JOURNAL OF BACTERIOLOGY, June 1978, p. 1039-1045
0021-9193/78/0134-1039$02.00/0

Vol. 134, No. 3
Printed in U.S.A.

Escherichia coli K-12 Mutants Deficient in Uracil-DNA Glycosylase†

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Received for publication 15 December 1977

A new assay specific for uracil-DNA glycosylase is described. *Escherichia coli* mutants partially and totally deficient in uracil-DNA glycosylase activity have been isolated by using this assay in mass-screening procedures. These have been designated *ung* mutants. The *ung* gene maps between *tyrA* and *nadB* on the *E. coli* chromosome. T4 phage containing uracil in their DNA grow on the most glycosylase-deficient hosts but are unable to grow on wild-type bacteria. This provides a simple spot test for the *ung* genotype. The *ung* mutants show slightly higher rates of spontaneous mutation to antibiotic resistance. Taken together, these results suggest a central role for uracil-DNA glycosylase in the initiation of an excision repair pathway for the exclusion of uracil from DNA.

Uracil is uniformly absent from the DNA of living cells. Lindahl and Nyberg studied the deamination of cytosine in DNA in vitro and concluded that uracil can be similarly produced in DNA at a significant rate in vivo and that its absence from DNA indicated the functioning of a uracil-specific repair pathway for the removal of these deaminated cytosine residues (14). Using uracil-containing DNA as substrate, Lindahl (11, 12) and Duncan et al. (7) identified uracil-DNA glycosylase activity in *Escherichia coli* and *Bacillus subtilis*, respectively. This activity was first named uracil *N*-glycosidase (11) and then uracil-DNA glycosidase (13), but has now been renamed uracil-DNA glycosylase in accord with current recommendations for carbohydrate nomenclature. Based on the properties of the purified enzyme (4, 13) and a consideration of the metabolic pathways (12, 22), several groups have proposed that uracil-DNA glycosylase is the first enzyme in a base excision repair pathway which functions to remove uracil from cellular DNA.

Recently Tye et al. (22) obtained indirect evidence for the in vivo synthesis of *E. coli* DNA containing small amounts of uracil. *E. coli dut* mutants deficient in the enzyme deoxyuridine-triphosphatase (dUTPase) apparently incorporate some dUTP into DNA, and this newly synthesized DNA was isolated as short Okazaki fragments in alkaline sucrose gradients. These short Okazaki fragments were presumed to arise during base excision repair initiated by the uracil-DNA glycosylase. The in vivo activity of this enzyme has also been observed by Duncan and Warner (8, 23) and Makino and Munakata (15).

This paper describes the isolation and some characteristics of *E. coli* mutants deficient in uracil-DNA *N*-glycosylase activity (*ung* mutants). These mutants should prove useful for characterizing base excision repair pathways in *E. coli* and for clarifying the origin of short DNA fragments produced during DNA replication (21). The isolation of a *B. subtilis* mutant (*urg*) deficient in uracil-DNA glycosylase activity has been described recently (15). A preliminary report of this work has appeared previously (Fed. Proc. **35**:1493, 1976).

MATERIALS AND METHODS

Bacterial and phage strains. The *E. coli* K-12 strains used in this study are listed in Table 1. Wild-type bacteriophages T4, T5, T7, and λ were from sources within the Biochemistry Department. P1*vir* and M13 were obtained from L. Rosner and D. Ray, respectively. The bacteriophage T4 mutant 56⁻ *denA denB alc-10* (19) used for the production of uracil-containing T4 phage was obtained from L. Snyder through E. Kutter. PBS2 phage and its host, *B. subtilis* SB19, were provided by A. Price; in this phage thymine is completely replaced by uracil.

Reagents and materials. [5-^{3}H]uracil was obtained from Schwarz/Mann. Nutritional supplements, antibiotics, and other chemicals were purchased from Sigma Chemical Corp. Microtiter trays and other equipment for mass screening were purchased from Linbro or Cooke Industries.

