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An Allele of *gyrA* Prevents *Salmonella enterica* Serovar Typhimurium from Using Succinate as a Carbon Source

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A mutant *gyrA* allele resulting in an A271E substitution in the DNA gyrase protein generated a strain unable to grow on the C_4-dicarboxylates succinate, malate, and fumarate. Bacteria harboring *gyrA751* displayed decreased negative supercoiling in cells. Expression of the *dctA* gene, which encodes the C_4-dicarboxylate transporter, was reduced in a *gyrA751* mutant, providing the first evidence that *dctA* expression is supercoiling sensitive and uncovering a simple metabolic screen for lesions in gyrase that reduce negative supercoiling.

DNA supercoiling is implicated in genome dynamics affecting processes such as DNA replication, gene expression, and phage genome integration (4, 20). DNA gyrase, an ATP-dependent enzyme encoded by the essential genes *gyrA* and *gyrB*, is the only known bacterial topoisomerase that introduces negative supercoils (13). Perturbing DNA gyrase via mutations or antibiotics results in reduced growth rates and altered gene expression and can lead to cell death as a result of aberrant DNA supercoiling (15, 27, 28). Many studies on *gyrA* have identified mutant alleles that confer resistance to specific antibiotics but few alleles with easily screened metabolic phenotypes. This work reports the identification of an allele of *gyrA* in *Salmonella enterica* serovar Typhimurium that results in a mutant gyrase (A271E) and generates a strong metabolic phenotype. Strains carrying the mutant gyrase (A271E) are defective in C_4-dicarboxylate transport mediated by DctA. The *dctA* gene encodes the aerobically expressed transporter for succinate, malate, and fumarate and is catabolite (cyclic AMP receptor protein) and anaerobically (ArcA) repressed (9). This is the first report of the strong influence of DNA supercoiling on *dctA* expression sufficient to generate a clear metabolic defect and provides a means to identify mutant strains with decreased negative supercoiling.

An allele of *gyrA* prevents growth on succinate, malate, and fumarate. In the course of other work, a mutant strain unable to utilize succinate (Fig. 1) as a sole carbon source for growth was isolated following mutagenesis by diethyl sulfate (11). Representative growth data are shown in Fig. 1. An otherwise wild-type strain carrying the causative lesion (*gyrA751*, as described below) was surveyed for growth on a variety of carbon sources. Nearly wild-type growth of the mutant strain was observed on the majority of carbon sources tested, including glucose, gluconate, galactose, glycerol, fructose, mannitol, acetate, and citrate (data not shown). However, similar to succinate, neither malate nor fumarate supported growth at 30, 37, or 42°C. The ability to use other specific tricarboxylic acid cycle intermediates (e.g., citrate and acetate) indicated that the causative lesion was not in a tricarboxylic acid cycle enzyme.

A Tn*10*d(Tc) insertion mutation linked by P22 to the causative mutation was identified with standard techniques (18). In a transductional cross using a donor pool of Tn*10*d(Tc) insertions, 36 of 18,000 Tc^r transductants had gained the ability to grow on succinate. The linkage of these insertions to the causative mutation was determined. The location of one insertion [*rcsC71*::Tn*10*d(Tc)], subsequently shown to be 56% linked to the succinate-negative phenotype, was identified by degenerate sequencing (5). A genetic map of the region surrounding *rcsC* is shown in Fig. 2A. When used as donors in transduction crosses, strains with insertions in *rcsB*, *yojN*, *apbE*, *ompC*, STM2273, STM2274, or STM2275 generated two phenotypic classes (Suc^+ and Suc^-) when the succinate-negative mutant was the recipient. Since sequence analyses determined that the relevant insertions were located internal to open reading frames (ORFs), they were assumed to eliminate gene function. Because these insertions neither eliminated nor caused the succinate-negative phenotype, it was concluded that the causative mutation was not an allele of the eight genes mentioned above.

