Sulfonamide Resistance in Clinical Isolates of *Campylobacter jejuni*: Mutational Changes in the Chromosomal Dihydropteroate Synthase

AMERA GIBREEL AND OLA SKÖLD*

Division of Microbiology, Department of Pharmaceutical Biosciences, Biomedical Center, Uppsala University, SE-751 23, Uppsala, Sweden

Received 2 February 1999/Returned for modification 17 March 1999/Accepted 28 June 1999

The characterization of the genetic basis of sulfonamide resistance in *Campylobacter jejuni* was attempted. The resistance determinant from a sulfonamide-resistant strain of *C. jejuni* was cloned and was found to show 42% identity with the *folP* gene (which codes for dihydropteroate synthase, the target of sulfonamides) of the related bacterium *Helicobacter pylori*. The sequences of the areas surrounding the *folP* gene in *C. jejuni* showed similarity to those of the areas surrounding the corresponding gene in *H. pylori*. The *folP* gene of *C. jejuni*, which mediates the resistance, was observed to show particular features when it was compared to other known *folP* genes. One of these features is the presence of two pairs of direct repeats (15 and 27 bp) within the coding sequence of the gene. Comparison of the *C. jejuni* *folP* genes that mediate susceptibility and resistance revealed the occurrence of mutations that changed four amino acid residues. Resistance of *C. jejuni* to sulfonamides could be associated with one or several of these four mutational substitutions, which all occurred in the five different resistant isolates studied. The codon for one of these changed amino acids was found to be located in the second direct repeat within the coding sequence of the gene. The change made the repeat perfect. The transformation of both the resistance and the susceptibility variants of the gene into an *Escherichia coli* *folP* knockout mutant was found to complement the dihydropteroate synthase deficiency, confirming that the characterized sulfonamide resistance determinant codes for the *C. jejuni* dihydropteroate synthase enzyme. Kinetic measurements established different affinities of sulfonamide for the dihydropteroate synthase enzyme isolated from the resistant and susceptible strains. In conclusion, sulfonamide resistance in *C. jejuni* was shown to be associated with mutational changes in the chromosomally located gene for dihydropteroate synthase, the target of sulfonamides.

Sulfonamides were once used successfully in the treatment of a variety of bacterial infections. However, the rapid emergence of sulfonamide resistance and the development of more potent drugs have limited their clinical use. The target of sulfonamides is the enzyme dihydropteroate synthase (DHPS), which catalyzes the formation of dihydropteroylpyruvate (DHP) from p-aminobenzoic acid (PABA), the substrate of the DHPS enzyme, and inhibits it competitively. It can also function as an alternative substrate for the production of a sulfonamide-containing pterate analog that cannot be used in the subsequent steps of the biosynthetic pathway. The folate cofactor pool in the bacterial cell is consequently depleted (29, 37), resulting in growth inhibition and cell death (15).

Chromosomal mutations in the *folP* gene that result in low levels of sulfonamide resistance can be isolated in the laboratory (15, 24). On the other hand, acquired resistance to high concentrations of sulfonamides has been observed in gram-negative bacteria. This resistance is plasmid borne and is due to the presence of genes that code for alternative drug-resistant variants of the DHPS enzyme (33, 39). Two such plasmid-borne resistance genes have been found (28, 35). One of them, *sulF*, is found almost exclusively on integron structures carried by large conjugative plasmids (35). The other sulfonamide resistance determinant, *sul2*, is frequently found on small mobilizable plasmids (28).

The susceptibilities of *Campylobacter jejuni* strains to sulfonamides, either alone or in combination with trimethoprim, were found to be variable according to the geographical source of the isolates (9, 10, 18, 36). The genetic basis of sulfonamide resistance in *C. jejuni* has not previously been investigated at the molecular level, however.

In the study described here, the DHPS gene of *C. jejuni* was characterized. Sulfonamide resistance was shown to be associated with the mutational substitution of four amino acid residues that resulted in a reduced affinity for sulfonamide of the resistant variant of DHPS. Some information about the surrounding areas of the *folP* gene on the chromosome of *C. jejuni* are also reported here.

**MATERIALS AND METHODS**

*Bacterial strains and plasmids*. The strains and plasmids involved in this study are listed in Table 1. Sulfonamide-resistant and susceptible clinical isolates of *C. jejuni* were obtained from the laboratory of clinical bacteriology in two Swedish hospitals (Department of Infectious Diseases, Uppsala University, and Department of Clinical Bacteriology, Göteborg University).

