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NOTES

Emergence of the Trimethoprim Resistance Gene *dfrD* in *Listeria monocytogenes* BM4293

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The sequence of the trimethoprim resistance gene of the 3.7-kb plasmid (pIP823) that confers high-level resistance (MIC, 1,024 μg/ml) to *Listeria monocytogenes* BM4293 was determined. The gene was identical to *dfrD* recently detected in *Staphylococcus haemolyticus* MUR313. The corresponding protein, S2DHFR, represents the second class of high-level trimethoprim-resistant dihydrofolate reductase identified in gram-positive bacteria. We propose that trimethoprim resistance in *L. monocytogenes* BM4293 could originate in staphylococci.

Listeria monocytogenes is a gram-positive pathogen responsible for severe food-borne infections which can lead to spontaneous abortion in pregnant women and to meningitis, meningoencephalitis, and septicemia primarily in newborns, immunocompromised patients, and elderly people (12). First-choice treatment of listeriosis generally consists of ampicillin combined with gentamicin or of co-trimoxazole (7, 11). Since 1988, more than 80 strains of *L. monocytogenes* resistant to one or more antibiotics have been isolated from food, the environment, or patients with sporadic cases of listeriosis (2).

Dihydrofolate reductase (DHFR) is a key enzyme in the tetrahydrofolic pathway, in which it catalyzes the NADPH-dependent reduction of dihydrofolate to tetrahydrofolate (10). Due to structural analogy with dihydrofolate, trimethoprim is a competitive inhibitor of this enzyme in bacteria (10). The most common mechanism of resistance to trimethoprim is plasmid-mediated production of an additional trimethoprim-resistant DHFR which can function in place of the susceptible host chromosomal enzyme. In gram-negative bacteria, a minimum of 17 different plasmid-encoded DHFRs have been described (10). By contrast, only two types of DHFR that confer resistance to trimethoprim have been described in gram-positive bacteria. The type S1 enzyme encoded by the *dfrA* gene located in transposon Tn*4003* has been found in *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Staphylococcus epidermidis*, and *Staphylococcus hominis* (3, 13), and recently, the type S2 DHFR encoded by *dfrD* carried by plasmid pABU17 has been detected in *S. haemolyticus* MUR313 (4).

In a previous study, we described the first strain of *L. monocytogenes* (strain BM4293) resistant to high levels of trimethoprim (MIC, 1,024 μg/ml). This strain, isolated from the environment in France, harbors plasmid pIP823, of 3.7 kb, which is responsible for the resistance (2). Since the resistance determinant did not hybridize with a *dfrA*-specific probe (2), we decided to clone and sequence this new gene.

(Part of this work was presented at the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy [1].)

Plasmid pIP823 DNA partially digested with *Sau*3A and pUC18 DNA digested with *Bam*HI were mixed, ligated, and introduced by transformation into *Escherichia coli* DH5α (Gibco BRL, Eragny, France). Cloning was performed with restriction endonucleases (Pharmacia Biotech, Saclay, France), T4 DNA ligase (Pharmacia), and alkaline phosphatase (Pharmacia) by standard methods (14). Transformants were selected on Mueller-Hinton agar (Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France) containing 100 μg of ampicillin (Laboratories Panpharma, Fougères, France) per ml and 5 μg of trimethoprim (Roche, Fontenay-sous-Bois, France) per ml and were screened for their plasmid contents by agarose gel electrophoresis of crude bacterial lysates. The smallest recombinant plasmid, pAT460, was found to contain a 1.1-kb insert. *E. coli* LH18 *thyA*Δ*fol*::*kan*, from which the gene for DHFR has been deleted, is a mutant auxotroph for thymine which is thus unable to grow on Mueller-Hinton agar (9). This strain, after acquisition of pAT460, was able to grow on Mueller-Hinton plates, whereas the same host containing pUC18 was not. This *trans*-complementation assay indicated that pAT460, which conferred resistance to the new host, encodes a functional DHFR.

