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Anaerobic Incubation Enhances the Colony Formation of a *polA recB* Strain of *Escherichia coli* K-12

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Escherichia coli strain E247 (*polA1 recB21*) has reduced colony formation (even at the permissive temperature of 30°C) because of a poor suppressor mutation (*sup-126*). The colony formation was enhanced in the absence of oxygen about 3-fold at 30°C and 10^6-fold at 43°C, suggesting that a *polA recB* strain was inviable due to oxygen toxicity. Colony formation was also increased by incubation in an agar medium containing the reducing agent thioglycolate and incubation in the presence of chloroform-killed *Saccharomyces cerevisiae pet*$^+$ cells, but not *pet* cells. Since the E247 strain viability was inversely dependent on the oxygen pressure and since the strain was more sensitive to superoxide radical than either the *polA* or the *recB* mutant, it seems likely that the *polA* and *recB* genes play a role in repairing DNA damage during respiration.

Since a DNA polymerase I-deficient strain of *Escherichia coli* was isolated by de Lucia and Cairns (6), various *polA* mutations have been shown to be lethal, but their reasons for lethality remain unsolved. One of the roles of DNA polymerase I in DNA replication is to fill gaps between DNA fragments (19), but its function is not essential because a *polA* strain is viable. Thus, it is reasonable to suppose that there must be a certain mutant with a double mutation which becomes lethal as a result of the failure of DNA fragments to complete the joining process. The most likely candidate was a *polA recA* strain because *polA* and *recA* functions are important for the repair of single-strand breaks induced by X rays (10, 16, 22) and UV light (15, 20, 21). Monk et al. (14) found that *polA recA* and *polA recB* strains could synthesize nascent DNA strands normally and join them as fast as a parental *polA* strain, an indication that they had no defects, at least in the elongation step of DNA replication. Monk and Kinross (13) showed that the temperature-sensitive *polA12 rec* mutant has a reduced colony-forming ability even at the permissive temperature of 30°C and forms nondividing filamentous cells. Its viability was enhanced by plating with chloroform-killed cells and sonic extracts of *E. coli*. This was reminiscent of *lon* cells irradiated with ionizing radiation (1, 7). Hence, they suggested that the enhanced colony formation of *polA rec* cells was due to a division-promoting substance in the cells. Recently Adler et al. (2) showed that cell fractions which stimulated septation in irradiated *lon* mutants had respiratory activity which removed oxygen from the plating medium. In this paper, I examine whether anaerobic incubation enhanced colony formation of a *polA recB* strain.

MATERIALS AND METHODS

Bacterial strains. All of the bacterial strains are derivatives of KN250, carrying a temperature-sensitive amber suppressor, *sup-126* (18), and are listed in Table 1. *Saccharomyces cerevisiae* S288c was kindly provided by T. Saeki (in my institute), and *S. cerevisiae pet* was isolated as a spontaneous mutant which could not grow on a plate containing glycerol as the sole carbon source.

Media. *E. coli* strains were cultured in L broth (LB) (10 g of tryptone [Difco Laboratories], 5 g of yeast extract, and 5 g of NaCl per liter) supplemented with 20 μg of thymine per ml and were plated on LG agar plates (5 g of glucose and 20 g of agar per liter of LB). *S. cerevisiae* cells were cultured in YEPD medium (20 g of tryptone [Difco], 10 g of yeast extract, and 20 g of glucose per liter) at 30°C for 2 to 3 days. When the viability at 43°C was examined, LG agar plates were freshly prepared in the morning and were used in the afternoon because the viability of strain E247 cells strongly depended on the freshness of the agar plates. The viability at 30°C was about 5% compared with the cell number measured by a Coulter Counter when agar plates were used on that day and then decreased with time. This decrease was relieved by an addition of chloroform-killed cells. Use of the same batch numbers of tryptone (Difco) and yeast extract was important to get reproducible results.

