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Genetics

# Mechanisms determining aerobic or anaerobic growth in the facultative anaerobe *Salmonella typhimurium*

(DNA gyrase/topoisomerase/mutation/superhelicity/gene regulation)

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**ABSTRACT** **We isolated mutant strains of the facultative anaerobe *Salmonella typhimurium* that grow either aerobically or anaerobically. Strict anaerobic mutants contained a defective DNA topoisomerase I gene (*topI*), while strict aerobic mutants contained a defective DNA gyrase subunit A gene (*gyrA*, also *nalA*). Topoisomerase I activity was detected in cell-free extracts of strict aerobic mutants but not of strict anaerobic mutant strains, whereas gyrase activity was detected in extracts of strict anaerobic mutants but not of strict aerobic mutants. Furthermore, extracts of wild-type cells, cultured under vigorous aerobic condition, contain topoisomerase I activity but no significant gyrase activity. In contrast, the extracts of anaerobically cultured wild-type cells contain gyrase activity but no significant topoisomerase I activity. Sucrose gradient centrifugation with ethidium bromide showed that chromosomal DNA in strict aerobic mutants and aerobically grown wild-type cells was relaxed, while the chromosomal DNA of strict anaerobic mutants and anaerobically grown wild-type cells was more supercoiled. Aerobic cultures of wild type and strict aerobic mutants produced both superoxide dismutase and catalase, whereas anaerobic cultures of wild type and strict anaerobic mutants did not. These results lead us to conclude that activity of topoisomerase I, associated with relaxation of chromosomal DNA, is necessary for expression of genes required for aerobic growth, whereas activity of gyrase, associated with supercoiling of chromosomal DNA, is necessary for expression of genes required for anaerobic growth.**

Although one can assume that strict aerobic and strict anaerobic bacteria express various common or similar enzymes to synthesize basic ingredients, such as amino acids and nucleotides for synthesis of proteins and nucleic acids, the respiratory enzymatic systems in these organisms appear to be different (1). Aerobic bacteria contain large amounts of Krebs cycle enzymes for aerobic respiration but low amounts of enzymes for glycolysis and soluble cytochrome *c* (1). In contrast, anaerobic organisms contain large amounts of glycolytic enzymes and soluble cytochrome *c* for anaerobic energy transduction but low activities of Krebs cycle enzymes (1). Moreover, aerobes and anaerobes possess different protective mechanisms and repair systems because types of toxic metabolites and DNA damage are different under these different environments. Strict aerobes carry protective enzymes, such as superoxide dismutase (2, 3) and catalase (3) and the SOS repair mechanism (4), whereas strict anaerobes carry other types of repair mechanisms (5).

Facultative anaerobes must carry genes for both aerobic and anaerobic energetic systems (1) and protective (3) and repair mechanisms (6) in order to replicate and survive in either environment. However, it is reasonable to assume that the genes for these environment-specific enzymes are expressed only when necessary under the stress of a specific environment. We have been able to demonstrate that the aerobic recombination and repair mechanisms (e.g., *recA*, *recBC*, and SOS repair system of *Salmonella typhimurium*) are expressed in the aerobic but not in the anaerobic environment (7). Since large numbers of specific enzymes are involved in each environment, a few or a limited number of regulatory genes may govern expression of large numbers of environment-specific genes, although some of these environment-specific genes also could be controlled individually. Isolation of mutants restricted to grow in either anaerobic or aerobic environments should not only unveil these regulatory mechanisms of expression of environment-specific genes but also provide invaluable information or evolutionary relationships between aerobes, anaerobes, and facultative anaerobes.

Using *S. typhimurium* as a representative facultative anaerobe, we isolated mutants restricted to grow aerobically (strict aerobic mutants), and mutants restricted to grow anaerobically (strict anaerobic mutants). Strict anaerobic mutants were found to carry a mutation in DNA topoisomerase I, while strict aerobic mutants carry a mutation in DNA gyrase. Each type of mutation appears to control expression of numerous operons and genes.