The nucleotide sequence of both strands of the pAT460 insert was determined by the dideoxynucleotide chain-termination method (15) with T7-modified DNA polymerase (Sequenase; United States Biochemicals, Cleveland, Ohio), [α-^{35}S]dATP (Amersham France, Les Ulis, France), and oligonucleotides complementary to the sequence synthesized by the methoxy phosphoamidite method (Unité de Chimie Organique, Institut Pasteur, Paris, France). This sequence has been submitted to the GenBank database (accession number U43152). A search for stop codons in the three reading frames of each DNA strand revealed the presence of a single open reading frame. Two putative translational initiation codons, ATG at coordinate 548 and TTG at coordinate 560, preceded by a typical Shine-Dalgarno sequence were identified. The

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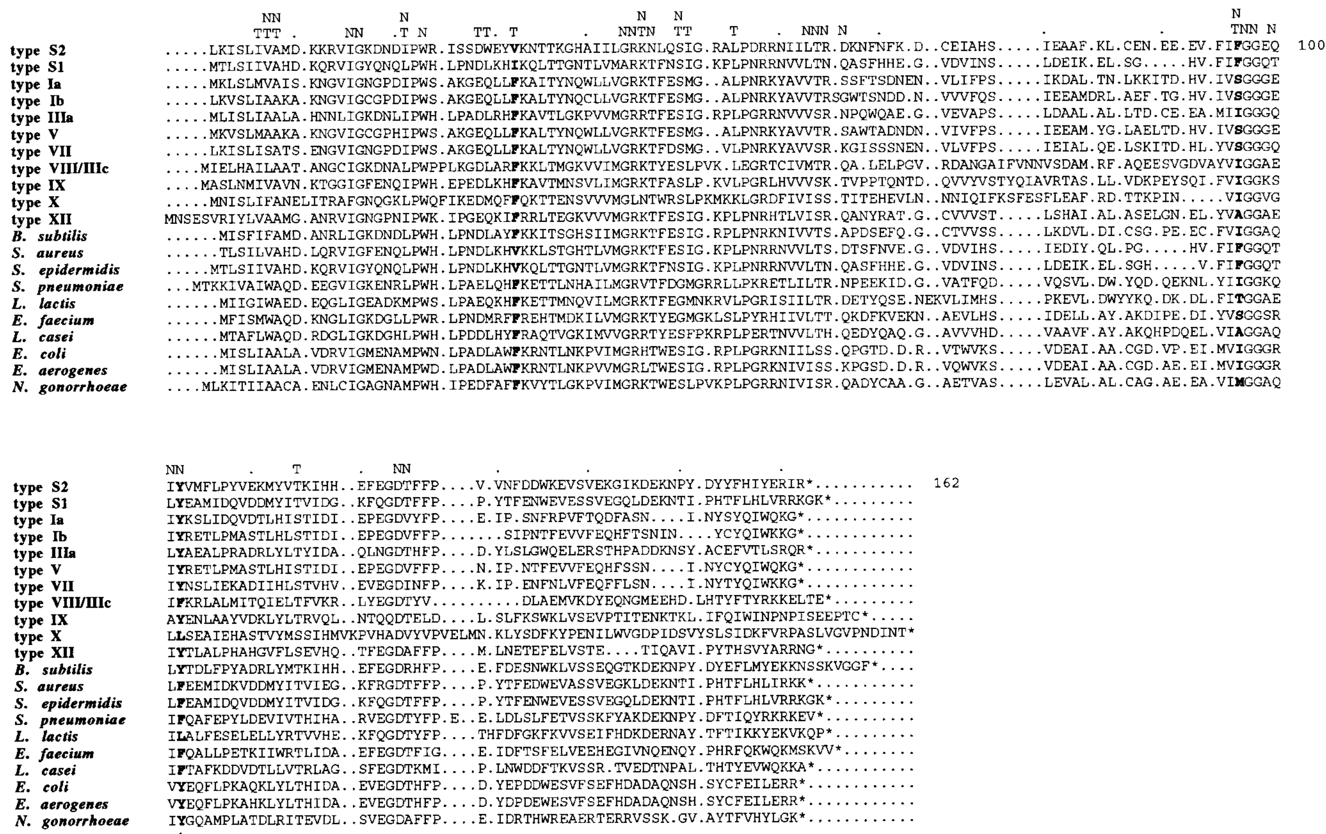