Media. The minimal medium described by Davis and Mingioli (6) was supplemented with the following nutrients as required: 0.4% glucose, 1.0 μg of thiamine per ml, 50 μg of L-amino acids per ml, 20 μg of adenine

† Scientific paper no. 9988 of the Minnesota Agricultural Experiment Station.

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TABLE 1. *E. coli strains used*

Strain	Genotype[a]	Source
BD10	W3110, *ung-1*	Mutagenesis
BD13 to BD28	W3110, *ung-2* through *ung-8*	Mutagenesis
BD1101	H677, *ung-1 his*$^+$	F15/BD10 × H677
BD1124	PA3306, *ung-6 nadB*$^+$ *purI*$^+$	Transduction
BD1125	PA3306, *ung*$^+$ *nadB*$^+$ *purI*$^+$	Transduction
BD1137	*thi-1 argH1 nadB4 purI66 pyrE41 lacY1 malA1 xyl-7 rha-6 ara-13 gal-7 rpsL9 tonA2* or *A22 supE44* T2^r *rel-1*?	CS101-4U1 × PA3306, transduction
BD1147	BD1137, *ung-1 nadB*$^+$ *tyrA2*	Transduction
BD1153	BD1137, *ung-1 nadB*$^+$ *purI*$^+$ *pyrE*$^+$	Transduction
BD1154	BD1137, *ung*$^+$ *nadB*$^+$ *purI*$^+$ *pyrE*$^+$	Transduction
CS101-4U1	Hfr, *metB1 pyrE41 tonA22* T2^r *rel-1*	CGSC[b]
H677	*thi-1 his-68 tyrA2 trp-45 purC50 lacY1 gal-6 xyl-7 mtl-2 malA1 rpsL125 tonA2 tsx-70* λ^- *supE44*?	CGSC (20)
KA197	Hfr, *thi-1 pheA97 relA1*	CGSC
PA3306	*thi-1 argH1 nadB4 purI66 lacY1 malA1 xyl-7 ara-13 mtl-2 gal-6 rpsL9 tonA2 supE44*	CGSC (20)
W3110	*thyA deo*-?	J. Fuchs (2)
BW212	BD10, *dut-11*	B. Weiss

[a] All strains are F$^-$ unless indicated otherwise.
[b] CGSC, *E. coli* Genetic Stock Center, Yale University School of Medicine, New Haven, Conn.

or uracil per ml, and 50 μg of thymine per ml. The rich nutrient medium contained 1.0% tryptone (Difco), 0.5% yeast extract, and 1.0% sodium chloride, pH 7.0 (TY medium). When necessary this was supplemented with 0.5% glucose (TYG medium) and 50 μg of thymine per ml (TYTG medium). Minimal or nutrient agar plates contained 1.5% agar (Difco) added to the above recipes.

DNA preparation. Radioactive PBS2 phage were prepared by adding [5-^{3}H]uracil (11 Ci/mmol; final concentration was 39 μCi/ml) to a *B. subtilis* SB19 culture in TYG medium 5 min after infection with PBS2 (infected with a multiplicity of infection of 4 at 2×10^8 bacteria per ml) and then shaking the culture at 37°C for 100 min. The lysate was centrifuged at 3,600 × *g* to remove debris and then for 3 h at 27,000 × *g* to sediment the phage. The resuspended phage were further purified by CsCl gradient centrifugation and dialyzed. The DNA was isolated by lysis of the virus with sodium dodecyl sulfate, repeated phenol extraction, and dialysis for 3 days against SSC buffer (0.15 M sodium chloride–0.015 M sodium citrate) and then against 0.1× SSC buffer. The DNA prepared in this way has radioactive label only in the uracil and the cytosine residues (8) and will be referred to as [5-^{3}H]Ura,Cyt-labeled DNA. Nonradioactive DNA was prepared by the same procedures (9). Heat-denatured DNA was prepared by incubation at 90°C for 5 min followed by rapid cooling in a water-ice slurry.

Genetics procedures. All bacterial genetics procedures were done as described by Miller (17), except as noted here. In transduction experiments bacteria with absorbed P1 phage were centrifuged after adding citrate. The culture was resuspended in minimal medium and spread on appropriate selective plates.

