JOURNAL OF BACTERIOLOGY, Oct. 2001, p. 6148–6149 Vol. 183, No. 20
0021-9193/01/$04.00+0 DOI: 10.1128/JB.183.20.6148–6149.2001

Reconstruction of *Escherichia coli mrcA* (PBP 1a) Mutants Lacking Multiple Combinations of Penicillin Binding Proteins

BERNADETTE M. MEBERG, FRANCES C. SAILER, DAVID E. NELSON,† AND KEVIN D. YOUNG*

Department of Microbiology and Immunology, University of North Dakota School of Medicine, Grand Forks, North Dakota 58202-9037

Received 9 July 2001 /Accepted 2 August 2001

Previously, we constructed a set of mutants from which eight penicillin binding protein (PBP) genes were deleted in 192 combinations from *Escherichia coli* (S. A. Denome, P. K. Elf, T. A. Henderson, D. E. Nelson, and K. D. Young, J. Bacteriol. 181:3981-3993, 1999). Although these mutants were constructed correctly as determined by restriction mapping and the absence of relevant protein products, we recently discovered by PCR mapping that strains from which *mrcA* (PBP 1a) was deleted were also missing two neighboring genes of unknown function (*yrfE* and *yrfF*). We created a new deletion mutation in *mrcA* and reconstructed 63 strains lacking PBP 1a and other PBP mutant combinations. The new *mrcA* mutants do not exhibit mucoidy, phage resistance, temperature sensitivity, growth rate defects, or antibiotic resistance, suggesting that these phenotypes require the loss of either *yrfE* or *yrfF* alone or in combination with the absence of multiple PBPs.

Four high-molecular-weight penicillin binding proteins (PBPs) of *Escherichia coli* (PBPs 1a, 1b, 2, and 3) are responsible for synthesizing and assembling the peptidoglycan sacculus that forms the rigid bacterial cell wall (5, 6). However, *E. coli* also possesses at least seven low-molecular-weight PBPs (PBPs 4, 5, 6, and 7 and DacD, AmpC, and AmpH), the biological functions of which are either poorly characterized or completely unknown (2, 5, 6).

To address this question of physiological function, we constructed a set of multiply mutated strains in which one to seven PBPs were deleted in every viable combination (2). At the time of construction, each strain was tested by restriction mapping and by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to confirm that the correct genes and protein products had been deleted. Recently, we found we were unable to PCR amplify the mutated *mrcA* gene (encoding PBP 1a) from chromosomal preparations when using oligonucleotide primers hybridizing to sequences just upstream and downstream of the putative deletion endpoints. Primers further away from the mutated site did give an amplification product (data not shown), suggesting that a larger fragment had been deleted than was reported previously. DNA sequencing confirmed that one open reading frame (ORF) (*yrfE*) and the promoter and 5′ end of a second open reading frame (*yrfF*) were deleted in addition to most of the *mrcA* gene (data not shown). The extent of the deletion is pictured schematically in Fig. 1B. Thus, every strain designated as Δ*mrcA* in our previous publication (2) is actually a Δ(*mrcA-yrfE-yrfF*) deletion. All other PBP gene deletions were correct as reported (data not shown).

The data in Fig. 1 illustrates why the deletion was not correctly characterized earlier. When creating the original *mrcA* mutation we observed a single *Xho*I-*Bsp*DI DNA fragment, leading us to believe that one *Bsp*DI site existed in the cloned segment. However, after the mutants were constructed, the complete genomic sequence of *E. coli* (1) revealed there were three *Bsp*DI sites—two so close together that they could not be distinguished as separate sites and the third in the *yrfF* gene. Because the lengths of the two *Xho*I-*Bsp*DI and *Bsp*DI-*Bsp*DI fragments are almost identical, they appeared as a single band on our gels. Thus, our original digestions actually created a deletion from the *Xho*I site in *mrcA* to the *Bsp*DI site in *yrfF* (Fig. 1B).