## MATERIALS AND METHODS

**Bacteria.** *S. typhimurium* LT-2, LT-7, and Q strains and their auxotrophic derivatives were used. Established mutants of *S. typhimurium* and *Escherichia coli* carrying defective DNA topoisomerase I and gyrase genes (designated *topI* and *gyr*, respectively) were kindly supplied by the following sources: *S. typhimurium* LT-2 *topI769* and *topIΔ765* (8) from P. Margolin, *E. coli* K-12 *top-10* and *top-250* (9) from J. C. Wang, *E. coli* K-12 *gyrAts43* (10) and its parent from N. R. Cozzarelli, and *E. coli* K-12 *gyrBtsN4177* (11) and its parent from M. Gellert. Culture media have been described (7).

**Anaerobic vs. Aerobic.** The anaerobic gas mixture consisted of 85% nitrogen, 10% carbon dioxide, and 5% hydrogen gas. Agar plates for anaerobic growth were placed in a Scott anaerobic jar (Scott Laboratories, Fiskerville, RI) and evacuated and flushed three times with anaerobic gas mixture. For liquid cultures, Hungate culture tubes with rubber seals (Bellco Glass, Vineland, NJ) containing medium equilibrated with anaerobic gas mixture were used. Reasurin indicator remained colorless under these conditions.

**Mutation.** Mutagenesis and detailed characterization of these mutants will be described elsewhere.

## RESULTS

**Isolation of *S. typhimurium* Mutants Restricted to Grow Under Aerobic or Anaerobic Conditions.** We were able to isolate two groups of environment-specific mutants: one unable to grow in the aerobic environment, designated strict anaerobic mutants, and the other unable to grow in the anaerobic environment, designated strict aerobic mutants (Table 1). Iso-

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Table 1. Isolation and characterization of *S. typhimurium* strict aerobic and strict anaerobic mutants

| Mutant strain* | Growth† | | Marker‡ retained | Sensitivity | |
|---|---|---|---|---|---|
| | Aerobic | Anaerobic | | P22 | ES18 |
| Q *trp-38 ana-1* | − | + | *trp* | + | + |
| Q *trp-38 ana-2* | − | + | *trp* | + | + |
| LT-2 *hisG46 ana-5* | − | + | *his* | + | + |
| LT-2 *hisG46 ana-6* | − | + | *his* | + | + |
| LT-2 *hisG46 ana-7* | − | + | *his* | + | + |
| LT-7 *proAB47 aer-1* | + | − | *pro* | − | + |
| LT-7 *proAB47 aer-2* | + | − | *pro* | − | + |
| LT-7 *proAB47 aer-3* | + | − | *pro* | − | + |
| LT-7 *proAB47 aer-16* | + | − | *pro* | − | + |

*The first genotype designation is for auxotrophic markers. The second genotype designation, *aer* or *ana*, is for strict aerobic or strict anaerobic mutants, respectively.
†Growth behavior under aerobic or anaerobic conditions: +, growth; −, no growth.
‡Demonstration of parental auxotrophic markers retained.

genicity of these mutants and the parent strains was demonstrated by the persistence of auxotrophic markers (see Table 1). Sensitivity of these mutants to infection by *S. typhimurium*-specific phage also was tested. Strict anaerobic mutants were able to support replication of both P22 and ES18, but strict aerobic mutants only supported replication of phage ES18 (Table 1). Since the tetrasaccharide repeating unit known as the O-4,5,12 antigens is the receptor for P22 phage adsorption (12), whereas a membranous protein is the receptor for ES18 (13), synthesis of some polysaccharides of the cell wall apparently is repressed in the strict aerobic mutants.