Preparation of chloroform-killed cells. Cells fully grown in a rich medium were treated with about 3% (vol/vol) chloroform for 30 min at 30°C, washed, and suspended in dilution buffer (5 g of NaCl, 1 ml of 1 M

TABLE 1. Bacterial strains used

Strain	Relevant genes	Source
K462	Wild type	Reference 17
K520	*polA1*	Reference 17
K614	*polA12*(Ts)	KN250 (reference 9) × P1 *polA12*
K619	*recA1*	KN250 (reference 9) × Hfr *recA1*
K621	*recF143*	KN250 (reference 9) × P1 *recF143*
K656	*recB21 recC22*	KN250 (reference 9) × P1 *recB21 recC22*
K700	*polA1 recA1*	K520 × Hfr *recA1*
E247	*polA1 recB21*	K520 × P1 *recB21*
E255	*uvrB59*	K462 × P1 *uvrB59*

$MgCl_2$, and 1 ml of 1% gelatin per liter of 0.01 M Tris-hydrochloride, pH 7.4) at about 10^{10} *E. coli* cells or 10^9 *S. cerevisiae* cells per ml. When stored at 4°C, they can be used for about 35 days without loss of the enhancing effect.

Viability test. Cells of strain E247 were cultured for 2 days in LB at 30°C and then plated on LG agar plates with soft agar containing chloroform-killed cells (about 10^9 *E. coli* cells or 2×10^8 *S. cerevisiae* cells). Plates were incubated for 2 to 3 days at 30°C in air or for 3 to 5 days in an anaerobic incubation jar, and the colony-forming units were counted. Viability was defined as the ratio of the number of colony-forming units to the number of cell particles measured by a Coulter Counter (model ZB1; Coulter Electronics, Inc.) equipped with an aperture 30 μm in diameter at an amplification of 1/4, an aperture current of 1 mA, a matching switch of 40 K, and thresholds from 7 to 100. An anaerobic incubation system, GasPak (BBL Microbiology Systems), uses a palladium catalyst and a (H_2 plus CO_2) generator envelope to deprive oxygen in a plastic jar containing an indicator, whose reaction from blue to colorless indicates an anaerobic condition. This requires several hours. When cells were incubated in this system at the nonpermissive temperature of 43°C, they were first placed at 30°C for 1 to 2 h during which time the temperature reached around 37°C due to catalytic reaction, and then they were incubated at 43°C for 3 to 5 days.

When the viability of cells cultured in oxygen was examined, a vented jar (GasPak 100 Holding Jar; BBL Microbiology Systems) was supplied with oxygen for 10 to 15 min at about 1 liter/min and then incubated for 3 to 5 days at 30°C.

Generation of superoxide radical. Superoxide radical was produced by illumination of a riboflavin solution containing methionine (11). The suspension of 10^7 cells per ml of 0.045 M phosphate buffer (pH 7.8) containing 4% glucose, 3×10^{-4} M riboflavin, 0.013 M methionine, and 0.22 mM EDTA was irradiated at 5,600 lx under three fluorescent lamps (15 W; Toshiba) with gentle stirring at 60 cycles per min in a 9-cm glass petri dish.

RESULTS

Effect of anaerobic conditions on the viability of strain E247. When strain E247 (*polA recB*) was incubated in air on an agar medium containing thioglycolate, colony formation was enhanced from 5 to 9% at 0.1 g of thioglycolate per liter. It decreased with increasing thioglycolate concentration (Fig. 1). In the anaerobic condition, the viability remained almost constant at 12% over the thioglycolate concentrations examined. The decreased colony formation at higher thioglycolate concentrations in air might be due to a toxic oxidation product of thioglycolate. Oxygen in the medium was also reduced by plating strain E247 with chloroform-killed *S. cerevisiae* cells (Table 2). The colony formation of strain E247 was 0.038 without chloroform-killed cells, and it was enhanced to 0.141 by plating with chloroform-killed *E. coli* cells. Chloroform-killed *S.*