Because of its location in the relevant region, the *gyrA* gene was PCR amplified and sequenced from strains isogenic for the causative lesion (i.e., phenotypically Suc^+ or Suc^-). Compared to the wild-type sequence, the mutant strain carried a C-to-A transversion at nucleotide 811 of the *gyrA* gene. This mutation resulted in the substitution of a glutamic acid for an alanine at residue 271 (A271E) in the gyrase protein. This residue is conserved in 26/41 gyrase homologs from diverse bacteria analyzed by standard BLAST analyses (1).

The A271E form of gyrase results in reduced negative supercoiling but not quinolone resistance. The majority of alleles of *gyrA* that have been described were identified because they resulted in resistance to the quinolone class of antibiotics, such as nalidixic acid and oxolinic acid, though a few *gyrA* alleles (*hisW*) were isolated as regulatory mutants of the *his* operon (2, 26). Numerous mutations in *gyrA* resulting in quinolone resistance have been sequenced, and a well-described quinolone resistance-determining region spans residues 51 to 106 (12, 29). Residue 271 does not fall in this region (Fig. 2B), so

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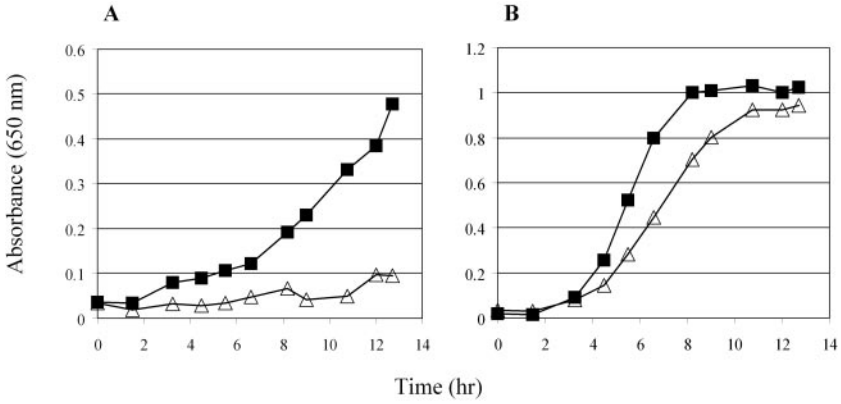

FIG. 1. A *gyrA271* mutant is unable to utilize succinate as a sole carbon source. Growth of *gyrA751* and *gyrA*$^+$ strains was measured as described previously (23, 24) in no-carbon E medium with either 24 mM succinate (A) or 11 mM glucose (B). Growth levels for DM8306 [*gyrA751 ompC396*::Tn*10*d(Tc)] (△) and DM8307 [*gyrA*$^+$ *ompC396*::Tn*10*d(Tc)] (■) in a representative experiment are shown.

the demonstration that the *gyrA751* allele did not affect sensitivity to nalidixic acid or oxolinic acid was not surprising (data not shown). In fact, residue 271 is in a region of the gyrase protein where no previous mutations have been reported.

The expression of a number of genes is affected by supercoiling, and therefore, aberrant expression of these genes occurs when DNA supercoiling has been altered by a *gyr* mutation(s) or by addition of various drugs, in particular quinolones and coumarins (15, 28). Transcriptional fusions in a number of genes (*hisD9953*::MudJ, *trp-3615*::MudJ, and *ilvD2654*::MudJ) were moved by P22 transduction into strains with and without the *gyrA751* allele. The resulting pairs of strains were assayed for β-galactosidase activity, and the results are shown in Table 1. The *gyrA751* mutation increased expression of the *his* operon fourfold under repressing conditions (e.g., Luria broth) and prevented the normal sevenfold derepression of the *ilv* operon under inducing conditions (defined medium with limiting, branched-chain amino acids). Conversely, no effect on expression of the *trp* operon was observed under growth conditions expected to either induce or repress the operon. Deregulation of the *his* and *ilv* operons, but not the *trp* operon, has been reported for cells with reduced negative supercoiling (e.g., the *hisW* class of alleles) (8, 10, 27). As predicted from the derepression of the *his* operon, strain DM8306 (*gyrA751*) formed wrinkled colonies on medium with 2% glucose and was resistant to the histidine analog amino triazole (22, 27). Both of these phenotypes are reported for *hisW* and *hisU* (alleles of *gyrB* that result in similar consequences) alleles (27). Together, these results suggest that the A271E substitution in GyrA, similar to the *hisW* allele class, decreases negative supercoiling and that the decreased supercoiling alters the transcription of the expected loci.