Enzyme assays. An adaptation of the standard glycosylase reaction described by Lindahl et al. (13) was devised to permit the screening of several hundred cultures per day during the search for mutants. Because it is based upon acid solubility of the uracil product, ethylenediaminetetraacetic acid (EDTA)-resistant endo- or exonucleases (such as exonuclease VII) might interfere with this assay. However, in the presence of EDTA, uracil is the only identifiable radioactive product released from [5-^{3}H]Ura,Cyt-labeled PBS2 DNA by both crude extracts and purified uracil-DNA glycosylase fractions from *E. coli* (data not shown). A second assay was used for the quantitative measurement of uracil-DNA glycosylase. This assay uses Dowex-1 to adsorb both the substrate and any acid-soluble nucleotide products formed and is therefore more specific and has a lower blank value because contaminating nuclease activities do not contribute to product formation. This Dowex-1 procedure should also be suitable for assaying any type of DNA-glycosidase activity provided that (i) a specific substrate can be made and (ii) the product is not adsorbed to the Dowex.

Screening assay based on acid solubility. Colonies were inoculated into 50 μl of TYTG broth in microtiter trays (96 cultures per tray) and grown to saturation at 25°C (48 h). The cultures were lysed by adding 25 μl of a buffered lysozyme-EDTA solution [0.33 M tris(hydroxymethyl)aminomethane (pH 8.0), 0.33 mg of lysozyme per ml, and 7.5 mM EDTA], incubating at room temperature for 15 min, and freeze-thawing three times. Then 25 μl of heat-denatured substrate ([5-^{3}H]Ura,Cyt-labeled DNA, 0.125 mM, 10^6 cpm of uracil per mmol) was added to each extract, and the culture tray was incubated at 45°C for 60 min (to select for heat-sensitive mutants). During this time, about 65% of the radioactivity was acid solubilized by extracts prepared from wild-type cells. The reaction was stopped by cooling the cultures at −20°C for 15 min and then adding 50 μl of 15% trichloroacetic acid. After 30 min at 0°C, the culture tray was centrifuged for 15 min at 3,000 rpm in an IEC model SBV centrifuge. The supernatant solution was removed and counted in 5 ml of scintillation fluid (0.6% 2,5-diphenyloxazole in toluene–Triton X-100, 2:1). With this pro-

cedure we analyzed between 400 and 800 colonies per day.

Quantitative enzyme assay with Dowex-1. The enzyme reaction mixtures consisted of 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid or tris(hydroxymethyl)aminomethane (pH 7.8), 1 mM EDTA, 1 mM dithiothreitol, and 20 μM heat-denatured substrate (3×10^6 to 3×10^7 cpm of uracil per mmol) in a total reaction volume of 0.15 ml. The reaction was initiated by adding 10^{-6} to 2×10^{-5} U of enzyme and transferring to a water bath at the desired temperature. After 30 min, the reaction was stopped by transferring the reaction mixture to an ice-water bath and applying it to a Dowex-1-$HCOO^-$ column (0.33 by 3 cm). The product, uracil, is eluted directly into a scintillation vial with the addition of 1.35 ml of water while the DNA substrate and any acid-soluble nucleotide products remain bound to the Dowex. Thus, endo- and exonucleases cannot contribute to apparent product formation as is possible with the acid solubility assay. Radioactivity was measured by adding 10 ml of scintillation fluid and counting. A recent modification of the assay is to add sodium dodecyl sulfate (0.3% final concentration) to stop the reaction before application to the Dowex column. A unit of enzyme activity is defined as 1 μmol of uracil formed per min at 37°C.

Phage spot test for *E. coli* containing the *ung* mutation. Colonies were picked with a toothpick and resuspended in 0.3 ml of broth in a depression dish. About 10^6 plaque-forming units of uracil-containing T4 phage were spread on a nutrient agar plate. These phage were prepared by allowing T4 (*56⁻ denA denB alc10*) to replicate in *dut ung E. coli* and contained about 30% uracil substituted in place of thymine (23). The bacteria in the depression dish were replica plated onto TYTG plates with and without phage. After 6 to 15 h at 30, 37, or 42°C, the bacterial growth spots on the plates were compared. Plaques were observed only in the spots corresponding to *ung* bacteria.

