SHORT COMMUNICATION

Construction of a Mutant of Escherichia coli That Has Deletions of Both the Penicillin-binding Protein 5 and 6 Genes

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A mutant of Escherichia coli has been constructed with deletions of the genes encoding penicillin-binding protein 5 (dacA) and penicillin-binding protein 6 (dac). The construction of this mutant establishes that the complete loss of the two most abundant species of penicillin-binding proteins can be tolerated by E. coli. Moreover, the double deletion mutant had the same growth rate and morphology as an isogenic dacA+ dacC+ strain.

INTRODUCTION

The penicillin-binding proteins (PBPs) of Escherichia coli catalyse the final stages of peptidoglycan biosynthesis (reviewed by Spratt, 1983). The high molecular weight PBPs 1A/1B, 2 and 3 are essential enzymes that catalyse both the polymerization and cross-linking of peptidoglycan precursors (Spratt, 1975; Nakagawa et al., 1979; Suzuki et al., 1980; Matsuhashi et al., 1982). The low molecular weight PBPs 5 and 6 are the two most abundant species of E. coli PBPs and they both possess D-alanine carboxypeptidase activity (Tamura et al., 1976; Amanuma & Strominger, 1980). PBPs 5 and 6 are known to be dispensable to E. coli, since a dacA deletion mutant (Spratt, 1980) and a dacC deletion mutant (Broome-Smith & Spratt, 1982) have been constructed. PBPs 5 and 6 are very similar in amino acid sequence (I. Ioannides, J. Broome-Smith & B. G. Spratt, unpublished), so it is possible that they perform such similar roles in vivo that the absence of one of them is tolerated because the other provides a compensatory function necessary for peptidoglycan biosynthesis. I describe here the construction of a strain of E. coli which totally lacks PBPs 5 and 6 due to deletions of both the dacA and dacC genes, and conclude that the absence of these two low molecular weight PBPs is not lethal to E. coli under laboratory conditions.

METHODS

Bacterial strains and bacteriophage. The strains of E. coli K12 used were JBS200 his supF srl::Tn10 recA56, JBS1001 his supF srl::Tn10 recA56 dacC1 (Broome-Smith & Spratt, 1982) and MM383 thy rha lac rpsL polA(ts)12 provided by Dr P. Meacock, Leicester Biocentre, University of Leicester, UK.

The bacteriophage used were P1 vir and λprecA (McEntee, 1976).

Media and growth conditions. L broth, containing 10 g tryptone, 5 g yeast extract, 5 g NaCl and 1 g glucose per litre, was used for the growth of liquid cultures. L broth was solidified with 1.2% (w/v) agar to make L agar. Bacteria carrying plasmids were selected on L agar plates containing kanamycin (Km), 25 µg ml⁻¹, or ampicillin (Ap), 25 µg ml⁻¹.

Genetic techniques. P1 transduction was done by standard methods (Miller, 1972). Strains were lysogenized with λprecA at 30 °C. Bacteria that had lost the thermoinducible prophage were identified as λ-sensitive survivors on L agar at 42 °C.

Abbreviation: PBP, penicillin-binding protein.
Isolation, manipulation and transformation of plasmid DNA. These techniques have been described previously (Stoker et al., 1982).

Assay of PBPs. PBPs were assayed in whole cells using [3H]benzylpenicillin [27 Ci (999 GBq) mmol\(^{-1}\); kindly provided by Dr P. Cassidy of Merck, Sharp and Dohme] as described previously (Broome-Smith & Spratt, 1982).

RESULTS

Construction of a \(\Delta dacA::\text{Km}^r\) mutant

The construction of a plasmid containing an inactive PBP 5 gene, in which an internal fragment of the gene was replaced by a kanamycin resistance cassette, is outlined in Fig. 1. Plasmid pJBS611 was constructed by cloning a \(\text{BamHI} - \text{EcoRI}\) fragment carrying the \(\text{dacA11191}\) allele (Broome-Smith & Spratt, 1984) into pAT153 (Twigg & Sherratt, 1980) digested with \(\text{BamHI}\) and \(\text{EcoRI}\). Plasmid pJBS611 was then digested with \(\text{PvuII}\) and \(\text{SmaI}\), and the largest fragment, which lacks the portion of the \(\text{dacA}\) gene that encodes amino acids 10 to 195, was purified. The purified fragment was ligated to pUC4K (Vieira & Messing, 1982) that had been digested with \(\text{HincII}\), yielding the plasmid pJBS612 in which the kanamycin resistance gene of pUC4K had replaced part of the \(\text{dacA}\) coding region.