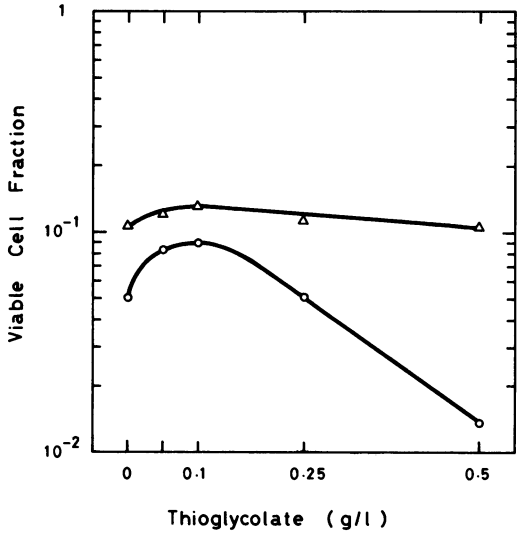

FIG. 1. Enhancement of strain E247 viability by incubation in thioglycolate-containing agar medium and in an anaerobic incubation jar. An overnight culture of E247 (1.44×10^9 cells per ml counted in a Coulter Counter) was plated on LG agar medium containing various concentrations of thioglycolate. Agar media were incubated in air (○) and in an anaerobic incubation jar (△) for 2 to 4 days at 30°C.

TABLE 2. Effect of chloroform-killed cells on the viability of strain E247[a]

Chloroform-killed cells	No. of colony formers	Viability[b]
None	5.8×10^7	0.038
AB1157 (*E. coli*)	2.2×10^8	0.141
S. cerevisiae pet$^+$	2.0×10^8	0.131
S. cerevisiae pet	6.4×10^7	0.042

[a] Number of cell particles of E247 determined in a Coulter Counter was 1.53×10^9/ml.

[b] Viability is the number of colony formers divided by 1.53×10^9.

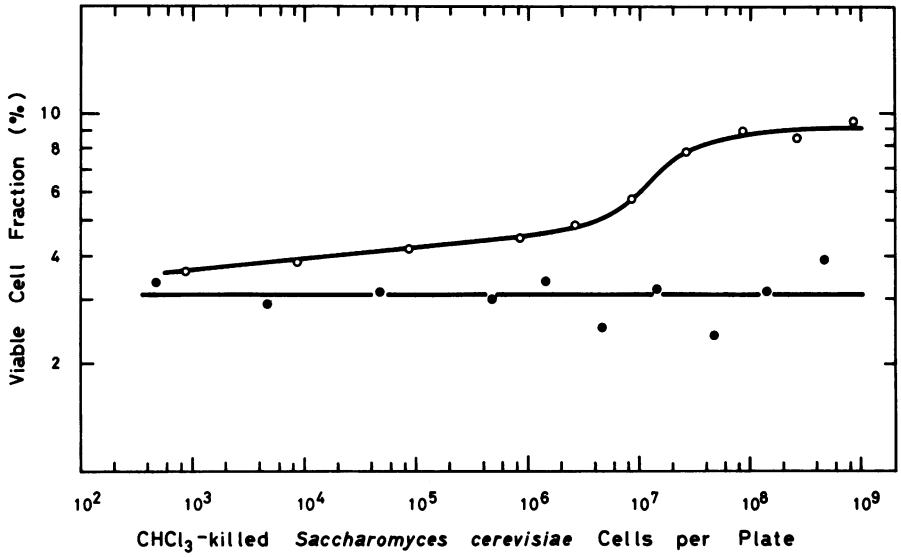

FIG. 2. Enhancement of the colony-forming ability of *E. coli* E247 by chloroform-killed *S. cerevisiae pet*$^+$ cells. E247 was cultured overnight in LB at 30°C and plated on LG agar with chloroform-killed *S. cerevisiae pet*$^+$ (○) and *pet* (●) cells. Cell particle numbers for *E. coli* and for *S. cerevisiae pet*$^+$ and *pet* cells were counted in a Coulter Counter (1.03×10^9, 8.64×10^8, and 4.70×10^8 cells per ml, respectively). Agar plates were incubated for 2 days at 30°C, and the colony-forming units were counted.