**Genetic Mapping of the Mutant Alleles Responsible for Strict Anaerobic Mutants.** Various auxotrophic mutants of *S. typhimurium* strains Q and LT-2 were used as recipients for transduction with P22 phage (14) previously grown in strict anaerobic mutants and transduced anaerobically. Over 100 prototrophic transductants for each auxotrophic marker were tested for their inability to grow aerobically. A strict anaerobic mutant allele (*ana-5*) cotransduced with tryptophan (*trp*) auxotrophic markers with a frequency of 47%. The results with other strict anaerobic mutants revealed that all strict anaerobic mutant alleles are located close to the tryptophan operon (*trp*) and that mutation in the same region is responsible for the restriction to anaerobic growth. We then used LT-2 *cysB* strain, which has a mutation near the *trp* operon, as a recipient for cotransduction analysis. The *cysB* marker cotransduced with the strict anaerobic mutant trait at a frequency of about 65%. From these results we conclude that the mutant gene responsible for strict anaerobic growth is located between *cysB* at 34 min and *trp* at 35 min of the *Salmonella* linkage map (15). This particular location suggests that mutation in *topI* may restrict cell growth to the anaerobic condition.

**Mapping and Characterization of Strict Aerobic Mutants.** The mutant gene responsible for strict aerobic growth was mapped by cotransduction analysis with phage ES18 (16) because of the insensitivity of strict aerobic mutants to phage P22 (Table 1). About 11% of the *metG* prototrophic marker contransduced with the strict aerobic mutant trait, suggesting that the strict aerobic alleles are near *metG* at 44 min on the *S. typhimurium* linkage map (15). Since the strict anaerobic mutant alleles were mapped to the region of *topI*, we reasoned that mutations restricted to grow aerobically are located possibly within *gyr*. The DNA gyrase subunit A gene (*gyrA*) is located at 45.5 min in the *S. typhimurium* map (15). Many mutants in the *gyrA* gene result in resistance to nalidixic acid, which binds to DNA gyrase subunit A (17, 18). All the strict aerobic mutant strains we isolated were at least

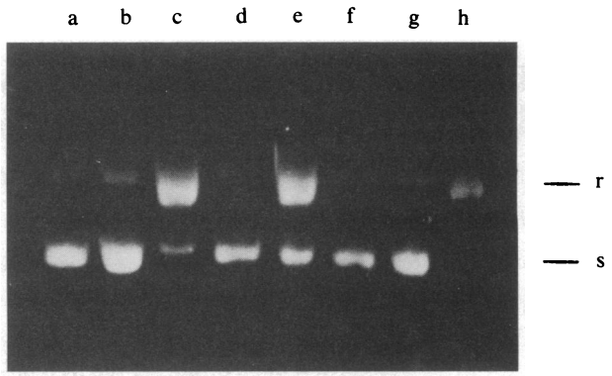


FIG. 1. DNA topoisomerase I activity in strict aerobic and strict anaerobic mutants and their wild-type parent cells as demonstrated by agarose gel electrophoresis. Cell-free extracts and enzyme assays were done by the method of Sternglanz *et al.* (9). Supercoiled PM2 DNA was used as substrate for assaying topoisomerase I activity. Lanes: a, control, 1.5 μg of PM2 DNA only; b, 1.5 μg of DNA and strict anaerobic mutant *ana-1*; c, 1.5 μg of DNA and strict aerobic mutant *aer-16*; d, 1.5 μg of DNA and anaerobically grown wild type; e, 1.5 μg of DNA and aerobically grown wild type; f, control, 0.3 μg of PM2 DNA only; g, 0.3 μg of DNA and anaerobically grown wild-type cells; h, 0.3 μg of DNA and aerobically grown wild-type cells. r, Relaxed; s, supercoiled.

10-fold more sensitive to nalidixic acid than were wild-type *S. typhimurium* strains.