The *gyrA751* mutant strain is deficient in extrachromosomal DNA maintenance and supercoiling. Plasmid DNA (pSU19) (3) was isolated with a commercial product (Promega, Madison, WI) from 10^9 CFU of strains isogenic for the *gyrA751* or *gyrA*$^+$ alleles. The yield of plasmid DNA was consistently lower from the *gyrA751* mutant than from the wild-type strain (1.1 ± 0.32 versus 5.1 ± 0.75 μg, respectively), though both strains were grown in the same medium. The plasmid DNA isolated from a *gyrA751* mutant was transformed into a wild-type strain. Subsequent isolation of the plasmid DNA confirmed that the

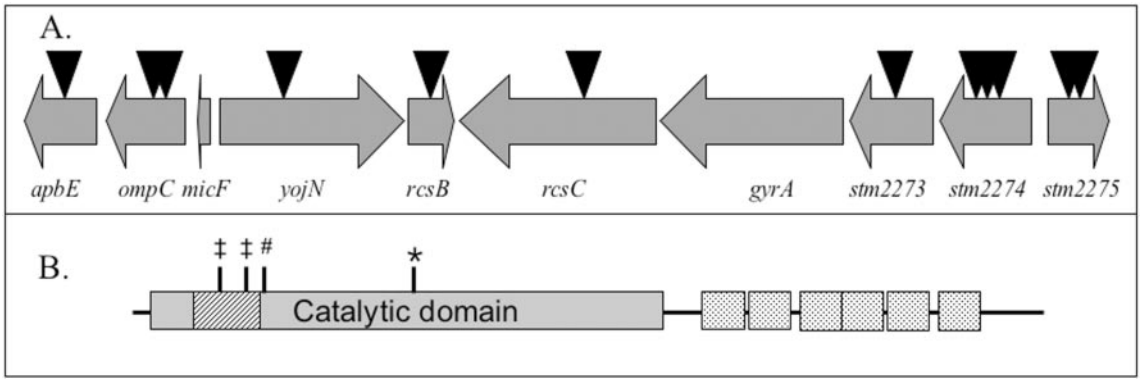

FIG. 2. Physical map of the *gyrA* region and GyrA topology. (A) Insertions linked to the mutation causing loss of growth on succinate are shown as triangles. Based on the location of the insertions in the ORFs, the insertions were considered to cause null mutations. Double mutants containing an insertion and the succinate growth defect demonstrated that the causative lesion was not in any of these ORFs. (B) A schematic of the primary sequence of GyrA is shown (drawn to scale). Regions relevant to function and/or phenotype are noted as follows: #, active-site Y122; ‡, hot spots for Nal^r mutations in *Escherichia coli* or *S. enterica* serovar Typhimurium; hatched box, quinolone resistance-determining area; dotted box, C-terminal chain; *, A271E (*gyrA751*).