cerevisiae enhanced colony formation as did chloroform-killed *E. coli*, whereas chloroform-killed *S. cerevisiae pet* cells could not enhance it. These results suggest that the enhanced colony formation of strain E247 by killed cells is likely due to an anaerobic condition, because *E. coli* and *S. cerevisiae pet*$^+$ cells deprive oxygen from the agar medium and possibly result in anoxic conditions, whereas *S. cerevisiae pet* cells, which lack mitochondria, cannot respire, and hence the agar medium remains aerobic. Figure 2 shows the effect of chloroform-killed *S. cerevisiae* cell density on the colony formation of strain E247. The colony formation of *E. coli* E247 began increasing at 10^7 chloroform-killed *S. cerevisiae* cells per plate and then reached a plateau at 9%, while it remained constant at 3% at 1×10^3 to 5×10^8 *S. cerevisiae pet* cells per plate. These results demonstrate that an anaerobic incubation enhanced the colony formation of strain E247.

Anaerobic incubation enhanced the colony formation of strain E247 at the nonpermissive temperature of 43°C (Table 3). When strain E247 was incubated at 43°C in the presence of air, it could not form colonies. When cells of strain E247 were incubated in the absence of oxygen, on the other hand, remarkably enhanced viabilities ranged from 2.4×10^{-3} to 1×10^{-2}.

Effect of chloroform-killed cells on the viability of various repair-deficient strains. The oxygen effect on colony formation of various repair-deficient strains was examined (Table 4). All of the single mutants, such as *polA*, *recA*, *recB*, *uvrB*, and *recF*, showed no increase in colony formation by the addition of chloroform-killed *S. cerevisiae pet*$^+$ cells, but their viabilities were as low as 40 to 90%. On the other hand, besides strain E247, strain K700 (*polA1 recA1*) showed a significantly enhanced viability upon the addition of chloroform-killed cells.

Oxygen, superoxide radical, and X-ray sensitivities of strain E247. The colony-forming ability of strain E247 cultured in anaerobic conditions, in

TABLE 3. Viability of strain E247 (*polA1 recB21*)[a]

Incubation	Chloroform-killed cells	Viability[b] when incubated at:	
		30°C	43°C
Air	*S. cerevisiae pet*$^+$	0.13	4.0×10^{-9}
	S. cerevisiae pet	0.072	4.0×10^{-9}
	None	0.063	4.0×10^{-9}
GasPak	*S. cerevisiae pet*$^+$	0.16	1.0×10^{-2}
	S. cerevisiae pet	0.12	4.0×10^{-3}
	None	0.097	2.4×10^{-3}
	None[c]	0.11[c]	4.0×10^{-9}[c]

[a] Number of E247 cell particles determined by a Coulter Counter to be 1.25×10^9/ml.
[b] Number of colony formers divided by 1.25×10^9.
[c] Plates containing air were sealed and incubated in the same GasPak.

TABLE 4. Effect of chloroform-killed *S. cerevisiae* cells on the viability of repair-deficient strains of *E. coli* K-12

Strain	Relevant gene	No. of cells[a] (10^9)	Viability[b]		
			Without cells	With chloroform-killed cells of	
				S. cerevisiae pet	*S. cerevisiae pet*$^+$
K462	Wild type	3.44	0.81	0.96	0.84
K619	*recA1*	2.42	0.69	0.61	0.77
K614	*polA1*	2.94	0.37	0.42	0.46
K656	*recB21 recC22*	2.56	0.36	0.36	0.42
K621	*recF143*	3.28	0.83	0.83	0.86
E255	*uvrB59*	3.10	0.86	0.86	0.89
K700	*polA1 recA1*	1.55	0.097	0.13	0.31
E247	*polA1 recB21*	2.32	0.049	0.064	0.11

[a] Determined in a Coulter Counter.
[b] Number of colony formers after 3 days at 30°C divided by the number of cells counted in the Coulter Counter.

air, and in oxygen was examined (Fig. 3). The viability of strain E247 was 14% in anaerobic conditions, 9% in air, and 0.1% in pure oxygen, while that of strains K520 and K656 was hardly affected by oxygen. This is an indication that strain E247 was killed by oxygen toxicity.