**Demonstration of DNA Gyrase and Topoisomerase I in Extracts of Strict Aerobic and Strict Anaerobic Mutants.** Cell-free extracts of aerobically cultured wild-type cells and strict aerobic mutants decreased the electrophoretic mobility of supercoiled PM2 DNA, whereas the extracts of strict anaerobic mutants and anaerobically cultured wild-type cells had no detectable effect on the DNA (Fig. 1). Thus, strict aerobic mutants and aerobically cultured wild-type cells contained DNA topoisomerase I activity, which relaxes the chromosomal DNA, but no detectable topoisomerase I activity was found in strict anaerobic mutants and anaerobically cultured wild-type cells (Fig. 1). From these observations it may be suggested that topoisomerase I activity is essential for aerobic growth but is not required for anaerobic growth.

When various extracts were incubated with relaxed, closed-circular ColE1 DNA, strict anaerobic mutants and anaerobically cultured wild-type cells increased the electrophoretic mobility of the DNA, but extracts of strict aerobic mutants and aerobically cultured wild-type cells did not affect ColE1 DNA (Fig. 2). These results indicate that strict

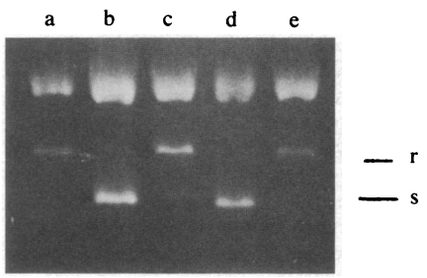


FIG. 2. DNA gyrase activity in strict aerobic and strict anaerobic mutants and their wild-type parent cells as demonstrated by gel electrophoresis. Preparation of the substrate for gyrase, the relaxed circular ColE1 DNA, and the gyrase assay were performed as described by Otter and Cozzarelli (19). Lanes: a, control, no extract; b, strict anaerobic mutant *ana-1*; c, strict aerobic mutant *aer-16*; d, anaerobically grown wild type; e, aerobically grown wild type. r, Relaxed; s, supercoiled. Linear DNA contaminants in the substrate can be seen as the top band.

Table 2. Growth behavior of species with established DNA topoisomerase I and DNA gyrase genetic markers

| Species | Mutation tested | Temperature, °C | Growth* | |
|---|---|---|---|---|
| | | | Aerobic | Anaerobic |
| *S. typhimurium* LT-2 | *topI* | 37 | + | + |
| | *topIΔ* | 37 | + | + |
| *E. coli* K-12 | *topI* | 37 | + | + |
| | *gyrAts43* | 42 | − | − |
| | | 37 | + | − |
| | | 30 | + | + |
| | *gyrBtsN4177* | 42 | − | - |
| | | 37 | + | − |
| | | 30 | + | + |

*+, Growth on nutrient agar; −, no growth on nutrient agar.

anaerobic mutants and anaerobically cultured wild-type cells contain a high level of gyrase activity, whereas this activity is low or deficient in strict aerobic mutants and aerobically cultured wild-type cells. Thus, we suggest that gyrase activity is essential for anaerobic growth of bacteria.

**Environmental Growth Pattern of Established *topI* and *gyr* Mutants.** The strict aerobic mutants we isolated contained mutations near and possibly in the *gyrA* gene. We then tested growth of an established *E. coli gyrA* mutant strain. As shown in Table 2, *E. coli* K-12 *gyrAts43*, a temperature-sensitive mutant (10), was unable to form colonies on nutrient agar in an anaerobic atmosphere at 37°C. However, it grew aerobically at 37°C and both anaerobically and aerobically at 30°C. Since gyrase is composed of two subunits, we also tested the growth behavior of a *gyrB* mutant. When the temperature-sensitive *E. coli* K-12 *gyrBts4177* mutant (11) was incubated in nutrient agar anaerobically at 37°C, no growth or extremely slow growth was observed; however, it grew well aerobically at 37°C and both anaerobically and aerobically at 30°C (Table 2). Thus, mutations with reduced gyrase activity are unable to grow anaerobically at 37°C and behave as strict aerobic mutants. This supports our hypothesis that gyrase function is essential for anaerobic growth (see Table 2).