To correct this situation, we deleted *mrcA* by using the λ

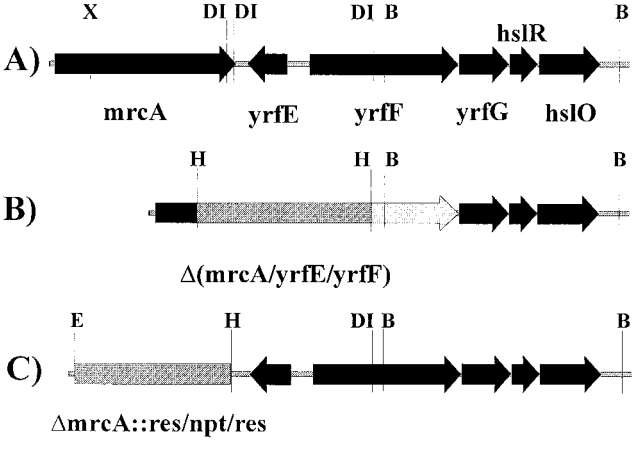

FIG. 1. Deletions of *mrcA* and neighboring genes in *E. coli*. (A)Gene order downstream of *mrcA* in the parental strain, *E. coli* CS109. (B) Extent of deletion created in *E. coli* CS13-2K, previously reported to encompass only *mrcA* (2). The segment from the *Xho*I site in *mrcA* to the *Bsp*DI site in *yrfF* was replaced by the *res-npt-res* cassette (4). (C) Extent of the new *mrcA* deletion in *E. coli* BMCS04-1K reported in this work. The entire *mrcA* gene from the initiation codon to the termination codon was replaced by the *res-npt-res* cassette (4). Abbreviations: B, *Bam*HI; E, *Eco*RI; DI, *Bsp*DI; H, *Hin*dIII; X, *Xho*I.

* Corresponding author. Mailing address: Department of Microbiology and Immunology, School of Medicine, University of North Dakota, Grand Forks, ND 58202-4037. Phone: (701) 777-2624. Fax: (701) 777-2054. E-mail: kyoung@medicine.nodak.edu.

† Present address: Division of Pulmonary, Allergy, Critical Care and Occupational Medicine, Indiana University School of Medicine, Indianapolis, IN 46202.

TABLE 1. New *mrcA* (PBP 1a) mutants[a]

Strain	PBPs Deleted	Parent	Strain	PBPs Deleted	Parent	Strain	PBPs Deleted	Parent
CS229-1K	1a 7	CS9-19	CS456-1K	1a 4 5 7	CS315-1	CS541-1K	1a 4 7 H C	CS441-1
CS230-1K	1a H	CS15-3	CS457-1K	1a 4 6 7	CS316-1	CS542-1K	1a 4 5 6 H	CS442-3
CS231-1K	1a 6	CS17-1	CS458-1K	1a 4 7 H	CS317-3	CS543-1K	1a 5 6 7 H	CS443-3
CS232-1K	1a 5	CS12-7	CS459-1K	1a 4 7 C	CS318-1	CS544-1K	1a 4 5 6 C	CS445-1
CS233-1K	1a 4	CS11-2	CS460-1K	1a 4 C H	CS320-1	CS545-1K	1a 4 5 6 7	CS446-1
CS234-1K	1a C	CS14-2	CS461-1K	1a 4 5 6	CS322-1	CS546-1K	1a 4 6 7 C	CS447-1
CS367-1K	1a 4 7	CS203-1B	CS462-1K	1a 4 6 H	CS323-3	CS547-1K	1a 4 5 7 H	CS448-3
CS368-1K	1a 4 H	CS221-3	CS463-1K	1a 4 6 C	CS324-1	CS548-1K	1a 4 5 7 C	CS449-2
CS369-1K	1a 4 5	CS219-1	CS464-1K	1a 4 5 H	CS326-1	CS549-1K	1a 5 6 7 C	CS450-1
CS370-1K	1a 4 6	CS220-1	CS465-1K	1a 4 5 C	CS327-1	CS550-1K	1a 5 7 C H	CS452-2
CS375-1K	1a 6 7	CS205-1	CS466-1K	1a 5 6 7	CS331-1	CS551-1K	1a 4 6 C H	CS453-1
CS376-1K	1a 6 H	CS213-1	CS467-1K	1a 6 7 H	CS332-3	CS552-1K	1a 4 5 C H	CS454-1
CS377-1K	1a 5 7	CS204-1	CS468-1K	1a 6 C H	CS334-1	CS553-1K	1a 6 7 C H	CS455-1
CS378-1K	1a 5 H	CS215-3	CS469-1K	1a 5 6 H	CS336-3	CS554-1K	1a 5 6 C H	CS470-1
CS379-1K	1a 7 H	CS206-3	CS470-1K	1a 5 6 C	CS337-1	CS614-1K	1a 4 5 6 7 H	CS531-3
CS380-1K	1a 4 C	CS222-1	CS471-1K	1a 7 C H	CS342-1	CS615-1K	1a 4 5 6 7 C	CS533-1
CS381-1K	1a 5 C	CS216-2	CS472-1K	1a 6 7 C	CS343-1	CS616-1K	1a 4 5 7 C H	CS534-1
CS382-1K	1a 6 C	CS213-1	CS473-1K	1a 5 7 H	CS345-3	CS617-1K	1a 4 6 7 C H	CS535-1
CS383-1K	1a 7 C	CS207-2	CS474-1K	1a 5 7 C	CS346-1	CS618-1K	1a 5 6 7 C H	CS536-1
CS384-1K	1a C H	CS209-1	CS475-1K	1a 5 C H	CS349-1	CS619-1K	1a 4 5 6 C H	CS539-1
CS385-1K	1a 5 6	CS211-2	CS540-1K	1a 4 6 7 H	CS440-3	CS703-1K	1a 4 5 6 7 C H	CS612-1