TABLE 1. *gyrA751* mutants have altered expression levels of amino acid biosynthetic operons[a]

Strain	*gyrA* allele	Fusion locus	Expression level (Miller units)	Growth	Expression ratio
DM9063	*gyrA751*	*hisD*	71.3 ± 5.4	Repressed	4.0
DM9065	*gyrA*$^+$	*hisD*	17.9 ± 1.5	Repressed	
DM9123	*gyrA751*	*ilvD*	11.6 ± 2.2	Repressed	0.53
DM9122	*gyrA*$^+$	*ilvD*	21.7 ± 1.9	Repressed	
DM9123	*gyrA751*	*ilvD*	52.9 ± 34	Induced	0.14
DM9122	*gyrA*$^+$	*ilvD*	390 ± 26	Induced	
DM9113	*gyrA751*	*trp*	6.1 ± 0.1	Repressed	0.84
DM9112	*gyrA*$^+$	*trp*	7.3 ± 0.3	Repressed	
DM9113	*gyrA751*	*trp*	25.0 ± 3.1	Induced	1.0
DM9112	*gyrA*$^+$	*trp*	25.2 ± 1.1	Induced	

[a] Operon fusions (MudJ) (6) in the indicated loci were transduced to strains with or without the *gyrA751* allele, and β-galactosidase assays were performed on the resulting strains. Repressing conditions represent growth in Luria broth, while inducing conditions indicate growth in no-carbon E medium supplemented with 11 mM glucose, 1 mM $MgSO_4$, and relevant amino acids at the following concentrations: Ile, Leu, Val, 60 μM each; Trp, 50 μM. The last column displays the ratio of transcription found in the mutant to that of the wild-type genetic background.

plasmid had reestablished itself to wild-type levels, indicating that the *gyrA751* mutant inefficiently maintained episomal DNA. Although it was formally possible that the *gyrA751* mutant was unable to maintain only pSU19, a similar result was obtained when pBR322 was isolated from isogenic strains. Since plasmids pSU19 and pBR322 have different origins of replication, it was concluded that the *gyrA751* mutant was in general unable to efficiently maintain plasmids. In a different study, gyrase inhibitors such as novobiocin were shown to promote plasmid curing, implicating a role for supercoiling in plasmid maintenance, which would be consistent with the interpretation of the data above (14). To measure directly the extent of plasmid supercoiling, the pSU19 topoisomers were separated by electrophoresis in a 1% agarose gel containing chloroquine (Fig. 3). The topoisomer mobility of plasmid DNA isolated from the *gyrA751* mutant was visibly decreased, confirming that the *gyrA751* mutant strain maintained a lower level of DNA supercoiling.

Decreased *dctA* expression is responsible for the succinate-negative phenotype of *gyrA751*. Since the *gyrA751* allele caused multiple transcriptional changes, it was hypothesized that the inability of the *gyrA751* mutant to grow on C_4-dicarboxylates was due to reduced expression of a gene(s) required for C_4-dicarboxylate utilization. Similar to the *gyrA751* mutant, strains lacking *dctA* are unable to grow with malate, fumarate, or succinate as a carbon source (17). DctA is an inner membrane permease responsible for transporting these three dicarboxylic acids during aerobic growth conditions (19, 25). To determine whether the growth behavior of a *gyrA751* mutant was consistent with a reduction in DctA activity, transport assays were performed. Cells were harvested from media containing 24 mM glycerol (as a carbon source) and 16 mM succinate (to induce transport), and the rates of [2,3-^{14}C]succinic acid transport in a pair of strains isogenic for the *gyrA*$^+$ or *gyrA751* allele are shown in Fig. 4. The rate of succinate uptake by the *gyrA751* mutant was significantly lower than that of the wild-type strain and in fact was not significantly higher than that of a *dctA* mutant. That the *gyrA751* mutant was strongly deficient

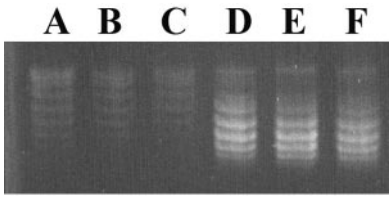

FIG. 3. The *gyrA751* mutant has lower DNA supercoiling levels than the wild type. Plasmid pSU19 was isolated from mutant strain *gyrA751* (A to C) and the wild type (D to F), and topoisomers were separated by agarose gel electrophoresis in the presence of 2.8 μg/ml chloroquine diphosphate. Mobility is from top to bottom.

in DctA activity was consistent with the succinate-negative growth phenotype.