The sensitivities of strains K462, K520, K656, and E247 to the superoxide radical, which is one of the active oxygens produced during respiration, were examined (Fig. 4). The superoxide radical was generated by the illumination of a methionine-containing riboflavin solution (11). The sensitivity to superoxide radical of a wild-type strain was not affected at all, while both strain K520 and strain K656 were sensitive to photochemically generated superoxide radical, but neither was as sensitive as strain E247, suggesting that the *polA* and *recB* functions are indispensable to protect *E. coli* against oxygen toxicity.

Since superoxide radical is also made by ionizing radiations (3, 4) and there is damage to tissue caused by increased pressures of oxygen (8), the X-ray sensitivity of strain E247 was examined (Fig. 5). The *polA* strain was two times, the *recB* strain was four times, and the *polA recB* strain was eight times more sensitive than a wild-type strain, confirming that there are *polA*-dependent and *rec*-dependent pathways to repair X-ray-induced DNA damages (23). These results indicate that there are *polA*-dependent and *recB*-dependent repair processes to protect *E. coli* against oxygen toxicity.

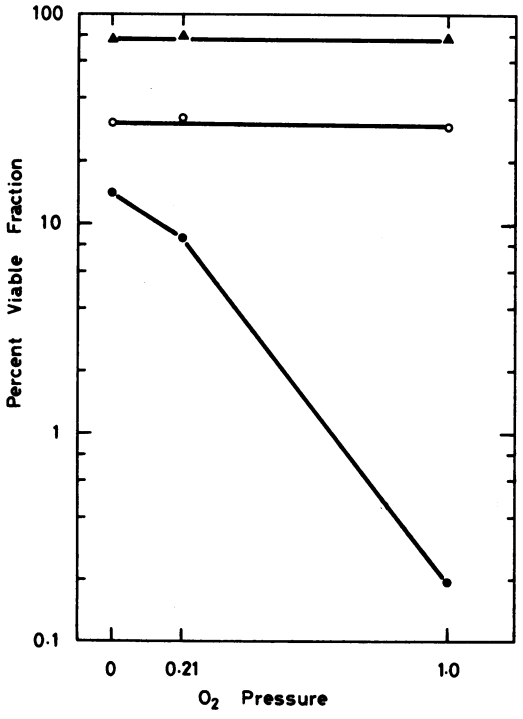

FIG. 3. Oxygen pressure-dependent viabilities of strains K520, K656, and E247. Cells cultured overnight in LB at 30°C were plated on LG agar and incubated for 3 days in a GasPak anaerobic incubation jar, in air, and in oxygen gas at 30°C for assaying colony-forming cells. The numbers of cell particles of strains K520, K656, and E247 were 1.52×10^9, 1.69×10^9, and 1.23×10^9/ml in a Coulter Counter, respectively, and the viability was calculated as the ratio of colony formers per number of cell particles. Symbols: K520 (▲), K656 (○), and E247 (●).

DISCUSSION

Depriving oxygen from the agar media enhanced the colony formation of strain E247, strongly suggesting that the *polA recB* mutations