*topI* point and deletion (*topIΔ*) mutants of *S. typhimurium* (8) and *E. coli* (9) grow aerobically and anaerobically on nutrient agar (Table 2). However, these *topI* mutants are known to carry a second mutation in *gyr*; thus, a reduced gyrase activity compensates for the lack of topoisomerase I activity (20–22). We transferred anaerobically *topI* and *topIΔ* mutant alleles from the *S. typhimurium topI* strains to a *cysB* mutant recipient of *S. typhimurium* via P22 cotransduction of *topI* and *cysB*$^+$ markers, and from a *E. coli* K-12 *topI* strain to *E. coli* B *trp* mutant via P1 cotransduction. When transduction plates were incubated aerobically for 4–6 days, extremely slow-growing colonies appeared, and these transductants had the *topI* mutant market as DiNardo *et al.* demonstrated (21). However, when we incubated transduction plates anaerobically, transductants carrying the *topI* mutant marker appeared in 2 days. This finding suggests that strains carrying the *topI* mutation grow at an extremely slow rate under aerobic condition. From these results with well-established *topI* and *gyr* mutations, we conclude that genes controlling tertiary structure of DNA are responsible for aerobic and anaerobic growth of microorganisms.

**Superhelicity of Chromosomal DNA in Strict Aerobic and Strict Anaerobic Mutants and Aerobically and Anaerobically Cultured Wild-Type Cells.** Levels of both topoisomerase I and DNA gyrase activities in the strict environment-specific mutants were also analyzed for the extent of DNA supercoiling *in vivo* (21–24). The deficiency or reduction of one enzyme should be demonstrated by an exaggerated effect of the other. To determine the degree of DNA supercoiling, sedimentation of chromosomal DNA was analyzed on sucrose gradients containing ethidium bromide by the method of Worcel and Burgi (25). The presence of ethidium bromide at a concentration of about 2 μg/ml converts the negatively supercoiled DNA to the relaxed form and the relaxed form to a positively supercoiled form (refs. 24 and 26; unpublished observation). These analyses of chromosomal DNA from mutant and wild-type cells under various conditions are summarized in Table 3. Chromosomal DNA in all the strict anaerobic mutants was supercoiled, suggesting that these mutants lack topoisomerase I activity but express gyrase activity. In contrast, DNA in the strict aerobic mutants was relaxed, thus suggesting that these mutants carry reduced DNA gyrase activity and consequently exhibit the effect of topoisomerase I activity. In the parental wild-type cells, the same analytical procedure showed that DNA in anaerobically grown cells was supercoiled, whereas the DNA in cells grown aerobically with vigorous shaking was more relaxed. These results support the finding that DNA topoisomerase I is not expressed or is present at reduced levels under anaerobic conditions and that DNA gyrase is not expressed or is present at reduced levels under aerobic conditions. When anaerobically grown wild-type cells were incubated for 2 hr under aerobic conditions without growth, chromosomal

Table 3. Chromosomal superhelicity of strict aerobic and strict anaerobic mutants and their parent wild-type strain of *S. typhimurium*

| Bacterial strain | Condition for growth | Incubation without growth | Relative sedimentation rates* (superhelical conversion by EtdBr) | Interpretation: superhelicity |
|---|---|---|---|---|
| Wild type | Anaerobic | — | Slower (negatively supercoiled to relaxed) | Supercoiled |
| | Anaerobic | Aerobic, 2 hr | Faster (relaxed to positively supercoiled) | Relaxed |
| | Aerobic | — | Faster (relaxed to positively supercoiled) | Relaxed |
| | Aerobic | Anaerobic, 3 hr | Slower (negatively supercoiled to relaxed) | Supercoiled |
| Anaerobic mutant | Anaerobic | — | Slower (negatively supercoiled to relaxed) | Supercoiled |
| | Anaerobic | Aerobic, 2 hr | Slower (negatively supercoiled to relaxed) | Supercoiled |
| Aerobic mutant | Aerobic | — | Faster (relaxed to positively supercoiled) | Relaxed |
| | Aerobic | Anaerobic, 3 hr | Faster (relaxed to positively supercoiled) | Relaxed |

