Lysis of *Escherichia coli* by β -Lactam Antibiotics: Deletion Analysis of the Role of Penicillin-binding Proteins 1A and 1B

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Deletions of the *ponA* and *ponB* genes of *Escherichia coli* have been constructed *in vitro* and recombined into the chromosome to produce strains that completely lack penicillin-binding protein 1A or penicillin-binding protein 1B. In each case a DNA fragment internal to the gene was replaced by a fragment encoding an antibiotic resistance. The *ponA* and *ponB* deletions can therefore be readily introduced into other *E. coli* strains by P1 transduction of the antibiotic resistance. Although the complete absence of penicillin-binding protein 1A or penicillin-binding protein 1B was tolerated, the absence of both of these proteins was shown to result in bacterial lysis.

INTRODUCTION

β -Lactam antibiotics kill bacterial cells by inhibiting a series of penicillin-binding proteins (PBPs) that catalyse the final stages of peptidoglycan synthesis (Spratt, 1975, 1983). In *Escherichia coli* inactivation of PBP 3 results in the inhibition of cell division and leads to growth of the bacteria as long filamentous cells, whereas inactivation of PBP 2 results in the growth of the bacteria as spherical cells (Spratt, 1975). The rapid lysis of *E. coli* by β -lactam antibiotics was originally suggested to result from the inactivation of PBP 1 (Spratt, 1975). Subsequently, PBP 1 was resolved into two components and the ability of β -lactams to lyse *E. coli* correlated with their affinity for PBP 1B but not with their affinity for PBP 1A (Spratt *et al.*, 1977).

Genetic studies have, however, shown that point mutants which appear to lack PBP 1A or PBP 1B activity are viable, but that loss of the activity of both of these PBPs is a lethal event, resulting in bacterial lysis (Spratt *et al.*, 1977; Suzuki *et al.*, 1978; Tamaki *et al.*, 1977). The situation is complicated by the report of a temperature-sensitive mutant of *E. coli* that lyses at the restrictive temperature and yet appears to be defective only in PBP 1B activity (Tamaki *et al.*, 1977). It is therefore unresolved whether inactivation of PBP 1B is sufficient to induce the rapid lysis of *E. coli* or whether the inactivation of both PBP 1A and PBP 1B is required.

Since point mutations may result in gene products that still retain substantial activity *in vivo*, we have re-investigated the role of PBP 1A and PBP 1B in bacterial growth by the construction of *E. coli* strains that contain precise deletions within the *ponA* and *ponB* genes.

METHODS

Bacterial strains, plasmids and bacteriophage. *E. coli* C600 (*thr leu thi lacY fhuA supE*) and ED3184 (*his lac tsx supF rpsL*) were used in most experiments. *E. coli* MM383 (*thy rha lac rpsL polA(ts)12*) has been described previously (Monk & Kinross, 1972). The plasmids pUC4K (Vieira & Messing, 1982), pHP45 Ω (Prentki & Krisch, 1984), pHSG415 (Hashimoto-Gotoh *et al.*, 1981), pSY42, pBS94 and pBS94C (Broome-Smith *et al.*, 1985) have been described. pBR328AAp is a derivative of pBR328 (Soberon *et al.*, 1980) that has a deletion which removes

Abbreviation: PBP, penicillin-binding protein.

most of the β -lactamase gene (B. G. Spratt, unpublished). λ NM627 has the genotype $\Delta(srI\lambda 1-2) cI857 srI\lambda 4^{+} nin5 srI\lambda 5^{+} Sam7$ and was obtained from Dr N. Murray, Department of Molecular Biology, University of Edinburgh, UK. Pl *vir* was used for transduction and λcI^{-} to distinguish λ lysogens from non-lysogens.

Media. L broth (Lennox, 1955) was used in most experiments and was solidified with 1.2% (w/v) Difco Bacto-agar to produce L agar. Minimum inhibitory concentrations (MICs) of antibiotics were determined on Penassay agar (Difco). Kanamycin was used at 50 μ g ml⁻¹ and spectinomycin was used either at 50–100 μ g ml⁻¹ (when the *Spc^r* gene was on a plasmid) or at 25–40 μ g ml⁻¹ (when the *Spc^r* gene was in the chromosome). In streptomycin-sensitive strains a cleaner background can be obtained by using streptomycin plus spectinomycin (both at 25 μ g ml⁻¹) to select *Spc^r* transformants or transductants (Prentki & Krisch, 1984).