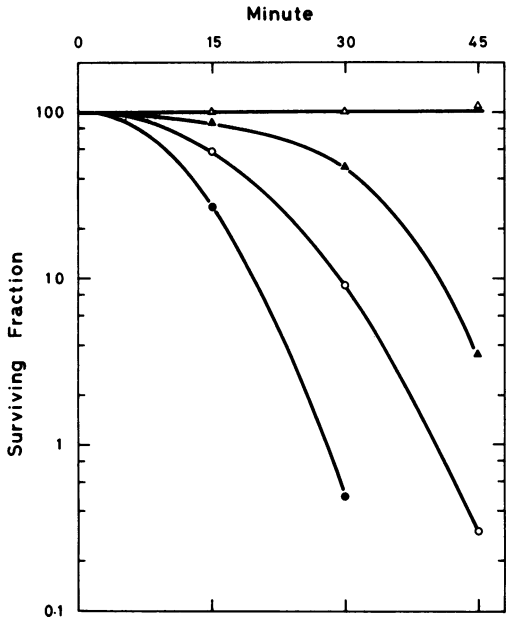

FIG. 4. Survival curves of strains K462, K520, K656, and E247 subjected to photochemically generated superoxide radical at 30°C. Cells cultured to a stationary phase in LB at 30°C were washed with and suspended in 0.01 M phosphate buffer (pH 7.8). They were diluted to 10^7 cells per ml in 0.045 M phosphate buffer (pH 7.8) containing 4% glucose, 3×10^{-4} M riboflavin, 0.013 M methionine, and 0.22 mM EDTA. They were irradiated at 5,600 lx under fluorescent lamps. At 15-min intervals, aliquots were plated on LG agar for colony formation. After incubation for 2 days at 30°C, viable cells were counted. Symbols: K462 (△), K520 (▲), K656 (○), and E247 (●).

were lethal due to oxygen toxicity. This was supported by the fact that the viability of strain E247 was inversely dependent on the pressure of oxygen (Fig. 3). Since strain E247 was more sensitive to oxygen and superoxide radical than were strains K520 and K656, it seems likely that the *polA* and *recB* genes are indispensable in repairing DNA damage during respiration for the protection of *E. coli* against oxygen toxicity. These results confirm the suggestion of Monk and Kinross (13) that the enhanced colony formation of *polA rec* mutants might be attributed to the division-promoting substance in cells.

It seems difficult to explain why strain E247 was sensitive to oxygen exposure but strains K520 and K656 were resistant (Fig. 3). Perhaps these results can be understood in terms of chronic effects of oxygen toxicity, since the viability test in oxygen took 3 to 4 days of exposure to oxygen gas. As strains K520 and K656 can repair DNA damage, they could completely recover when exposed to oxygen at a low dose rate. On the other hand, strain E247 has such a poor ability to repair DNA damage that it could not recover from oxygen toxicity during the viability test.

The viability of strain E247 at 30°C was only 14 to 20% at best as so far examined. Since the *polA* viability was 80% and that of *recB* was 30% (Fig. 3), the expected viability of strain E247 was 24% (80% × 30%), which is about two times larger than the viabilities of E247 obtained in Table 4 and Fig. 2 (10%) and those in Tables 2 and 3 and Fig. 1 and 3 (15%). It seems likely that the anaerobic incubation used in this communication was not strictly anaerobic but nevertheless enhanced the colony formation of strain E247.

The toxicity of tryptophan photoproduct on *rec* mutants of *Salmonella typhimurium* and *E. coli* (24, 25) was later identified as the toxicity of hydrogen peroxide (12), and recently Carlsson and Carpenter (5) showed that the *recA* gene is

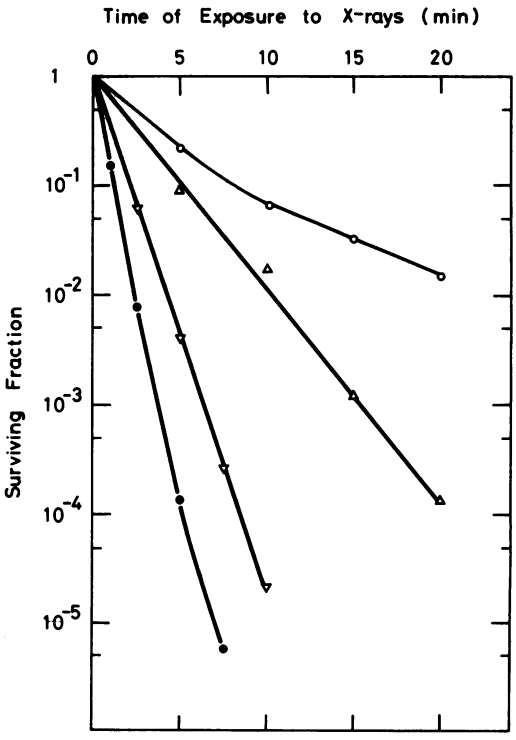

FIG. 5. X-ray survival curves of strains K462, K520, K656, and E247. Cells were cultured overnight in LB at 30°C and diluted into M9 buffer at 10^7 cells per ml. Cell suspensions were irradiated at 50 kV and 20 mA with stirring. Cells were plated on LG agar with about 10^8 chloroform-killed *S. cerevisiae* cells. After incubation for 2 days at 30°C, viable cells were counted. Symbols: K462 (○), K520 (△), K656 (▽), and E247 (●).