Mutation frequencies. Mutation frequencies were determined by using a modified fluctuation test. Cultures were started from a single colony in 1.5 ml of TY medium and grown overnight at 37°C to reach stationary phase. Ten tubes containing 1.5 ml of TY medium were each inoculated with about 150 to 200 cells and incubated for 18 h at 37°C. Then 0.1 ml from each tube was plated on a TY plate supplemented with nalidixic acid (20 μg/ml) or rifampin (100 μg/ml) and incubated for 36 h to measure spontaneous mutation frequencies (17). Viable cell count was measured by combining 0.1 ml of each of the 10 cultures, diluting, and plating on TY plates without antibiotic. Frequencies were then calculated as the average number of resistant colonies per 10^7 viable cells. "Jackpots" were excluded by omitting from the calculations any plates with more than twice the mean number of mutants.

RESULTS

Mutant isolation. Because we could not devise a selection procedure for the isolation of uracil-DNA glycosylase *E. coli* mutants, we decided to use a large-scale nonselective procedure to isolate mutants by direct enzyme assay of single colony isolates from a heavily mutagenized culture (16, 24). A wild-type F^- strain, W3110, was mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine according to the method of Adelberg et al. (1). The mutagenized culture was divided into 10 portions, and these were outgrown for 13 h to segregate mutations. At this point 35% of the viable cells contained new auxotrophic requirements and could not grow on minimal plates. About 150 bacteria were spread on minimal plates and grown at 25°C. After 3 days, 3,000 colonies were transferred to microtiter trays, grown to saturation in TYTG broth at 25°C, and assayed for uracil-DNA glycosylase activity by the acid solubility assay. About 4% of the mutagenized cultures appeared to contain less than 40% of the activity of *E. coli* W3110. When the enzyme-deficient cultures were reassayed, 28 isolates were still at least 60% enzyme deficient. These 28 isolates were assayed by the quantitative enzyme assay procedure, and 8 had decreased enzyme specific activities.

Five of these mutants are known to be of independent origin because they were isolated from different cultures after the mutagenesis. Furthermore, the growth and enzymatic properties of the possible siblings are sufficiently different to confirm that all are of independent origin (see Table 2). Two mutants, BD10 and BD13, have essentially no enzyme activity detectable under the most sensitive in vitro assay conditions. The most enzyme-deficient strain, BD10, has at most 0.02% of the enzyme specific activity found in the parental strain, W3110. The remaining mutants have detectable, but reduced enzyme activity. When compared to wild-type bacteria, the ratio of enzyme specific activities at 42°C relative to 25°C (Q 42°/25°) is decreased in five of these mutants (Table 2). Thus, most of the mutant enzymes are also heat sensitive.

The *ung-6* allele, which produces the most heat-sensitive enzyme, was transduced from BD21 into *E. coli* PA3306 to produce BD1124 (*ung-6*). When BD1124 was grown at 25°C and assayed at 42°C, the residual enzyme activity relative to BD1125 (*ung⁺*) was then 30%, rather than <1% (Table 2). This indicates that the uracil-DNA glycosylase corresponding to the *ung-6* allele is inactivated in vivo at 42°C. Attempts to purify the mutant enzyme from BD1124 were unsuccessful due to instability of the enzyme. In vitro incubation of this enzyme for 5 min at 45°C resulted in a 98% loss of activity, whereas the wild-type enzyme activity retained over 90% of its activity (Fig. 1). Lindahl et al. (13) have shown that *E. coli* uracil-DNA glycosylase consists of a single polypeptide of molecular weight 25,000. Thus, it is likely that

TABLE 2. *Uracil-DNA glycosylase activity in extracts of E. coli ung mutants*[a]

