitive phenotype to the reagents is one approach to analyze mechanisms controlling the activity of DnaA proteins.

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