very important in the protection of DNA against hydrogen peroxide toxicity. Hydrogen peroxide is an intermediate product of respiration, as is the superoxide radical, which is also made by ionizing radiation (3, 4). Two types of repair processes, designated as type II, requiring *polA* function, and type III, requiring *rec* functions, operate in *E. coli* for the rejoining of X-ray-induced single-strand breaks (21). The X-ray sensitivity of E247 was equal to the addition of that of a *polA* strain and that of a *recB* strain (Fig. 5), and strain E247 was more sensitive to oxygen and superoxide radical than either the *polA* or the *recB* strain. From these results, I speculate that strain E247 did not grow due to the defective repair of X-ray-type DNA damage during respiration. If this assumption is correct, strain E247 would be viable even at the nonpermissive temperature of 43°C if it was cultured in a completely anaerobic condition. To get a strictly anaerobic condition before temperature rise, an anaerobic incubation jar was first placed at 30°C for 1 to 2 h and then transferred to 43°C. The viability of E247 at 43°C was enhanced about 10^6-fold in the absence of oxygen. Incubation at 30°C must be long enough to get a strictly anaerobic condition but sufficiently short that the cells do not begin to make microcolonies. Incubation of a tightly sealed agar plate containing air in the same anaerobic incubation jar did not show any enhanced viability (the last line of Table 3). These results indicate that a *polA recB* strain remained viable when cultured in a completely anaerobic condition. However, a more severe test, with all of the treatments of the bacteria including dilution, plating, and incubation done in a strictly anaerobic condition, would be interesting because the enhanced viability of 10^6-fold obtained at 43°C was still not complete.