Strain[b]	Genotype	Generation time (min)		Sp act (nmol/min per mg)		Q 42°/25°[c]	% Residual activity at 42°C
		25°C	42°C	25°C	42°C		
W3110	*ung*$^+$	92	27	3.10	12.8	4.2	100
BD10	*ung-1*	88	27	<0.003	0.003		0.02
BD13	*ung-2*	82	31	0.003	0.08		0.7
BD15	*ung-3*	94	73	0.31	0.88	2.8	6.9
BD17	*ung-4*	98	>200	0.58	0.93	1.6	7.3
BD20	*ung-5*	105	48	0.14	0.15	1.1	1.2
BD21	*ung-6*	197	69	0.04	0.05	1.5	0.4
BD24	*ung-7*	113	37	1.81	7.72	4.2	60.0
BD28	*ung-8*	170	54	0.26	0.25	0.9	2.0

[a] All extracts were prepared from cultures grown at 42°C in TYTG broth to late-log phase. The bacteria were centrifuged, treated with lysozyme-EDTA, and assayed at the indicated temperatures. Up to 20 μg of protein containing approximately 10^{-6} to 10^{-5} U of enzyme activity was assayed by the standard procedure.

[b] Isolates from the same flasks were BD10 and BD28, BD13 and BD21, BD15 and BD17.

[c] *Q* 42°/25° is the ratio of enzyme specific activities at 42 and 25°C.

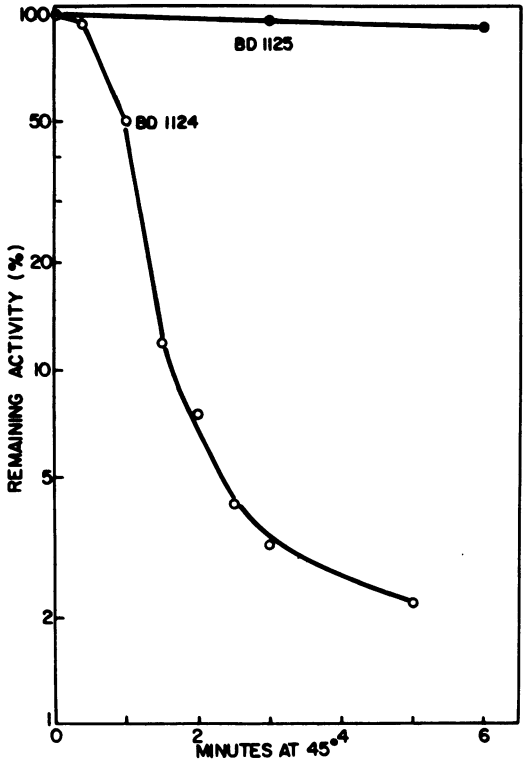

FIG. 1. *Heat inactivation of uracil-DNA glycosylase from strains BD1124 (ung-6) and BD1125 (ung*$^+$*). Cultures were grown at 25°C; extracts were prepared by the lysozyme-EDTA procedure described in the text. After adjusting the protein concentration to 5 mg/ml, nucleic acids were precipitated by the addition of streptomycin. The supernatant fractions were incubated at 45°C; samples were removed at the indicated times and assayed immediately at 30°C. The activity remaining is relative to the zero-time sample (100%).*

the *ung-6* mutation occurs in the structural gene for the enzyme.

Although several of the mutants grow quite slowly, the tightest mutants show the shortest generation times, and BD10 has a generation time comparable to that of the parent. Thus, we assume that the impairment of growth is due to secondary mutations having nothing to do with the glycosylase deficiencies and that the glycosylase is not essential for growth. One of the strains, BD21, is also extremely sensitive to UV light, but this sensitivity was lost after transduction of the *ung-6* allele.

Mapping of *ung* gene. F_{15} was introduced into BD10 by conjugation, and a *thy*$^+$, M13-sensitive colony was selected. This strain was used to transfer the *ung* gene into the well-marked F$^-$ recipients PA3306 and H677 (Table 1) by conjugation. The *ung* gene was transferred early and found to be tightly linked to the *tyrA* and *purI* loci. A *tyrA ung-1* double mutant, BD1101, prepared by such a conjugation was used as a transduction donor with a *nadB purI* recipient, BD1137, and the *purI*$^+$ and *nadB*$^+$ transductants were selected and analyzed for coinheritance of *tyrA, ung, nadB,* and *purI*. The cotransduction frequencies of the markers for this experiment are shown in Table 3 and indicate the gene order on the *E. coli* genetic map (3) to be *tyrA-ung-nadB-purI* (Fig. 2). Other transduction matings confirm this gene order (data not shown).