FIG. 1. Alignment of the deduced amino acid sequences of S2DHFR from *L. monocytogenes* BM4293, additional bacterial trimethoprim-resistant DHFRs (types S1 to XII), and prokaryotic chromosomal DHFRs. The sequence numbering is based on that of S2DHFR. The stop codons are indicated by asterisks. The amino acids at positions 32, 96, and 102 in S2DHFR are indicated in boldface type. The amino acid positions involved in the binding of trimethoprim (T) and NADPH cofactor (N), based on studies of the *E. coli* K-12 enzyme (3, 4), are indicated. The Swissprot and GenBank accession numbers of the additional and chromosomal DHFRs are as follows: type S1, P13355; type Ia, P00382; type IIIa, P12833; type V, P11731; type VII, P27422; *Bacillus subtilis*, P11045; *S. aureus*, P10167; *Enterococcus faecium*, P00380; *Lactobacillus casei*, P00381; *E. coli*, P00379; *Enterobacter aerogenes*, P31074; and *Neisseria gonorrhoeae*, P04174 in the SwissProt database and type Ib, Z50805; type IIIc, U09273; type VIII, U10186; type IX, X57730; type X, L06418; type XII, Z21672; *S. epidermidis*, Z48233; *Streptococcus pneumoniae*, Z74778; and *Lactococcus lactis*, X60681 in the GenBank database.

486-bp sequence from the TTG codon at coordinate 560 to the TAA codon at coordinate 1046, designated *dfrD*, could code for a protein of 162 amino acid residues with a calculated molecular mass of 19,273 Da, designated S2DHFR. The G+C content of *dfrD* (31.5%) was more similar to that of *Staphylococcus* DNA (34%) than to that of DNA from *L. monocytogenes* (38%) and *Enterococcus* (38%). The deduced amino acid sequence of type S2 DHFR encoded by pIP823 was aligned with those of known DHFRs in the SwissProt and GenBank databases by using the Genetics Computer Group program (Fig. 1) (5, 8). This sequence was found to be identical to that of the recently described S2DHFR encoded by *dfrD* of pABU17 from *S. haemolyticus* MUR313 (4). The two enzymes differ by their number of amino acid residues since the ATG translational initiation codon was chosen for the S2DHFR from *S. haemolyticus*. On the basis of the structural and kinetic properties, the active site of S2DHFR is very closely related to that of the resistant and susceptible DHFRs already described in staphylococci (3, 4, 13). The S2DHFR was more distantly related to the S1DHFR from *S. aureus*, with 38.1% amino acid identity, and it showed less similarity with trimethoprim-resistant DHFRs from gram-negative bacteria (from 22.2% with type X DHFR to 37.7% with type IIIa DHFR).

In contrast to plasmid pABU17 (3.8 kb) from *S. haemolyticus*, plasmid pIP823 (3.7 kb) from *L. monocytogenes* does not possess a *Bam*HI site, and the three *Eco*RI sites present in the two plasmids yield restriction fragments of different sizes (Fig. 2). These plasmids therefore appear to be different. However, the 81-bp sequence upstream and the 203-bp sequence downstream from *dfrD* are identical in pIP823 and pABU17 (data not shown). Plasmid pIP823 is able to replicate and is stable in *E. coli* HB101 and *S. aureus* RN4220, where it confers high-level trimethoprim resistance (2; data not shown). It is therefore likely that pIP823 belongs to the family of rolling-circle replicating plasmids, common in staphylococci, that possess a broad host spectrum including gram-positive and gram-negative bacteria (2, 6).

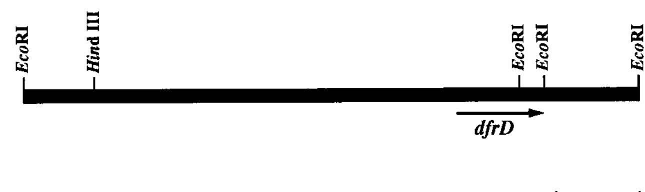

FIG. 2. Map of 3.7-kb plasmid pIP823 from *L. monocytogenes* BM4293. Only relevant restriction sites are indicated. The open arrow represents the direction and extent of translation of *dfrD*. The bar at the bottom represents 500 bp.

Our results suggest that the trimethoprim resistance gene *dfrD* from *L. monocytogenes* BM4293 could originate in the genus *Staphylococcus*. However, it would be interesting to characterize the trimethoprim-susceptible chromosomal *dfr* gene of *L. monocytogenes* to obtain additional information on the origin of *dfrD* and on the divergence and the evolution of DHFRs from gram-positive bacteria. The emergence of trimethoprim resistance in *L. monocytogenes* is of particular interest since the trimethoprim-sulfamethoxazole combination is a successful alternative treatment for human listeriosis (7). Dissemination of plasmid pIP823 from BM4293 to other strains of *L. monocytogenes* and to other species of *Listeria* is likely. Acquisition by *L. monocytogenes* of other resistance genes carried by this family of plasmids can also be anticipated.

Nucleotide sequence accession number. The nucleotide sequence of the pAT460 insert has been submitted to the GenBank database and has been given accession number U43152.

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