Measurement of MIC values of antibiotics. Overnight cultures of the bacteria were diluted 100-fold and 5 μ l amounts were spotted on Penassay agar plates containing various concentrations of antibiotic. The plates were incubated at 37 °C for 18–24 h. The MIC was defined as the lowest concentration of antibiotic that prevented bacterial growth.

Assay of PBPs. PBPs were assayed in washed cell envelopes with [³H]benzylpenicillin (27 Ci mmol⁻¹; 999 GBq mmol⁻¹) as described previously (Spratt *et al.*, 1977).

Isolation of lysogens. Lysogens of λ pSY1 were obtained by mixing 0.1 ml of dilutions of the phage with 0.1 ml of an overnight culture of strain ED3184. After 20 min at room temperature, 2.5 ml L broth was added and the mixture was incubated for 60 min at 30 °C and dilutions were plated on L agar containing spectinomycin. The lysogenic state of the spectinomycin-resistant colonies was indicated by their inability to grow at 42 °C due to induction of the prophage.

Curing of lysogens. *E. coli* ED3184(λ pSY1) was grown at 30 °C overnight in L broth and appropriate dilutions were plated at 42 °C on L agar containing EDTA (1 mM) to select bacteria that had spontaneously lost the prophage. The loss of λ pSY1 from the bacteria was confirmed by demonstrating their sensitivity to λcI^{-} at 30 °C.

Preparation of plasmid and bacteriophage DNA. Plasmid DNA was prepared as described previously (Stoker *et al.*, 1982). Small amounts of plasmid DNA for restriction mapping and transformation were prepared by the method of Holmes & Quigley (1981). Bacteriophage λ DNA was prepared as described previously (Spratt *et al.*, 1980).

DNA restriction and ligation. Restriction enzymes and DNA ligase were purchased from Amersham or Boehringer-Mannheim and were used under the conditions recommended by the suppliers. DNA fragments were analysed by electrophoresis in agarose gels as described previously (Spratt *et al.*, 1980). DNA fragments were isolated from agarose gels by the method of Dretzen *et al.* (1981).

Chemicals. [phenyl-4-³H]Benzylpenicillin (27 Ci mmol⁻¹) was a generous gift from Merck, Sharp and Dohme, Rahway, NJ, USA. The sources of the β -lactam antibiotics were as follows: ampicillin, Sigma; benzylpenicillin, cephalixin and cephaloridine, Glaxo Laboratories; mecillinam, Leo Laboratories; cephaloridine and cefsulodin, Ciba-Geigy.

RESULTS

Deletion of the *ponA* gene

The *ponA* gene was subcloned on a 3.5 kb *Bam*HI-*Sph*I fragment from pSY42 (Broome-Smith *et al.*, 1985) into pBR328 Δ Ap to produce pBS81 (Fig. 1). The 1148 bp *Xho*I-*Pst*I fragment internal to the *ponA* gene of pBS81 (nucleotides 614–1761 in the *ponA* sequence, encoding amino acid residues 161–541 of PBP 1A; Broome-Smith *et al.*, 1985) was replaced with a 1.4 kb *Sal*I-*Pst*I fragment from pUC4K that encodes kanamycin resistance to produce pSY101 (Fig. 1). The structure of pSY101 was confirmed by restriction mapping.

The chromosomal copy of the *ponA* gene was replaced by the inactivated gene (Δ *ponA* : : Km^r) by recombination between the *ponA* sequences flanking the kanamycin resistance fragment of pSY101 and the homologous sequences in the chromosome. pSY101 was introduced into *E. coli* MM383 (*polA(ts)*) and the bacteria were grown at 30 °C and then transferred to 42 °C for 20 generations to inhibit plasmid replication and allow the segregation of plasmid-free cells. After this treatment only 1 : 10⁵ of the bacteria remained kanamycin-resistant. Of 250 kanamycin-resistant colonies, 247 were also chloramphenicol-resistant, indicating that they still carried pSY101; the other three were chloramphenicol-sensitive.

PBPs were analysed in the three kanamycin-resistant, chloramphenicol-sensitive derivatives and in each case PBP 1A was lacking. Pl phage was grown on one of the latter strains (MM383 Δ *ponA* : : Km^r) and was used to transduce *E. coli* ED3184 to kanamycin resistance. All of the transductants examined lacked PBP 1A activity (Fig. 2).

Fig. 1

Fig. 2

Fig. 1. Construction of an inactivated *ponA* gene. B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; P, *Pst*I; S, *Sal*I; Sp, *Sph*I; X, *Xho*I; X/S, hybrid *Xho*I/*Sal*I junction. The chromosomal DNA from the *ponA* region is shown as a solid block; the open block shows the kanamycin resistance cassette. In pUC4K only the *Sal*I and *Pst*I sites are shown.