Because crude extracts of strains carrying the *ung-1* allele have no detectable enzyme activity, the *ung-1* may not be a structural gene mutation. To localize the structural gene for uracil-DNA glycosylase, the *ung-3* and *ung-6* alleles were tested for cotransduction with the *nadB* locus and were found to map on the *tyrA* side of *nadB*.

TABLE 3. *Transduction analysis of ung relative to tyrA, nadB, and purI*

Selected marker[a]	Unselected markers[a]		No. of transductants
purI$^+$	*nadB*	*ung*$^+$	115
	nadB$^+$	*ung*$^+$	17
	nadB	*ung-1*	0
	nadB$^+$	*ung-1*	16
nadB$^+$	*ung*$^+$	*tyrA*$^+$	42
	ung-1	*tyrA*$^+$	29
	ung$^+$	*tyrA*	1
	ung-1	*tyrA*	12

[a] *nadB*$^+$ and *purI*$^+$ genes from a *tyrA-ung-1* donor were transduced into a *nadB-purI* recipient.

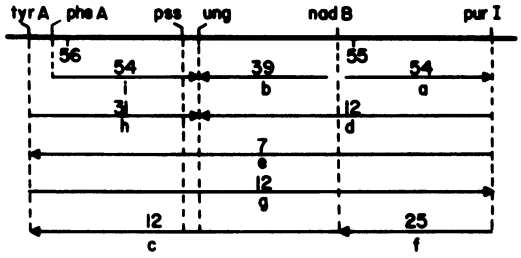

FIG. 2. *Transduction mapping of ung relative to other nearby genes. Each P1 transduction cross is represented by an arrow extending from the selected to the unselected marker. Numerical values are percent cotransduction frequencies. At least 75 recombinants were scored in each cross. The crosses were BD1137 × BD1101 (a-f), BD1147 × W3110 (g-h), KA197 × BD10 (i).*

We conclude that both *ung-1* and the structural gene for *ung* are located between the *tyrA* and *nadB* loci on the *E. coli* map.

General characteristics of *ung* mutants. BD10, which carries the most defective uracil-DNA glycosylase mutation and grows as well as its parent in minimal or TY media, was selected for further characterization of the *ung* mutation. The bacteriophages T4, T5, T7, λ, P1, and M13 plate equally well on BD10 and W3110 hosts. When the mutant and its parent were compared in their sensitivity to various lethal treatments, their survival was the same after exposure to methylmethane sulfonate, mitomycin C, nalidixic acid, UV light, and thymine starvation. However, the *ung* mutant appeared to be slightly more sensitive to nitrous acid than the parental strain (5). Because nitrous acid can deaminate cytosine residues in DNA to uracil, the mutant might be expected to have difficulty repairing such damaged DNA.

Sensitivity to uracil-containing phage. In concurrent experiments we discovered that bacteriophages which do not induce a dUTPase activity incorporate substantial amounts of uracil into their DNA in place of thymine when grown on *dut ung E. coli.* Mutant T4 phage in which 30% of the thymine in the phage DNA has been replaced by uracil are viable in *ung-1* hosts and replicate normally (23). The phage abortively infect wild-type bacteria, presumably due to uracil-DNA glycosylase-initiated breakdown of the parental phage DNA.

The plating behavior of these uracil-containing phage has proved useful for determining the *ung* genotype of bacteria because the spot test correlates with enzyme assay results. In testing the various *ung* mutants with uracil-containing T4 phage, only the most enzyme-deficient alleles, *ung-1* and *ung-2,* allow plaque formation at 30, 37, or 42°C (Fig. 3), whereas all of the strains allow plaque formation by uracil-free T4 phage. Thus, it appears that even low levels of uracil-DNA glycosylase are sufficient for restricting the uracil-containing T4 phage used.