*Sulfonamide susceptibility testing of *C. jejuni* strains*. Susceptibility testing was initially carried out with commercially available sulfonamide disks (AB Biodisk, Solna, Sweden). The procedures were as described previously (11). Zone sizes were interpreted according to the guidelines of the National Committee for Clinical Laboratory Standards (22). The MICs of sulfonamide were determined by the agar dilution method, as described previously (17).

**Cloning of the sulfonamide resistance determinant from a clinical isolate of *C. jejuni***. The chromosomal DNA from a sulfonamide-resistant *C. jejuni* strain (strain CJ9; Table 1), which was isolated from a patient with gastroenteritis, was cleaved with the *HindIII* (this restriction enzyme and the other enzymes men-
SULFONAMIDE RESISTANCE IN CAMPYLOBACTER JEJUNI

TABLE 1. Bacterial strains and plasmids involved in this study

| Strain or plasmid | Relevant genotype or description | Reference or source |
|-------------------|---------------------------------|---------------------|
| **Strains** | | |
| *C. jejuni* | | |
| C3-1 | Clinical isolate, Tp' Su' | This study |
| C3-13 | Clinical isolate, Tp' Su' | This study |
| C4 | Clinical isolate, Tp' Ap' | This study |
| C9 | Clinical isolate, Tp' Na' Sm' Su' | This study |
| C14 | Clinical isolate, Tp' Ap' De' Na' Su' | This study |
| C17 | Clinical isolate, Tp' De' Su' | This study |
| E. coli | | |
| DH5α | F' endA thi-1 hsdR17 supE44 relA1 gyrA96 recA1 Δ(oacU169 thi-1 thrl-1 leuB6 lacY1 tonA2I supE44 ΔfolP::Km') | 30 |
| C600Δ(folP::Km') | | 8 |
| **Plasmids** | | |
| pUC19 and pUC18 | Ap' cloning vectors | 42 |
| pCS1 | 2.4-kb HindIII fragment from CJP in HindIII-cleaved pUC19 | This study |

ª Tp', Ap', De', Na', Sm', and Km', resistance to trimethoprim, ampicillin, doxycycline, nalidixic acid, sulfonamide, streptomycin, and kanamycin, respectively.

amplified by PCR from four other sulfonamide-resistant strains, strains CJ1, CJ3, CJ14, and CJ17 (Table 1). They were resistant to 256 μg/ml sulfathiazole per ml, was used for cloning of the sulfonamide resistance determinant. The chromosomal folP gene was further amplified by PCR from four other sulfonamide-resistant strains, strains CJ1, CJ3, CJ14, and CJ17 (Table 1). They were resistant to 128 to 256 μg of sulfathiazole per ml for 4 h.

**Determination of DHPS activity.** Host bacteria (*E. coli* DH5α) with folP-carrying vectors were grown at 37°C to the late exponential phase in 400 ml of Iso-Sensitest broth (Oxoid) supplemented with the appropriate antibiotic. The cells were harvested by centrifugation, washed once in 0.1 M potassium phosphate buffer (pH 7.0), and then resuspended in 3 ml of the same buffer. The cell-free extract was prepared by sonicating the cells three times, for 20 s each time, followed by centrifugation for 20 min. The DHPS activity was determined by the incorporation of 3H-labelled PABA into dihydropteridine acid as described previously (37). Enzyme kinetics were analyzed by varying the concentration of the PABA substrate with the other substrate, 2-hydroxy-4-amin-6-hydroxymethylpyrophosphate, in excess. The Km was determined by measuring the apparent Km in the presence of different concentrations of the inhibitor.

**Other methods.** Cleavage with restriction endonucleases and agarose gel electrophoresis were carried out by standard procedures (31). Chromosomal DNA was prepared from *C. jejuni* strains as described by Pitcher et al. (27).

**Nucleotide sequencing.** The dideoxynucleotide chain termination method of Sanger et al. (32) was used. Double-stranded templates were prepared by cloning the sequences into pUC19 or pUC18 as described previously (31). The commercially available universal 17-nucleotide primer and the reverse 16-nucleotide primer specific for the pUC cloning vector were used for sequencing. All oligonucleotide primers derived from the sequence of the cloned *folP* gene that was determined were designed and were also used for sequence walking. These primers include 5'-GGAGTGAGGTTAACACACG-3' (nucleotides 175 to 195), 5'-GGCTTAAATCCTCCGATATTG-3' (nucleotides 450 to 472), and 5'-GGAAAGTGGGACGCGTAAAAATTG-3' (nucleotides 907 to 930). The nucleotide sequences of both strands of the susceptible or the resistant variant of the *folP* gene were determined for two different clones. Double-stranded templates were prepared for sequencing by the method of Wong et al. (41). The modified T7 DNA polymerase (Amersham, Cleveland, Ohio) was used for elongation. [α-35S]dATP from New England Nuclear, Dreieich, Germany, was the labelling component.