Fig. 2. Assay of PBPs in *ponA* and *ponB* deletion mutants. PBPs were assayed in washed cell envelopes prepared from (a) ED3184; (b) ED3184 Δ *ponA* :: *Km^r* and (c) ED3184 Δ *ponB* :: *Spc^r*.

The structure of the *ponA* lesion in ED3184 Δ *ponA* :: *Km^r* was confirmed by re-cloning the inactivated gene from the chromosome and subjecting it to restriction mapping. pBS81 was introduced into ED3184 Δ *ponA* :: *Km^r* and an overnight culture was plated on L agar containing kanamycin (1 mg ml⁻¹). Under these conditions 0.01% of the bacteria formed colonies and these should have been enriched for bacteria in which the Δ *ponA* :: *Km^r* lesion had been transferred from the chromosome onto pBS81 by homologous recombination to provide increased levels of kanamycin resistance. Plasmid DNA was prepared from several thousand of the pooled colonies and was used to transform C600 to both chloramphenicol and kanamycin resistance to produce pSY101-1. Restriction mapping of pSY101-1 showed that it was indistinguishable from pSY101 (Fig. 1) and established that the organization of the inactivated *ponA* gene in the chromosome was identical to that in pSY101.

Deletion of the *ponB* gene

The strategy of Joyce & Grindley (1984) was used to obtain a deletion of the *ponB* gene. The *ponB* gene was obtained on a 3.5 kb *EcoRV* fragment from pBS94 (Broome-Smith *et al.*, 1985) and was inserted into the *Hinc*II site of pteH9 (Hedge & Spratt, 1984) to produce pSY121 (Fig. 3). The three small *Bcl*I fragments internal to the *ponB* gene were replaced with a 2.0 kb *Bam*HI fragment encoding spectinomycin resistance obtained from pHP45 Ω (Fig. 3). The spectinomycin resistance fragment in the resulting plasmid (pSY122) replaced nucleotides 675–2501 in the sequence of *ponB* (Broome-Smith *et al.*, 1985) which encode amino acid residues 203–808 of PBP 1B. The structure of pSY122 was confirmed by restriction mapping.

Fig. 3. Construction of an inactivated *ponB* gene and construction of λ pSY1. Abbreviations are as in the legend to Fig. 1, plus Bc, *Bcl*I; HII, *Hinc*II; RV, *Eco*RV; Bc/B, hybrid *Bcl*I/*Bam*HI junction; HII/RV, hybrid *Hinc*II/*Eco*RV junction. The open block shows the spectinomycin resistance cassette. The solid block is chromosomal DNA from the *ponB* region, except that the small segment clockwise from the *Eco*RI site downstream of the *ponB* gene of pSY121 and pSY122 is derived from the tetracycline resistance region of pBR322. In pHP45 Ω only the *Bam*HI sites are shown.

The modified *ponB* gene (Δ *ponB* : : Spc^r) was cloned on an *Eco*RI fragment from pSY122 into λ NM627 to produce the Δ (*att-int*) recombinant λ pSY1 (Fig. 3). *E. coli* ED3184 was lysogenized with λ pSY1 at 30 °C and was plated on L agar containing EDTA (1 mM) at 42 °C to select for bacteria that had been spontaneously cured of the prophage. Approximately equal numbers of large and small colonies were obtained under these conditions. The large colonies were spectinomycin-sensitive and arose by the excision of λ pSY1, whereas the small colonies were spectinomycin-resistant and presumably arose by a crossover that released λ *ponB*⁺ and left the inactive Δ *ponB* : : Spc^r allele in the chromosome. Analysis of the PBPs confirmed that the large spectinomycin-sensitive colonies had normal levels of PBP 1B and that the small spectinomycin-resistant colonies completely lacked PBP 1B activity (Fig. 2).

P1 phage was grown on one of the latter derivatives (ED3184 Δ *ponB* : : Spc^r) and the resulting phage lysate was used to transduce *E. coli* C600 to spectinomycin resistance. All of the transductants examined lacked PBP 1B activity. The structure of the *ponB* lesion in ED3184 Δ *ponB* : : Spc^r was confirmed by re-cloning the inactivated gene from the chromosome onto pBS94 to produce pBS94-1, using the same strategy as that used to re-clone the *ponA* lesion (see above). Restriction mapping of pBS94-1 confirmed that the organization of the inactivated *ponB* gene in the chromosome was identical to that in pSY122 (Fig. 3).