Strict aerobic and strict anaerobic mutants and wild-type bacteria in logarithmic phase ($1 \times 10^8$ cells per ml) were labeled in the appropriate growth condition for 2 hr with 2 μCi (1 Ci = 37 GBq) of [$H^3$]thymidine per ml (4.5 Ci/mmol). For some experiments cells were labeled aerotically after anaerobic growth or vice versa. Labeled DNA was prepared by the lysozyme-detergent method of Worcel and Burgi (25). DNA was layered gently on the top of the 10–30% (wt/wt) sucrose gradient containing 2 μg of ethidium bromide (EtdBr) as described by Drlica and Snyder (24) and Steck *et al.* (26). The gradients were centrifuged at 17,000 rpm for 35 min in a SW 50 swinging bucket, then collected from the bottom of the tube (5 drops per vial), and assayed in a scintillation counter. Sedimentation profiles of these mutants and parental chromosomes will be reported elsewhere.

*Rates of sedimentation are compared with linear $C^{14}$-labeled P22 marker DNA.

Table 4. Activity of superoxide dismutase and catalase in *S. typhimurium* wild type and strict anaerobic and aerobic mutants under aerobic and anaerobic conditions

| Bacteria strain | Growth condition | Incubation after growth | Catalase units* | Superoxide dismutase units† |
|---|---|---|---|---|
| Wild type | Aerobic | — | 3.6 | 4.8 |
| Wild type | Anaerobic | — | 0 | <0.2 |
| Wild type | Anaerobic | Aerobic, 2 hr | 3.1 | 6.0 |
| *aer-16* | Aerobic | — | 3.9 | 5.0 |
| *ana-1* | Anaerobic | — | 0 | <0.2 |
| *ana-1* | Anaerobic | Aerobic, 2 hr | 0 | 0.6 |

Cells were grown to late logarithmic phase ($1 \times 10^9$ cells per ml) disrupted with a Bronsom sonicator, centrifuged for 5 min at $3000 \times g$ to remove debris, and assayed immediately. Superoxide dismutase was assayed by inhibition of superoxide-mediated oxidation of pyrogallol as described by Marklund and Marklund (27). Catalase was assayed by the potassium permanganate method of Goldblith and Proctor (28). These enzymes were assayed with 0.1 mg of bacterial protein and were expressed in terms of activity per 1 mg of protein.

*One unit of catalase activity will decompose 1 μmol of $H_2O_2$ per min at pH 7.0 at 25°C per mg of protein.

†One unit of superoxide dismutase is equal to 50% inhibition of progallol oxidation per mg of protein.

DNA became relaxed. Similarly when aerobically cultured wild-type cells were incubated anaerobically for 3 hr, the DNA became highly supercoiled. Strict anaerobic mutants were unable to relax their DNA when incubated aerobically for 2 hr (Table 3). Conversely strict aerobic mutants were unable to supercoil their DNA when incubated anaerobically for 3 hr. These results (Table 3), together with enzymatic analyses of topoisomerase I and gyrase activities, further show that the DNA topoisomerase I is reduced or deficient in the strict anaerobic mutants and that DNA gyrase is reduced or deficient in the strict aerobic mutants.