**Data bank analyses.** For analysis of the sequencing data, software from the University of Wisconsin Genetics Computer Group (7) was used. Nucleotide sequences were compared to those in the EMBL and GenBank databases by using the FASTA algorithm (25), and the derived protein sequences were analyzed for similarity to protein sequences in the databases by using the BLAST algorithm (1).

**RESULTS AND DISCUSSION**

Sulfonamide susceptibility of *C. jejuni*. Sulfonamide-resistant *C. jejuni* C39 (Table 1), which was resistant to 256 μg of sulfathiazole per ml, was used for cloning of the sulfonamide resistance determinant. The chromosomal *folP* gene was further amplified by PCR from four other sulfonamide-resistant strains, strains CJ1, CJ3, CJ14, and CJ17 (Table 1). They were resistant to 128 to 256 μg of sulfathiazole per ml. For compar-
Characterization of the sulfonamide resistance trait of a clinical isolate of *C. jejuni*. Cloning of the sulfonamide resistance determinant from a sulfonamide-resistant isolate of *C. jejuni* (isolate C39 [Table 1]; see the Materials and Methods section) resulted in four sulfonamide-resistant clones. The four sulfonamide-resistant transformants, for which MICs were >560 μg/ml, were found to contain a HindIII fragment of about 2.4 kb. A restriction map of the inserted fragment carried on plasmid pCS1 (Table 1) was made with different restriction enzymes and is shown in Fig. 1.

For further characterization of the resistance determinant, the cloned fragment was cleaved with HindIII, AvaI, and BglII into three smaller fragments (Fig. 1). Subcloning of each of the resulting fragments into the pUC19 cloning vector and transformation into *E. coli* DH5α resulted in the loss of the sulfonamide resistance phenotype. The nucleotide sequence of each of the subcloned fragments was determined in order to further localize the sulfonamide resistance determinant within the 2.4-kb HindIII fragment and to characterize its surroundings.

Analysis of the resulting sequence data suggested that the 2.4-kb HindIII fragment carries a gene that codes for the DHPS enzyme. The gene was found to show 54% (at the nucleotide level) and 42% (at the amino acid level) identities to the corresponding gene of *H. pylori* when it was compared to the corresponding folP gene of *C. jejuni* by PCR. Abbreviations: holB, gene coding for DNA polymerase III delta prime subunit; folP, gene coding for DHPS; and lig, gene coding for DNA ligase in the genome of *H. pylori*.

FIG. 1. Restriction map of the DNA fragment carrying the sulfonamide resistance determinant located on plasmid pCS1 (Table 1). The sizes of the three fragments resulting from cleavage with HindIII, BglII, and AvaI are indicated. The arrows below the map show the direction of the transcription of the open reading frames located on the inserted HindIII fragment. SUP1 and SUP2 refer to a primer pair designed to amplify the folP gene of *C. jejuni* by PCR. Abbreviations: holB, gene coding for DNA polymerase III delta prime subunit; folP, gene coding for DHPS; and lig, gene coding for DNA ligase in the genome of *H. pylori*.

It should be mentioned that the folP gene of *C. jejuni* has special characteristics in comparison to the corresponding genes of other bacterial species. First, it is the largest folP gene characterized so far. Its product consists of 390 amino acid residues, compared to the 380 residues of the corresponding DHPS of the related organism *H. pylori*. The other, previously characterized folP genes of *E. coli* (6), *Staphylococcus aureus* (13), *Streptococcus pneumoniae* (20), and *Bacillus subtilis* (34) recognize seven nucleotides of the consensus −10 and −35 sequences of *E. coli* recognized by the σ70 factor (TATAAT and TTGACA [14]), respectively. The existence of the proposed promoter sequences could be consistent with a low level of expression of the DHPS. Alternatively, other −10 and −35 promoter sequences, starting at positions −44 and −70 of the start codon of the gene, respectively, could also be detected. The latter promoter regions are less close to the consensus sequence, and their presence may therefore also be consistent with low level expression.