Table 1. MIC values of antibiotics for strains ED3184 Δ *ponA* : : Km^r and ED3184 Δ *ponB* : : Spc^r

MIC values (μ g ml⁻¹) were determined as described in Methods. The results are from a typical experiment.

Strain	Ampicillin	Benzylpenicillin	Mecillinam	Cephalexin	Cephalexidine	Cefsulodin	Tetracycline	Chloramphenicol
ED3184	2	20	0.1	4	2	15	0.5	3
ED3184 Δ <i>ponA</i> : : Km ^r	2	20	0.1	4	2	15	0.5	3
ED3184 Δ <i>ponB</i> : : Spc ^r	1	5	0.05	1	0.05	1	0.5	3

Construction of strains carrying deletions of *ponA* and *ponB*

The Δ *ponA* : : Km^r and Δ *ponB* : : Spc^r lesions could be introduced into *E. coli* strains by P1 transduction selecting for kanamycin resistance or spectinomycin resistance respectively. Attempts to introduce the *ponA* deletion into ED3184 Δ *ponB* : : Spc^r to produce a double chromosomal deletion of *ponA* and *ponB* were unsuccessful. The double chromosomal deletion could, however, be constructed if the recipient contained a plasmid expressing either PBP 1A or PBP 1B. *E. coli* therefore cannot survive in the absence of both PBP 1A and PBP 1B, but can survive with only PBP 1A, or with only PBP 1B.

PBP 1B exists in *E. coli* in two forms which differ at their amino-termini due to the presence of two in-frame translation starts in the *ponB* gene (Kato *et al.*, 1984; Broome-Smith *et al.*, 1985). A plasmid (pBS94C) has been constructed that produces only the small form of PBP 1B due to an alteration of the reading frame between the two translation starts (Broome-Smith *et al.*, 1985). An *E. coli* strain with a deletion of both the *ponA* and *ponB* genes could be constructed in a strain carrying pBS94C. The small form of PBP 1B is therefore sufficient for bacterial growth in the absence of both PBP 1A and the large form of PBP 1B.

To examine the physiological effects of the complete absence of both PBP 1A and PBP 1B we cloned the *ponB* gene into the low copy number vector, pHSG415, which is temperature-sensitive for plasmid replication, to produce pSY123. The Δ *ponB* : : Spc^r lesion was P1-transduced into ED3184 Δ *ponA* : : Km^r(pSY123) at 30 °C to obtain a strain with a chromosomal deletion of both *ponA* and *ponB*. The presence of both chromosomal deletions was confirmed by demonstrating that P1 phage grown on the strain could transduce both the *ponA* and *ponB* deletions. *E. coli* ED3184 Δ *ponA* : : Km^r Δ *ponB* : : Spc^r(pSY123) was grown in L broth at 30 °C and was then shifted to 42 °C and grown at this temperature with periodic dilution of the culture with pre-warmed media to maintain a low cell density. At 42 °C the replication of pSY123 will cease, and further growth will result in the dilution of the level of PBP 1B, to produce bacteria that completely lack both PBP 1A and PBP 1B. The bacteria grew rapidly for about 3 h and then lysed. Examination of the lysing bacteria by phase contrast microscopy showed the presence of spheroplasts and 'rabbit ears' that were indistinguishable from those produced by the growth of *E. coli* in the presence of high concentrations of penicillin.

Properties of strains lacking PBP 1A or PBP 1B

Strains ED3184 and ED3184 Δ *ponA* : : Km^r grew in L broth at 37 °C with indistinguishable growth rates but ED3184 Δ *ponB* : : Spc^r grew 12% slower than its isogenic parent (results not shown). The sensitivity of ED3184 Δ *ponA* : : Km^r to β -lactam antibiotics, and to tetracycline and chloramphenicol, was very similar to that of its parent strain, ED3184 (Table 1). ED3184 Δ *ponB* : : Spc^r was substantially more sensitive to β -lactams than ED3184 but showed normal sensitivity to tetracycline and chloramphenicol (Table 1). The morphology of strains ED3184 Δ *ponA* : : Km^r and ED3184 Δ *ponB* : : Spc^r was indistinguishable from that of strain ED3184.

DISCUSSION

PBP 1A and PBP 1B are bifunctional enzymes that catalyse both a penicillin-insensitive peptidoglycan transglycosylase reaction and a penicillin-sensitive peptidoglycan transpeptidase reaction (Matsuhashi *et al.*, 1982). The amino acid sequences of PBP 1A and PBP 1B have been deduced from the nucleotide sequences of the *ponA* and *ponB* genes and show a low level of similarity throughout most of their lengths (Broome-Smith *et al.*, 1985). The enzymes appear to be sufficiently similar to have interchangeable roles in peptidoglycan synthesis since either PBP 1A or PBP 1B (or the small form of PBP 1B) is sufficient for bacterial growth.

