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Expression in *Escherichia coli* of a Staphylococcal Gene for Resistance to Macrolide, Lincosamide, and Streptogramin Type B Antibiotics

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Plasmid pBD9, which comprises two plasmids from *Staphylococcus aureus*, pE194 and pUB110, was joined to plasmid pBR322 by in vitro recombination to form plasmid pKH80. The *ermC* gene of plasmid pE194 confers inducible resistance to macrolide, lincosamide, and streptogramin type B antibiotics. When pKH80 was transferred to *Escherichia coli* K-12, the bacteria became resistant to several of these antibiotics.

Plasmid pE194 of *Staphylococcus aureus* has a molecular weight of 2.3×10^6 and confers resistance to macrolide, lincosamide, and streptogramin B (MLS) antibiotics (14, 24). Resistance is induced, in both *S. aureus* and *Bacillus subtilis*, by subinhibitory concentrations (10 to 100 nM) of the macrolide antibiotic erythromycin (24). This antibiotic induces the synthesis of an RNA methylase (molecular weight, 29,000) encoded by the *ermC* gene (19). The methylase brings about a specific N^6, N^6 dimethylation of adenine in 23S rRNA (24) which is responsible for the resistant phenotype (16). The nucleotide sequence of the *ermC* gene (and of adjacent DNA) has been determined, and a model for post-transcriptional induction by erythromycin has been proposed (10, 13).

Several drug resistance genes on plasmids originally found in *S. aureus* or in *B. subtilis* are expressed in *Escherichia coli*. Examples include genes conferring resistance to tetracycline, penicillin, or kanamycin (6, 7, 18). In contrast, genes found on plasmids from gram-negative bacteria, such as the pBR322 gene which confers resistance to penicillin, are not expressed in *B. subtilis* (7, 9).

Plasmid-specified resistance to MLS antibiotics among natural isolates of members of the *Enterobacteriaceae* has not been reported, although Malke and Holm (16) recently found that a streptococcal plasmid confers erythromycin and lincomycin resistance in *E. coli*. A plasmid specifying MLS resistance was found in a *Bacteroides fragilis* strain by Welch et al. (25); this is the only report so far of such a plasmid in a natural isolate of a gram-negative bacterium. Gram-negative bacteria are inherently more resistant than gram-positive bacteria to MLS antibiotics. This may be because they are more impermeable to the antibiotics (22), and it has been suggested that the presence of N^6-monomethyl adenine in the 23S rRNA of gram-negative bacteria might also protect them against MLS antibiotics (21).

The work reported here shows that the *ermC* gene of pE194 is expressed in *E. coli*. This gene is of particular interest because of its novel induction mechanism (10, 13) and because it can be used to promote expression in *B. subtilis* of cloned DNA (12). Expression of the *ermC* gene in *E. coli* thus allows the detection of *E. coli* transformants having plasmids with inserts which inactivate the *ermC* gene. It is often convenient to obtain recombinants in *E. coli* and then to transfer them into *B. subtilis*.

To determine whether the *ermC* gene is expressed in *E. coli*, pBD9 (9, 11) and pBR322 (2) were ligated together to form plasmid pKH80 (12). Plasmid pBD9 is composed of two staphylococcal plasmids, pE194 and pUB110, and confers resistance to MLS antibiotics and to kanamycin in *B. subtilis*. Plasmids pBD9 and pBR322 were cleaved at their single *Eco*RI-sensitive sites and were ligated, by using T4 DNA ligase, under conditions previously described (1). Recombinant plasmids were isolated from the ligation mixture by transforming (4) *E. coli* K-12 strain HB101 (3) and selecting for transformants growing on plates of L-agar containing ampicillin (40 μg/ml), tetracycline (25 μg/ml), and kanamycin (5 μg/ml).

E. coli K-12 is inherently more resistant to erythromycin than is *B. subtilis*. Erythromycin is normally used at a concentration of 5 μg/ml to inhibit sensitive strains of *B. subtilis*. To determine whether the *ermC* gene is expressed in *E. coli*, erythromycin was included in L-agar plates at a concentration of 60 μg/ml. Strain

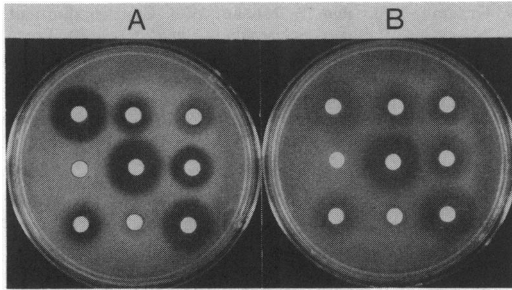

FIG. 1. Resistance of *E. coli* to MLS antibiotics. *E. coli* DB11 and DB11(pKH80) were inoculated on L-agar and tested for their response to disks containing 10 μg of antibiotic. (A) DB11; (B) DB11(pKH80). Antibiotics (left to right): top row, erythromycin, carbomycin, tylosin; middle row, chalcomycin, cirramycin, kitasamycin; bottom row, maridomycin, ostreogrycin B, clindamycin.