To determine whether the transcriptional expression of the *dctA* gene was reduced in a *gyrA751* mutant background, a *lacZ* operon fusion (MudJ) in *dctA* was isolated from a pool of random MudJ insertions. The *dctA81*::MudJ insertion was transduced into strains with or without the *gyrA751* mutation, and the resulting strains were assayed for β-galactosidase activity. Expression of *dctA* was twofold lower in the *gyr* mutant than in the wild-type strain (163 ± 5 versus 358 ± 22 Miller units, respectively), indicating that the *dctA* promoter is sensitive to supercoiling. The relatively small effect on transcription was surprising given the large effect of the *gyrA751* mutation on

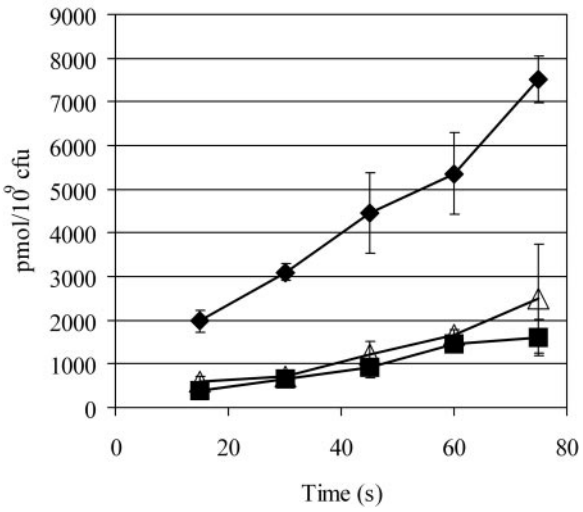

FIG. 4. The *gyrA751* mutant is unable to transport succinate. Succinate uptake was measured with radiolabeled [2,3-^{14}C]succinate. Cultures were grown in minimal no-carbon E medium supplemented with 1 mM $MgSO_4$, 24 mM glycerol, and 16 mM fumarate. Cells were harvested in mid-exponential phase and washed twice in 3 ml no-carbon E buffer. Succinate transport assays were performed at 37°C and contained the following: 100 mM potassium phosphate (KP) buffer (pH 7.4), 100 mM $MgSO_4$, 0.2 mM succinate (2.4 nCi/nmol), and approximately 7×10^7 CFU in a total volume of 0.520 ml. Aliquots of 0.1 ml were taken every 15 seconds after the addition of succinate, applied to a 25-mm, 0.4-μm-pore-size nitrocellulose membrane, and washed twice in rapid succession with 3 ml KP · $MgSO_4$ buffer over a vacuum manifold. Filters were dried, and radioactivity retained on filters was measured by standard methods. Representative transport levels for DM8306 [*gyrA751 ompC396*::Tn*10*d(Tc)] (△), DM8307 [*gyrA*$^+$ *ompC396*::Tn*10*d(Tc)] (◆), and FM2 (*dct-71*) (16) (■) in a representative experiment are shown.

both the phenotype and DctA-dependent transport. It had previously been noted that inactivation of *dctA* with a *lacZ* fusion caused constitutive expression of *dctA* and suggested that DctA might be autoregulatory (9), thus complicating the interpretation of the fusion data. Total cellular RNA was isolated (RNeasy; QIAGEN) from *gyrA751* or *gyrA*$^+$ isogenic strains cultured in 24 mM glycerol, 16 mM fumarate in midlogarithmic phase, and reverse transcription-PCR was performed as previously described (7). The levels of *dctA* mRNA were determined in at least two isolates of isogenic strains and were normalized to *trp* mRNA levels as a control. The amount of *dctA* mRNA was 13-fold lower in the *gyrA751* strain than in the wild-type strain (2,896 ± 4,007 versus 38,298 ± 2,922 arbitrary units, respectively), demonstrating that *dctA* expression is strongly regulated by the extent of DNA supercoiling.