![FIG. 2. (A) Comparison of the deduced amino acid sequence of the folP gene of sulfonamide-resistant *C. jejuni* C39 (Table 1) with that of the corresponding gene of *H. pylori*. Data are taken from the complete genome sequence of *H. pylori* (40). Dots within the sequence indicate gaps that give optimal similarity. Vertical lines between two amino acids indicate identical residues. The other, previously characterized folP genes of *E. coli* (6), *Staphylococcus aureus* (13), *Streptococcus pneumoniae* (20), and *Bacillus subtilis* (34) recognize seven nucleotides of the consensus −10 and −35 sequences of *E. coli* recognized by the σ70 factor (TATAAT and TTGACA [14]), respectively. The existence of the proposed promoter sequences could be consistent with a low level of expression of the DHPS. Alternatively, other −10 and −35 promoter sequences, starting at positions −44 and −70 of the start codon of the gene, respectively, could also be detected. The latter promoter regions are less close to the consensus sequence, and their presence may therefore also be consistent with low level expression.](image-url)
are relatively smaller. Second, the coding sequence of the \textit{C. jejuni} \textit{folP} gene was found to have two relatively long direct repeats (Fig. 2A).

The rest of the sequence of the \textit{BglII-AviI} fragment upstream of the characterized \textit{folP} gene and the sequence of the \textit{HindIII-BglII} fragment (Fig. 1) were also analyzed. These sequences are similar to another gene in \textit{H. pylori}, known as the \textit{holB} gene, which codes for the DNA polymerase III delta prime subunit (Fig. 1) (40).

Downstream of the coding sequence of the \textit{folP} gene, the existence of a starting codon of a new open reading frame was observed (Fig. 1). Analysis of this sequence revealed that it is similar to a part of a gene (\textit{lig}) that codes for DNA ligase in \textit{H. pylori} (40); these genes exhibited 59.2 and 51.8% similarity and identity, respectively. The complete genome sequence of \textit{H. pylori} (40) showed that the \textit{lig} gene codes for 656 amino acid residues. In this study, only 245 amino acid residues of the \textit{lig} gene coding sequence were detected up to the \textit{HindIII} end of the chromosomal insert in pCS1 (Fig. 1).

The areas surrounding the \textit{folP} gene on the chromosome of \textit{C. jejuni} were thus found to show similarity to the corresponding parts of the chromosome of \textit{H. pylori}. The gene coding for the DNA polymerase III delta prime subunit, detected upstream of the \textit{folP} gene, was found to have the same location in both the \textit{C. jejuni} and the \textit{H. pylori} genomes (40). The two organisms differ, however, in the type of gene located downstream of the \textit{folP} gene. In \textit{C. jejuni}, a gene that codes for DNA ligase was detected at this position, while in \textit{H. pylori}, the gene located downstream of the \textit{folP} gene was defined as an unknown gene (40). The gene that codes for DNA ligase was detected at another location in the \textit{H. pylori} genome (40). Data from the available sequencing database for \textit{C. jejuni} at the Sanger Center (16) confirmed our results regarding the location of the \textit{lig} and the \textit{holB} genes flanking the \textit{folP} gene.

Detection of the \textit{folP} gene from a sulfonamide-susceptible and other sulfonamide-resistant isolates of \textit{C. jejuni} by PCR.

By using the sequence obtained for the \textit{folP} gene, which mediates sulfonamide resistance, and by PCR amplification (see the Materials and Methods section), the \textit{folP} genes from a sulfonamide-susceptible \textit{C. jejuni} strain (strain CJ4; Table 1) and four other sulfonamide-resistant strains of \textit{C. jejuni} (strains CJ1, CJ3, CJ14, and CJ17; Table 1) were amplified. The PCR products were then cloned into pUC19 and were transformed into \textit{E. coli} DH5\(\alpha\). For transformants carrying \textit{folP} genes from resistant isolates, MICs were >560 \(\mu\)g/ml, while MICs of 14 \(\mu\)g/ml were found for the corresponding susceptible transformants. The sequences of the amplified \textit{folP} genes from the sulfonamide-resistant isolates were found to be identical to that of the corresponding gene cloned from isolate CJ9. The sequence of the \textit{folP} gene amplified from the susceptible strain, however, showed differences in four amino acid residues compared to the sequences of the corresponding resistance genes from all five isolates studied. These differences involve (susceptibility to resistance) L186F, D238N, N245K, and F246Y (Fig. 2B). The mutation of Leu to Phe (amino acid residue 186) was found to participate in the formation of the 27-bp perfect direct repeats within the coding sequence of the resistant \textit{folP} gene (Fig. 2A). The amino acid substitutions detected in the present study do not coincide with the mutations in the \textit{folP} genes from other sulfonamide-resistant organisms (13). In our study, three of the amino acid substitutions associated with resistance were observed to be located close to two conserved amino acid residues known to be involved in the binding of hydroxymethylpteridine pyrophosphatase (Fig. 2B) (13).