Effect of *ung* on mutation rates. One of the possible functions of uracil-DNA glycosylase is to specifically suppress cytosine to thymine transition mutations by initiating repair of deaminated cytosine residues in DNA. Mutation to resistance to nalidixic acid and rifampin was measured to observe any effect of the *ung* gene on spontaneous mutation frequencies. When the mutation frequencies were measured in isogenic *ung* and *ung*$^+$ strains, resistance to either antibiotic was about fivefold higher in the *ung* bacteria (Table 4). Similar results were found with other isogenic strain pairs in other genetic backgrounds, indicating that the effect is due to the *ung* gene and that the *ung* allele is a weak mutator. The rather small effect (fivefold) may be because spontaneous mutation to nalidixic acid or rifampin resistance occurs at a high frequency; a wide range of mutational events can lead to antibiotic resistance. Further experiments to elucidate the kinds of base changes occurring in *ung* bacteria will establish whether or not uracil-DNA glycosylase is responsible for the specific repair of guanine-uracil base pairs that might otherwise lead to mutation.

DISCUSSION

A nonselective mass-screening approach has been successfully used to isolate mutants deficient in uracil-DNA glycosylase. This technique allows the isolation of mutants without presupposing their phenotypic characteristics in the design of a selection procedure. We have isolated eight strains defective in uracil-DNA glycosylase activity. The *ung* gene may be nonessential because no activity (less than 0.02%) has been observed in the *ung-1* mutant extracts. However, Lindahl has purified the extracts of W3110 and BD10 cells through two purification steps and finds detectable, although very low (about 0.5%

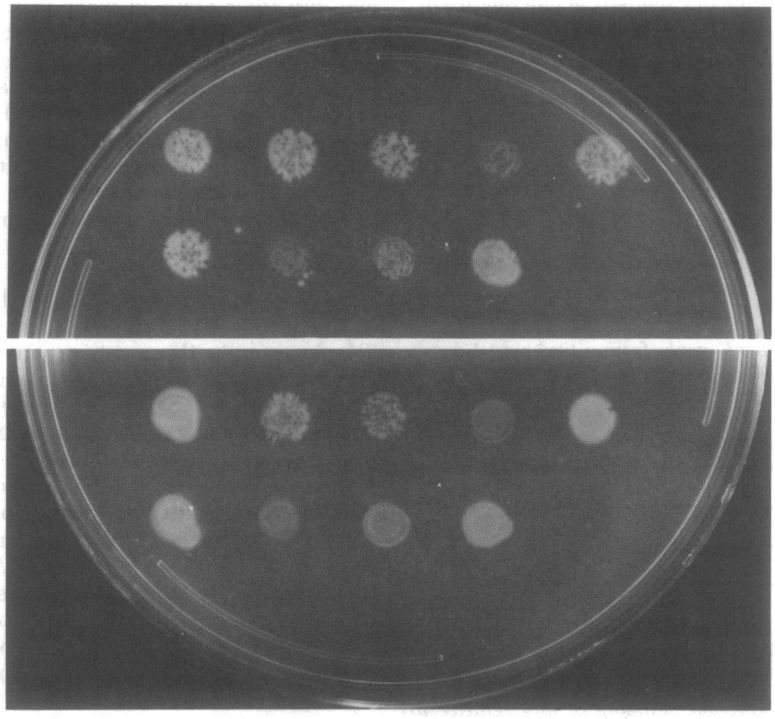

FIG. 3. *Sensitivity of ung mutants to uracil-containing T4. The upper frame shows the plaques formed in bacterial spots by uracil-free T4. The lower frame shows plaque formation by uracil-containing phage. These phage had about 30% of their thymine replaced by uracil and a relative plating efficiency of 3% on ung⁺ compared with ung-1. From left to right, upper row: W3110, ung-1, ung-2, ung-3, ung-4; lower row: ung-5, ung-6, ung-7, ung-8. The plates were incubated for 14 h at 30°C.*