HB101(pKH80) grew well on plates during 24 h of incubation, whereas strain HB101 without a plasmid grew poorly. HB101 containing pKH80 which had hepatitis B viral DNA inserted at the *BclI* cleavage site within the *ermC* gene also grew poorly on L-agar plates containing erythromycin (60 μg/ml) (12).

Some growth of strain HB101 occurred on plates containing erythromycin, and the use of concentrations greater than 60 μg/ml did not permit an unequivocal distinction to be made between *E. coli* HB101 and *E. coli* HB101(pKH80). However, when *E. coli* K-12 strain DB11, of genotype *hsdR met thi-1* and phenotype Ery^s Nal^r, (a derivative of strain K802) was used as host strain, plasmids carrying an intact *ermC* gene could be unambiguously distinguished from those which did not. Strain DB11 (provided by J. Davies) was obtained by mutagenesis and screening for enhanced sensitivity to several antibiotics. It did not grow on L-agar plates containing erythromycin at 5 μg/ml after incubation for 48 h. However, strain DB11(pKH80) grew on these plates after 24 h of incubation.

MLS-resistant strains of *Staphylococcus*, *Streptococcus*, and *Streptomyces* often show the phenomenon of "dissociated resistance" (8, 23). The product of the *ermC* gene confers resistance, in both *S. aureus* and *B. subtilis*, to antibiotics of the lincosamide and streptogramin B types, as well as to various macrolides, such as erythromycin. However, not all of these MLS antibiotics can induce resistance, so unless cells are also treated with an antibiotic which induces resistance, they remain sensitive to certain MLS antibiotics. This effect can be readily detected using antibiotic-containing disks.

Plasmid pKH80 increased the resistance to *E. coli* DB11 to all nine MLS antibiotics tested (Fig. 1). These antibiotics were also tested in all possible combinations for antagonistic effects by placing disks close together on L-agar plates inoculated with strain DB11(pKH80). The only marked effect observed was the apparent antagonism of the lincosamide clindamycin (2 μg/ml) toward the effect of the macrolide erythromycin (5 μg/ml); the inhibition zone around erythromycin was reduced in size at the side closest to the clindamycin disk (Fig. 2). The opposite interaction is observed with *B. subtilis* and *S. aureus*, in which erythromycin apparently antagonizes the effect of clindamycin (20). The apparent antagonism indicates that the *ermC* gene in *E. coli* may be more efficiently induced by clindamycin than by erythromycin. Possible reasons for the different abilities of MLS antibiotics to induce genes such as *ermC* have been discussed by Fujisawa and Weisblum (8). These include different effects of antibiotics on the induction process itself and differences in antibiotic transport or in ribosome structure. It is not known which of these may be responsible for the opposite effects of clindamycin and erythromycin on *B. subtilis* and *E. coli*. High-copy-number mutants of pE194 express MLS resistance constitutively in *B. subtilis* (24). It is possible that the high copy number of the pBR322 derivatives used here might also result in some constitutive expression of the *ermC* gene in *E. coli*.

To determine whether a protein corresponding to the *ermC* gene product is synthesized in *E. coli*, polypeptides synthesized by *E. coli* minicells (produced by strain DS410 [5]) carrying pKH80 were examined. Three pBD9-specified proteins were labeled in significant amounts in minicells of *B. subtilis* CU403 (18a). These had molecular weights of 38,000, 27,500, and 13,500; they presumably correspond to proteins K2, E3

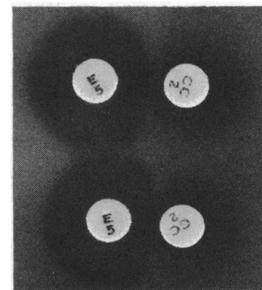

FIG. 2. Interaction between erythromycin and clindamycin. The distorted inhibition zone surrounding the disks containing 5 μg of erythromycin (left) is caused by the disks containing clindamycin (2 μg). This indicates that clindamycin, which diffuses from the disks on the right, induces resistance to erythromycin.

(the product of the *ermC* gene), and E6 (or K5), respectively, which have been detected by Shivakumar et al. (20) in pBD9-containing minicells of *B. subtilis* CU403. A protein of molecular weight 27,500 was also labeled in minicells prepared from cultures of strain DS410(pKH80) which had been induced with erythromycin (5 μg/ml). The 27,500-dalton protein was absent from extracts of minicells produced by strain DS410(pBR322) and from uninduced cultures of strain DS410(pKH80), indicating that the 27,500-dalton protein seen in extracts of erythromycin-induced DS410 (pKH80) is the *ermC* gene product. However, although the 27,500-dalton protein was invariably seen in extracts of strain DS410(pKH80), it always occurred as a faint band, indicating that little of the protein was present in *E. coli*, even after induction. Numerous polypeptides having molecular weights of less than about 20,000 were always seen in extracts of DS410(pKH80) minicells. These were never found in extracts of minicells produced by strain DS410(pBR322) or strain CU403(pBD9), suggesting that some of the polypeptides specified by pBD9 might be more unstable in *E. coli* than in *B. subtilis*.

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