Determination of DHPS enzyme kinetics. To test whether the amino acid differences between the susceptible and the resistant variants of the DHPS enzyme are reflected in the kinetic parameters of the enzyme, the inhibitory constant for sulfonamide was determined. A dramatic difference in the \(K_{i}\) for sulfathiazole was detected (500 \(\mu\)M for the resistant enzyme and 0.5 \(\mu\)M for the susceptible one), indicating that the amino acid substitutions detected in the present study have resulted in a reduced binding affinity of the resistant DHPS enzyme for sulfonamides. A difference in the \(K_{i}\) for PABA was also apparent (0.85 \(\mu\)M for the resistant enzyme and 0.25 \(\mu\)M for the susceptible one), indicating that the amino acid changes in the resistant enzyme have also resulted in a less efficient enzyme. This is in parallel to the situation in \textit{Neisseria meningitidis} (8) and \textit{Streptococcus pyogenes} (38), in which similar differences in \(K_{i}\) values for PABA for susceptible and resistant variants of the enzyme were observed. In all these cases, sulfonamide resistance seems to persist, even though the use of this drug has all but ceased in clinical practice in Sweden (cf. reference 26 though). This is in contradiction to the expected selection pressure against the less efficient enzyme.

Transformation of the \textit{C. jejuni} \textit{folP} gene into an \textit{E. coli} \textit{folP} knockout mutant.

The transformation of the cloned \textit{folP} gene from \textit{C. jejuni} strains (susceptible or resistant variant of the gene) was found to complement the DHPS deficiency so that the mutant could grow on Iso-Sensitest agar plates (see the Materials and Methods section). This led to the conclusion that the cloned sulfonamide resistance determinant as well as the amplified \textit{folP} gene from the sulfonamide-resistant strain are coding for the chromosomal DHPS enzyme of \textit{C. jejuni}. The G+C content of the \textit{folP} gene that was detected (about 30%) is also in agreement with the low G+C content of \textit{Campylobacter} species. The promoter sequences (−35 and −10 sequences) detected upstream of the \textit{folP} gene were also found to closely resemble the sequence of the \(\sigma^{70}\) promoter, which is known to be the main sigma factor involved in the transcription of the housekeeping genes (14, 30). It seems likely that \(\sigma^{70}\) promoters of the housekeeping genes of \textit{C. jejuni} will function in \textit{E. coli}, as indicated previously (4).

ACKNOWLEDGMENTS

We are grateful to Carl Pålthson and Eva Jøgjren for kindly providing the clinical isolates of \textit{C. jejuni} used in this study. We also thank students Leena Sahlström and Monica Johansson for help with the sequence determinations, Maria Ohagen for transforming the \textit{folP} gene into the \textit{folP} knockout mutant, and Tina Olsson and Rikard Pehrson for carrying out enzyme assays.