To exchange the chromosomal \(\text{dacA}\) gene for the \(\Delta dacA::\text{Km}^r\) allele, MM383(pJBS612) was grown overnight in L broth at 30 °C and then diluted into pre-warmed L broth and grown for 20 generations at 43 °C. At this temperature replication of the plasmid pJBS612 is inhibited and plasmid-free cells segregate. Cells that retained kanamycin resistance were selected at 30 °C, and these were further tested for their ability to grow in the presence of ampicillin (25 µg ml\(^{-1}\)).
Of the kanamycin resistant isolates, 1% failed to grow in the presence of ampicillin, suggesting that they were plasmid-free cells in which reciprocal recombination between the chromosomal dacA gene and the ΔdacA::Km allele of pJBS612 had preceded loss of the plasmid. PBP assays were done on two such isolates and they were found to lack PBP 5 activity completely (data not shown).

Construction of a ΔdacA::KmΔdacC mutant

The construction of a ΔdacC mutant, JBS1001, was previously reported (Broome-Smith & Spratt, 1982). This strain, and the isogenic parent JBS200, were lysogenized with λprecA (McEntee, 1976) so that they could serve as recipients for P1-mediated transduction. P1 phage were grown on MM383 ΔdacA::Km and the resulting lysate was used to transduce JBS1001(λprecA) and JBS200(λprecA) to kanamycin resistance: the transductants were obtained at similar frequencies. A kanamycin resistant transductant of each strain was cured of the prophage, yielding JBS1002 and JBS202 respectively. PBP assays were done on these derivatives, and on their isogenic parents, and, as shown in Fig. 2, this confirmed that JBS1002 lacked both PBP 5 and PBP 6, whilst JBS202 lacked PBP 5.

Properties of the ΔdacA::KmΔdacC mutant

The growth rate, morphology and β-lactam sensitivity of the ΔdacA::KmΔdacC mutant, JBS1002, and of the three isogenic strains JBS200(dacA+ dacC+), JBS202(ΔdacA::Km· dacC+), and JBS1001(dacA+ ΔdacC) were examined. JBS1002 grew with a mean generation time of 32 min in L broth at 37 °C and identical doubling times were found for each of the three isogenic strains. All four strains grew as rod-shaped cells with indistinguishable morphologies. Mutants that lack PBP 5 activity show slightly increased sensitivity to β-lactam antibiotics, whilst the absence of PBP 6 has virtually no effect (Spratt, 1980; Broome-Smith & Spratt, 1982). The minimum inhibitory concentration of ampicillin that abolished colony-forming ability on agar was 0.8 μg ml⁻¹ for JBS200(dacA+ dacC+) and JBS1001(dacA+ ΔdacC) whereas it was 0.4 μg ml⁻¹ for both JBS202(ΔdacA::Km· dacC+) and the double mutant JBS1002.

DISCUSSION

The chromosomal dacA gene of E. coli has been inactivated by replacing the region encoding amino acids 10 to 195 of PBP 5 with a kanamycin resistance cassette. Since this region includes the active site serine residue (serine-44; Broome-Smith et al., 1983; Glauner et al., 1984) the mutant is totally defective in PBP 5 activity. Furthermore, the insertion of the kanamycin resistance cassette facilitates the transfer of the ΔdacA mutation to other strains as it is rendered 100% cotransducible with kanamycin resistance.

JBS1002(ΔdacA::Km· ΔdacC) was constructed by transducing the ΔdacA::Km· mutation into a ΔdacC mutant. Kanamycin resistant transductants were obtained at the same frequency as when an isogenic dacC+ recipient was used, so the double mutant does not exhibit reduced viability (compared to the mutant that only lacks PBP 5 activity).

The construction of a ΔdacA ΔdacC mutant demonstrates that the absence of the two most abundant species of penicillin-binding proteins (PBPs 5 and 6) can be tolerated by E. coli cells, at least under laboratory conditions. However, in addition to PBPs 5 and 6, PBP 4 also possesses D-alanine carboxypeptidase activity in vitro (Iwaya & Strominger, 1977) and the effect of the combined loss of PBPs 4, 5 and 6 is unknown.

The double deletion mutant has a similar growth rate and morphology to the isogenic strain with normal levels of PBPs 5 and 6. Whilst it is slightly ampicillin-sensitive, it is indistinguishable in this respect from a mutant lacking only PBP 5 activity.

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REFERENCES