**Assays of Superoxide Dismutase and Catalase in Strict Aerobic and Strict Anaerobic Mutants.** Organisms that utilize aerobic respiration require superoxide dismutase and catalase for removal of the toxic oxygen byproducts of aerobic respiration. Therefore, the presence of these two enzymes provides an indication of expression or repression of many aerobic respiratory functions and has been used as a taxonomic criterion for anaerobes. Strict aerobic mutants of *S. typhimurium* contained a level of superoxide dismutase equal to that of aerobically grown wild-type cells (Table 4). When wild-type cells were cultured anaerobically, no dismutase activity was detectable, but 2 hr of subsequent aerobic incubation induced a high level of superoxide dismutase activity. None of the strict anaerobic mutants contained detectable superoxide dismutase activity; even after 2 hr of aerobic incubation, the superoxide dismutase activity in the strict anaerobic mutants was only 10% of that in wild-type cells. Similarly, strict aerobic mutants of *S. typhimurium* contain a level of catalase activity equal to that in wild-type cells. Neither the anaerobically cultured wild-type cells nor the strict anaerobic mutants had significant catalase activity. Even after 3 hr of aerobic incubation, strict anaerobic mutants showed no detectable catalase activity.

## DISCUSSION

We have shown that DNA topoisomerase I activity is reduced or absent in strict anaerobic mutants and that DNA gyrase is reduced or absent in strict aerobic mutants. These mutant studies imply that topoisomerase I activity is not required for anaerobic growth, while significant gyrase activity is not required for aerobic growth. This proposition was confirmed by direct assay of the enzymes. The results of the assays were consistent with the finding that chromosomal DNA of anaerobically grown wild-type cells was supercoiled, whereas the DNA of aerobically grown wild-type cells was more relaxed.

Although gyrase activity in aerobically grown cells was not detectable in electrophoretic analysis of the enzyme assay, conditional lethal mutants in *gyrA* and *gyrB* suggest that some gyrase activity may be required for growth under aerobic environment (see Table 2). Evaluation of this possibility requires quantitative analysis of gyrase activity. Since dissociation of the covalent complex formed between DNA gyrase and DNA during enzymatic action can be inhibited by nalidixic acid, a filter-binding assay of gyrase–radiolabeled DNA complexes formed in the presence of nalidixic acid should quantitate gyrase activity (19). By this procedure we detected gyrase activity in anaerobically cultured wild-type cells but not in aerobically cultured wild-type cells (unpublished results). Since DNA gyrase activity is required for DNA replication (24), gyrase activity in aerobically cultured cells may be present but below the detectable level of this assay.

Since expression of some genes is known to be controlled by topoisomerase I and gyrase (22, 23, 29–31), aerobic or anaerobic condition may determine the extent of supercoiling of chromosomal structures, which in turn regulate promoter activities of genes specific for aerobic or anaerobic respiration and growth. An example of such environmental regulation was demonstrated by the observation that superoxide dismutase and catalase are efficiently produced in aerobically cultured cells but not in anaerobically cultured cells.

In ordinary cultivation conditions, such as a liquid stand culture rather than extremely aerobic or anaerobic environments, *S. typhimurium* and *E. coli* cells contain both gyrase and topoisomerase I activities. The coexistence of these enzymes is advantageous not only for growth of these organisms but also for their defense and viability upon encountering fully aerobic or anaerobic environments (surface or bottom of culture medium). However, we have no information on whether these coexisting enzymes act on chromosomal DNA simultaneously or independently under the moderate or intermediate environment. Studies of wild-type cells cultured under extreme environmental conditions and the strict aerobic and strict anaerobic mutants we have isolated should provide invaluable information on roles of topoisomerase I and gyrase in the expression of environment-specific genes.

**Note Added in Proof.** Strict anaerobic mutants are unstable due to high reversion rates. Recently we isolated stable double mutants by mutagenesis of strains already containing the *topI* deletion. Strict anaerobic mutation sites other than topoisomerase genes may also need to be considered, since Boling *et al.* (32) have demonstrated that an *E. coli ts* mutant deficient in the 5′ → 3′ exonuclease of DNA polymerase I, *polA(EX)*, does not grow aerobically but grows normally under anaerobic condition at restrictive temperature.

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