TABLE 4. *Mutation frequencies in ung-1 and ung⁺ strains*

Mutation	Mutants per 10^7 viable cells		*ung-1/ung⁺*
	BD1154 (*ung⁺*)	BD1153 (*ung-1*)	
$Rif^s \rightarrow Rif^r$	1.5	6.9	4.5
$Nal^s \rightarrow Nal^r$	2.1	11	5.4

of normal), glycosylase activity in the BD10 cells (personal communication). Genetic tests have located the structural gene between *nadB* and *tyrA* on the *E. coli* K-12 linkage map. The results indicate the close proximity of *ung* to the phosphatidylserine synthase (*pss*) gene described by Raetz et al. (18). We have recently obtained additional results which indicate that the *pss* marker is located between *ung* and *tyrA* and close to *ung* as shown in Fig. 2 (unpublished data).

The properties of uracil-DNA glycosylase and in vivo studies with *ung* and *urg* mutants indicate that the enzyme is involved in the repair of uracil-containing DNA. We have previously observed that uracil-containing coliphage T4 DNA is broken down in vivo in *ung⁺* cells, whereas *ung* mutants are permissive hosts for uracil-containing T4 phage (23). PBS2 phage normally contain uracil instead of thymine in their DNA and induce an inhibition of uracil-DNA glycosylase early in infection, before progeny phage DNA synthesis commences. When chloramphenicol was added to a *B. subtilis* culture before infection, the induction of the inhibitor was prevented, and the parental phage DNA was degraded to acid-soluble material (8). This antibiotic-dependent degradation does not occur if *urg B. subtilis* mutants are used (15). Makino and Munakata have also implicated uracil-DNA glycosylase in the repair of uracil-containing transforming DNA (15).

Previous studies (14, 22) suggest that *E. coli* DNA may transiently contain uracil. If uracil-DNA glycosylase is the major enzyme responsible for excision of uracil from DNA, then *ung* mutants might contain uracil in their DNA. Analysis of *E. coli* DNA labeled with [6-^{3}H]-uridine has failed to detect differences between the uracil content of DNA from *ung⁺* and *ung* cells (unpublished data). The direct methods used were sufficient to detect uracil at about 1% of the total pyrimidine content of the DNA. It

seems highly likely that dUTPase excludes all but trace quantities of uracil from the DNA under normal growth conditions. We have recently obtained indirect evidence that uracil occurs at a frequency of at least 0.1% in the DNA of *ung* mutants (21). One other enzyme, endonuclease V, has been shown by Gates and Linn (10) to be very active on PBS2 DNA and might substitute for the uracil-DNA glycosylase in the exclusion of uracil from the host genome in the absence of the *ung* gene product. However, this enzyme does not appear to be present in levels sufficient to restrict the growth of uracil-containing T4 phage in an *ung-1* host.

The properties of the *E. coli ung* mutants described here and those of the *B. subtilis urg* mutants described by Makino and Munakata (15) are very similar. In both cases the defective glycosylase activity does not alter normal growth, and neither mutant is sensitive to UV irradiation, methylmethane sulfonate, or mitomycin C. The *urg* mutant was reported to be insensitive to sodium nitrite (15), whereas the *ung* mutant is sensitive (5). However, the conditions for observing this sensitivity are very critical, suggesting that the mutants may not be different for this property.

At least one other DNA *N*-glycosylase activity has been discovered in *E. coli* (12). This activity releases 3-methyladenine from DNA in a reaction analogous to that catalyzed by uracil-DNA glycosylase. The uracil- and 3-methyladenine-DNA glycosylases are probably different enzymes because BD10 has normal 3-methyladenine-DNA glycosylase activity (T. Lindahl, personal communication). Other glycosylases may exist which should be detectable by the quantitative Dowex-1 procedure described, provided that the appropriate radioactive DNA substrate can be made.

ACKNOWLEDGMENTS

This work was supported by Public Health Service research grant GM 21464 to H.R.W. and postdoctoral fellowship GM 05401 to B.K.D. from the National Institute of General Medical Sciences.

We thank Elizabeth Kutter and Larry Snyder for the gift of the T4 *alc* mutant phage used for preparing uracil-containing T4 phage. We also thank James Fuchs and Lorraine Wyman for advice on the bacterial genetics experiments.

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