Conclusion. The data herein identify *dctA* as a locus whose transcription is sensitive to levels of DNA supercoiling. Further, a temperature-insensitive allele of the essential *gyrA* gene that decreased supercoiling yet did not influence the sensitivity of the strain to growth inhibition by quinolones was identified. Visualization of the relevant A271 residue in the crystal structure of the breakage-reunion domain of GyrA (GyrA59) (21) reveals that this residue is in the α11 helix that is proximal to, but not in, the quinolone resistance-determining region and catalytic tyrosine region. To the best of our knowledge, no mutations in the α11 helix have been reported, and the importance of the α11 helix for protein function remains to be determined biochemically. The proximity of the α11 helix to the catalytic site might suggest that the A271 residue has a role in DNA recognition or catalysis. This work has identified a simple metabolic screen (the inability to grow on succinate) for the isolation of *gyrA* alleles and other genes that result in reduced supercoiling.

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REFERENCES

1. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
2. **Anton, D. N.** 1968. Histidine regulatory mutants in *Salmonella typhimurium*. V. Two new classes histidine regulatory mutants. J. Mol. Biol. **33:**533–546.
3. **Bartolomé, B., Y. Jubete, E. Martinez, and F. de la Cruz.** 1991. Construction and properties of a family of pACYC184-derived cloning vectors compatible with pBR322 and its derivatives. Gene **102:**75–78.
4. **Brahms, J. G., O. Dargouge, S. Brahms, Y. Ohara, and V. Vagner.** 1985. Activation and inhibition of transcription by supercoiling. J. Mol. Biol. **181:** 455–465.
5. **Caetano-Annoles, G.** 1993. Amplifying DNA with arbitrary oligonucleotide primers. PCR Methods Appl. **3:**85–92.
6. **Castilho, B. A., P. Olfson, and M. J. Casadaban.** 1984. Plasmid insertion mutagenesis and *lac* gene fusion with mini-Mu bacteriophage transposons. J. Bacteriol. **158:**488–495.
7. **Cowles, K. N., and Goodrich-Blair, H.** 2005. Expression and activity of a *Xenorhabdus nematophila* haemolysin required for full virulence towards *Manduca sexta* insects. Cell. Microbiol. **7:**209–219.
8. **Davidson, J. P., D. J. Wilson, and L. S. Williams.** 1982. Role of a hisU gene in the control of stable RNA synthesis in *Salmonella typhimurium*. J. Mol. Biol. **157:**237–264.
9. **Davies, S. J., P. Golby, D. Omrani, S. A. Broad, V. L. Harrington, J. R. Guest, D. J. Kelly, and S. C. Andrews.** 1999. Inactivation and regulation of the aerobic C_4-dicarboxylate transport (*dctA*) gene of *Escherichia coli*. J. Bacteriol. **181:**5624–5635.
10. **Davis, L., and L. S. Williams.** 1982. Altered regulation of isoleucine-valine biosynthesis in a *hisW* mutant of *Salmonella typhimurium*. J. Bacteriol. **151:** 860–866.
11. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Friedman, S. M., T. Lu, and K. Drlica.** 2001. Mutation in the DNA gyrase A gene of *Escherichia coli* that expands the quinolone resistance-determining region. Antimicrob. Agents Chemother. **45:**2378–2380.