[a] The parental strain from which individual and multiple PBP genes were deleted was *E. coli* CS109 (W1485 *rpoS rph*). Each mutant ("Strain" columns) was created by P1 transduction of *mrcA*::*res-npt-res* from BMCS04-1K (CS109 *mrcA*::*res-npt-res*) into the respective parental strain ("Parent" columns) and selection for kanamycin resistance. The parental strains are described by Denome et al (2). PBPs deleted from individual *E. coli* strains are abbreviated as follows (PBP, followed by gene name): 1a, = PBP 1a, *mrcA*; 4 = PBP 4, *dacB*; 5 = PBP 5, *dacA*; 6 = PBP 6, *dacC*; 7 = PBP 7, *pbpG*; C = AmpC, *ampC*; H = AmpH, *ampH*.

recombination system described by Yu et al. (8). The *res-npt-res* cassette of plasmid pCK155 (4) was amplified by PCR using two primers homologous to each end of the cassette and containing at their 5′ ends chromosomal sequences homologous to those preceding the AUG start codon of *mrcA* or sequences following the UGA stop codon of *mrcA*. The primer sequences were, respectively, ACCGCGCGTTTGTTTATAAACTGCC CAAATGAAACTAAATGGAATTCGAGCTCTGCAGTCCC and CACTTTGTCAGCAAACTGAAAAGGCGCCGAAGC GCCTTTTTAAGATAAGCTTGCATGCCTGCAG. The resulting PCR product was electroporated into *E. coli* DY329 (8), and the cells were plated onto Luria-Bertani agar plates plus kanamycin (50 μg/ml) and incubated for 2 days at 32°C. Kanamycin-resistant colonies were screened for the correct mutation by PCR amplification, and the new *mrcA* deletion was confirmed by PCR amplification with combinations of internal and external primers and by SDS-PAGE of ^{125}I-labeled PBPs (3) (data not shown). The mutation was moved into *E. coli* CS109 by P1 transduction to form strain BMCS04-1K. The extent of this new *mrcA* deletion is pictured schematically in Fig. 1C.

The new Δ*mrcA*::*res-npt-res* mutation was moved into selected *E. coli* strains to recreate the set of multiple mutants lacking PBP 1a in combination with every possible combination of six other PBPs (Table 1). Therefore, these strains are replacements for and should be used instead of the *mrcA* mutants described previously (2).

After screening the original *mrcA* mutants (now known to be Δ*mrcA-yrfEF*) (2), we reported preliminary observations that deletion of PBP 1a alone or in combination with other PBP mutations resulted in expression of a colanic acid capsule, phage resistance, temperature sensitivity, and resistance to lysis by certain β-lactams (7). The new mutant combinations described in this work exhibited none of these phenotypes (data not shown), suggesting that loss of either *yrfE* or *yrfF* alone or in combination with the absence of multiple PBPs is responsible for these characteristics. We are currently examining the relationship among these genes and phenotypes.

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