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LITERATURE CITED

1. **Adler, H. I.** 1966. Repair of radiation-induced damage to the cell division mechanism of *Escherichia coli*. J. Bacteriol. **91**:737–742.
2. **Adler, H. I., A. Carrasco, W. Crow, and J. C. Gill.** 1981. Cytoplasmic membrane fraction that promotes septation in an *Escherichia coli lon* mutant. J. Bacteriol. **147**:326–332.
3. **Ander, S.** 1967. Untersuchungen an UV- und rontgenbestrahltem Wasser. Strahlentherapie **132**:135–142.
4. **Bielski, B. H. J., and A. O. Allen.** 1967. Radiation chemistry of aqueous tetranitromethane solutions in the presence of air. J. Phys. Chem. **71**:4544–4549.
5. **Carlsson, J., and V. S. Carpenter.** 1980. The *recA* gene product is more important than catalase and superoxide dismutase in protecting *Escherichia coli* against hydrogen peroxide toxicity. J. Bacteriol. **142**:319–321.
6. **de Lucia, P., and J. Cairns.** 1969. Isolation of an *E. coli* strain with a mutation affecting DNA polymerase. Nature (London) **1224**:1164–1166.
7. **Fisher, W. D., H. I. Adler, F. W. Shull, Jr., and A. Cohen.** 1969. Properties of a cell fraction that requires damage to the cell division mechanism of *Escherichia coli*. J. Bacteriol. **97**:500–505.
8. **Gerschman, R.** 1964. Biological effects of oxygen, p. 475–495. *In* F. Dickens and E. Neil (ed.), Oxygen in the animal organisms. Macmillan, New York.
9. **Horiuchi, T., and T. Nagata.** 1973. Mutations affecting growth of the *Escherichia coli* cell under a condition of DNA polymerase I deficiency. Mol. Gen. Genet. **123**:89–110.
10. **Kapp, D. S., and K. C. Smith.** 1970. Repair of radiation-induced damage in *Escherichia coli*. II. Effect of *rec* and *uvr* mutations on radiosensitivity and repair of X-ray-induced single-strand breaks in deoxyribonucleic acid. J. Bacteriol. **103**:49–54.
11. **Massey, V., S. Strickland, S. G. Mayhew, L. G. Howell, P. C. Engel, R. G. Matthews, M. Schumann, and P. A. Sullivan.** 1969. The production of superoxide anion radicals in the reaction of reduced flavines and flavoproteins with molecular oxygen. Biochem. Biophys. Res. Commun. **36**:891–897.
12. **McCormick, J. P., J. R. Fisher, J. P. Pachlatko, and A. Eisenstark.** 1976. Characterization of a cell-lethal product from the photooxidation of tryptophan; hydrogen peroxide. Science **191**:468–469.
13. **Monk, M., and J. Kinross.** 1972. Conditional lethality of *recA* and *recB* derivatives of a strain of *Escherichia coli* K-12 with a temperature-sensitive deoxyribonucleic acid polymerase I. J. Bacteriol. **109**:971–978.
14. **Monk, M., J. Kinross, and C. Town.** 1973. Deoxyribonucleic acid synthesis in *recA* and *recB* derivatives of an *Escherichia coli* K-12 strain with a temperature-sensitive deoxyribonucleic acid polymerase I. J. Bacteriol. **114**:1014–1017.
15. **Monk, M., M. Peacey, and J. D. Gross.** 1971. Repair of damage induced by ultraviolet light in DNA polymerase-deficient *Escherichia coli* cells. J. Mol. Biol. **58**:623–630.
16. **Morimyo, M., Z. Horii, and K. Suzuki.** 1968. Appearance of low molecular weight DNA in a *rec*$^-$ mutant of *Escherichia coli* K-12 irradiated with X-rays. J. Radiat. Res. **9**:19–25.
17. **Morimyo, M., and Y. Shimazu.** 1976. Evidence that the gene *uvrB* is indispensable for a polymerase I-deficient strain of *Escherichia coli* K-12. Mol. Gen. Genet. **147**:243–250.
18. **Nagata, T., and T. Horiuchi.** 1973. Isolation and characterization of a temperature-sensitive amber suppressor mutant of *Escherichia coli* K-12. Mol. Gen. Genet. **123**:77–88.
19. **Okazaki, R., M. Arisawa, and A. Sugino.** 1971. Slow joining of newly replicated DNA chains in DNA polymerase I-deficient *Escherichia coli* mutants. Proc. Natl. Acad. Sci. U.S.A. **68**:2954–2957.
20. **Rupp, W. D., and P. Howard-Flanders.** 1968. Discontinuities in the DNA synthesized in an excision-defective strain of *Escherichia coli* following ultraviolet irradiation. J. Mol. Biol. **31**:291–304.
21. **Smith, K. C., and D. H. C. Meun.** 1970. Repair of radiation-induced damage in *Escherichia coli*. I. Effect of *rec* mutations on post-replication repair of damage due to ultraviolet radiation. J. Mol. Biol. **51**:459–472.
22. **Town, C. D., K. C. Smith, and H. S. Kaplan.** 1971. DNA polymerase required for rapid repair of X-ray-induced DNA strand breaks *in vivo*. Science **172**:851–854.
23. **Town, C. D., K. C. Smith, and H. S. Kaplan.** 1973. Repair of X-ray damage to bacterial DNA. Curr. Top. Radiat. Res. **8**:351–399.

24. **Yoakum, G., and A. Eisenstark.** 1972. Toxicity of L-tryptophan photoproduct on recombinationless (*rec*) mutants of *Salmonella typhimurium*. J. Bacteriol. **112**:653–655.

25. **Yoakum, G., W. Ferron, A. Eisenstark, and R. B. Webb.** 1974. Inhibition of replication gap closure in *Escherichia coli* by near-ultraviolet light photoproducts of L-tryptophan. J. Bacteriol. **119**:62–69.