13. **Gellert, M., K. Mizuuchi, M. H. O'Dea, and H. A. Nash.** 1976. DNA gyrase: an enzyme that introduces superhelical turns into DNA. Proc. Natl. Acad. Sci. USA **73:**3872–3876.
14. **Hooper, D. C., J. S. Wolfson, G. L. McHugh, M. D. Swartz, C. Tung, and M. N. Swartz.** 1984. Elimination of plasmid pMG110 from *Escherichia coli* by novobiocin and other inhibitors of DNA gyrase. Antimicrob. Agents Chemother. **25:**586–590.
15. **Jovanovich, S. B., and J. Lebowitz.** 1987. Estimation of the effect of coumermycin A_1 on *Salmonella typhimurium* promoters by using random operon fusions. J. Bacteriol. **169:**4431–4435.
16. **Kay, W. W., and M. Cameron.** 1978. Citrate transport in *Salmonella typhimurium*. Arch. Biochem. Biophys. **190:**270–280.
17. **Kay, W. W., and H. L. Kornberg.** 1971. The uptake of C4-dicarboxylic acids by *Escherichia coli*. Eur. J. Biochem. **18:**274–281.
18. **Kleckner, N., R. M. Chalmers, D. Kwon, J. Sakai, and S. Bolland.** 1996. Tn10 and IS10 transposition and chromosome rearrangements: mechanism and regulation *in vivo* and *in vitro*. Curr. Top. Microbiol. Immunol. **204:** 49–82.
19. **Lo, T. C., M. K. Rayman, and B. D. Sanwal.** 1972. Transport of succinate in *Escherichia coli*. I. Biochemical and genetic studies of transport in whole cells. J. Biol. Chem. **247:**6323–6331.
20. **Mizuuchi, K., and H. A. Nash.** 1976. Restriction assay for integrative recombination of bacteriophage lambda DNA *in vitro*: requirement for closed circular DNA substrate. Proc. Natl. Acad. Sci. USA **73:**3524–3528.
21. **Morais Cabral, J. H., A. P. Jackson, C. V. Smith, N. Shikotra, A. Maxwell, and R. C. Liddington.** 1997. Crystal structure of the breakage-reunion domain of DNA gyrase. Nature **388:**903–906.
22. **Murray, M. L., and P. E. Hartman.** 1972. Overproduction of *hisH* and *hisF* gene products leads to inhibition of cell cell division in *Salmonella*. Can. J. Microbiol. **18:**671–681.
23. **Petersen, L., and D. M. Downs.** 1996. Mutations in *apbC* (*mrp*) prevent function of the alternative pyrimidine biosynthetic pathway in *Salmonella typhimurium*. J. Bacteriol. **178:**5676–5682.
24. **Petersen, L. A., J. E. Enos-Berlage, and D. M. Downs.** 1996. Genetic analysis of metabolic crosstalk and its impact on thiamine synthesis in *Salmonella typhimurium*. Genetics **143:**37–44.
25. **Rayman, M. K., T. C. Lo, and B. D. Sanwal.** 1972. Transport of succinate in *Escherichia coli*. II. Characteristics of uptake and energy coupling with transport in membrane preparations. J. Biol. Chem. **247:**6332–6339.
26. **Roth, J. R., D. N. Anton, and P. E. Hartman.** 1966. Histidine regulatory mutants in *Salmonella typhimurium*. I. Isolation and general properties. J. Mol. Biol. **22:**305–323.
27. **Rudd, K. E., and R. Menzel.** 1987. *his* operons of *Escherichia coli* and *Salmonella typhimurium* are regulated by DNA supercoiling. Proc. Natl. Acad. Sci. USA **84:**517–521.
28. **Sanzey, B.** 1979. Modulation of gene expression by drugs affecting deoxyribonucleic acid gyrase. J. Bacteriol. **138:**40–47.
29. **Yoshida, H., M. Bogaki, M. Nakamura, and S. Nakamura.** 1990. Quinolone resistance-determining region in the DNA gyrase *gyrA* gene of *Escherichia coli*. Antimicrob. Agents Chemother. **34:**1271–1272.