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. Bertani, G. 1951. Studies on lysogeny. I. The mode of phage liberation by lysogenic \textit{Escherichia coli}. J. Bacteriol. 62:293–300.
3. Brown, G. M. 1962. The biosynthesis of folic acid. II. Inhibition by sulfonamides. J. Biol. Chem. 237:536–540.
4. Chan, V. L., and H. L. Bingham. 1991. Complete sequence of the \textit{Campylobacter jejuni} glyA gene encoding serine hydroxymethyltransferase. Gene 101:51–58.
5. Dagert, M., and S. D. Ehrlich. 1979. Prolonged incubation in calcium chloride improves the competence of \textit{Escherichia coli} cells. Gene 6:23–28.
6. Dallas, W. S., J. E. Gowen, P. H. Ray, M. J. Cox, and I. K. Dev. 1992. Characterized sequencing and determined expression of the \textit{dihydropteroate} synthase gene from \textit{Escherichia coli} MC4100. J. Bacteriol. 174:5961–5970.
7. Devereux, J., P. Haebeli, and O. Smithies. 1984. A comprehensive set of
sequence analysis programs for the VAX. Nucleic Acids Res. 12:387–395.
8. Fermé, C., and G. Swedberg. 1997. Adaptation to sulfonamide resistance in
Nesseria meningitidis may have required compensatory changes to retain
enzyme function: kinetic analysis of dihydropteroate synthase from N. men-
ingitidis expressed in a knockout mutant of Escherichia coli. J. Bacteriol.
179:831–837.
9. Fliegelman, R. M., R. M. Petrak, L. J. Goodman, J. Segreti, G. M. Tren-
holme, and R. L. Kaplan. 1985. Comparative in vitro activities of twelve
antimicrobial agents against Campylobacter species. Antimicrob. Agents
Chemother. 27:429–430.
10. Garcia, M. M., H. Lior, R. B. Stewart, G. M. Ruckerbauer, J. R. Trudel, and
A. Skljarevski. 1985. Isolation, characterization and serotyping of Campy-
lobacter jejuni and Campylobacter coli from slaughter cattle. Appl. Environ.
Microbiol. 49:667–672.
11. Gibreel, A., and O. Sköld. 1998. High-level resistance to trimethoprim in
clinical isolates of Campylobacter jejuni by acquisition of foreign genes (dfr1
and dfr9) expressing drug-insensitive dihydrofolate reductases. Antimicrob.
Agents Chemother. 42:3059–3064.
12. Gold, L. 1988. Post-transcriptional regulatory mechanisms in Escherichia
coli. Annu. Rev. Biochem. 57:199–233.
13. Hampele, I. C., A. D’Arcy, G. E. Dale, D. Kostrewa, J. Nielsen, C. Oefner,
Karmali, M. A., S. de Grandis, and P. C. Fleming. 1991. Antimicrobial
susceptibility of Streptococcus pneumoniae (UA580). Microbiology
144:2177–2183.
14. Helman, J. D., and M. J. Chamberlin. 1988. Structure and function of
bacterial sigma factors. Annu. Rev. Biochem. 57:839–872.
15. Huovinen, P., L. Sundström, G. Swedberg, and O. Sköld. 1995. Tri-
methoprim and sulfonamite resistance. Antimicrob. Agents Chemother. 39:
279–289.
16. Karlyshev, A. V., J. Henderson, J. M. Ketley, and B. W. Wren. 1998. An
improved physical and genetic map of Campylobacter jejuni NCTC 11608
(UA580). Microbiology 144:503–508.
17. Karmali, M. A., S. de Grandis, and P. C. Fleming. 1981. Antimicrobial
susceptibility of Campylobacter jejuni with special reference to resistance
patterns of Canadian isolates. Antimicrob. Agents Chemother. 19:593–597.
18. Larivière, L., A. C. L. Gaudreau, and F. F. Turgeon. 1986. Susceptibility of
clinical isolates of Campylobacter jejuni to twenty-five antimicrobial agents. J.
Antimicrob. Chemother. 18:681–685.
19. Lineweaver, H., and D. Burk. 1934. The determination of enzyme dissocia-
tion constants. J. Am. Chem. Soc. 56:558.
20. Lopez, P., M. Espinosa, B. Greenberg, and S. A. Lacks. 1987. Sulfonamide
resistance in Streptococcus pneumoniae: DNA sequence of the gene encoding
dihydropteroate synthase and characterization of the enzyme. J. Bacteriol.
169:4320–4326.
21. Müller-Hill, B., L. Crapo, and W. Gilbert. 1968. Mutants that make more lac
repressor. Proc. Natl. Acad. Sci. USA 59:1259–1264.
22. National Committee for Clinical Laboratory Standards. 1981. Performance
standards for antimicrobial disk susceptibility tests, vol. 1. p. 141–156. Ap-
proved standard. National Committee for Clinical Laboratory Standards,
Wayne, Pa.
23. Öfverstedt, L. G., K. Hammarström, N. Balgobin, S. Hjertén, U. Pettersson,
and J. Chattopadyaya. 1984. Rapid and quantitative recovery of DNA frag-
ments from gels by displacement electrophoresis (isotachophoresis). Bio-
chem. Biophys. Acta 782:120–126.
24. Pata, M. L., and G. M. Brown. 1963. Mechanisms of resistance of Escherichia
coli to sulfonamides. Arch. Biochem. Biophys. 103:443–448.