The *ponA* and *ponB* deletions remove sequences that encode amino acid residues 161–541 and 203–808 respectively of PBP 1A and PBP 1B and presumably result in the complete loss of both their transglycosylase and transpeptidase activities. The *ponA* and *ponB* deletions will result in the expression of fragments comprised of the amino-terminal 160 and 203 amino acid residues of PBP 1A and PBP 1B respectively. These fragments will not possess transpeptidase activity as the active site serine residue essential for this function has been identified as serine-465 in PBP 1A and serine-510 in PBP 1B (Keck *et al.*, 1985). It is not possible to state categorically that the fragments will lack transglycosylase activity as the location of this domain has not been firmly established. The transglycosylase domain of these homologous PBPs is, however, likely to be located in a region of amino acid sequence similarity. Since the similarity between PBP 1A and PBP 1B is not apparent at the amino-termini and only occurs from residues 84 and 231 respectively (Broome-Smith *et al.*, 1985), we believe it unlikely that a functional transglycosylase domain is contained within the fragments expressed from the deleted genes.

The deletion of the *ponA* gene had no significant effects on cell growth rate, cell morphology, or the sensitivity of the bacteria to a range of β -lactam antibiotics. The deletion of the *ponB* gene was, however, slightly deleterious to the bacteria and resulted in a slight reduction of the growth rate compared to the isogenic parent strain. The deletion of the *ponB* gene also resulted in supersensitivity to a variety of β -lactam antibiotics. This phenomenon has been observed previously with a point mutant that lacks PBP 1B activity and is believed to be a consequence of the much greater affinity of PBP 1A for β -lactams compared with PBP 1B (Tamaki *et al.*, 1977; Suzuki *et al.*, 1978). Thus in a wild-type strain of *E. coli*, cell lysis will occur at the concentration of β -lactam antibiotic that inactivates both PBP 1A and PBP 1B, whereas in a *ponB* mutant only the β -lactam-sensitive PBP 1A needs to be inactivated. The slightly increased sensitivity of the *ponB* deletion to mecillinam, which has no significant affinity for PBP 1A or PBP 1B, may result from a general increase in the susceptibility of the strain to lysis by cell wall inhibitors resulting from the synthesis of partially defective peptidoglycan by PBP 1A.

Our results with *ponA* and *ponB* deletions confirm those obtained with point mutants by Suzuki *et al.* (1978) and establish rigorously that inactivation of both PBP 1A and PBP 1B results in cell lysis, whereas inactivation of either PBP 1A or PBP 1B is tolerated, at least under laboratory conditions. The lysis of the *ponB* mutant of Tamaki *et al.* (1977) at 42 °C may reflect the influence of genetic background on the severity of the lack of PBP 1B or the presence of additional mutations in this strain.

Rapid lysis of *E. coli* by β -lactam antibiotics therefore requires the inactivation of both PBP 1A and PBP 1B. In practice, almost all β -lactam antibiotics have a higher affinity for PBP 1A than for PBP 1B and the ability of β -lactams to lyse *E. coli* is limited by their affinity for PBP 1B.

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REFERENCES

- BROOME-SMITH, J. K., EDELMAN, A., YOUSIF, S. & SPRATT, B. G. (1985). The nucleotide sequences of the *ponA* and *ponB* genes encoding penicillin-binding proteins 1A and 1B of *Escherichia coli*. *European Journal of Biochemistry* **147**, 437–446.
- DRETZEN, G., BELLARD, M., SASSONE-CORSI, P. & CHAMBON, P. (1981). A reliable method for the recovery of DNA fragments from agarose gels. *Analytical Biochemistry* **112**, 295–298.
- HASHIMOTO-GOTOH, T., FRANKLIN, F. C. H., NORDHEIM, A. & TIMMIS, K. N. (1981). Specific purpose cloning vectors. I. Low copy number, temperature-sensitive mobilization-defective pSC101-derived containment vectors. *Gene* **16**, 227–235.
- HEDGE, P. J. & SPRATT, B. G. (1984). A gene fusion that localises the penicillin-binding domain of penicillin-binding protein 3 of *Escherichia coli*. *FEBS Letters* **176**, 179–184.
- HOLMES, D. S. & QUIGLEY, M. (1981). A rapid boiling method for the preparation of bacterial plasmids. *Analytical Biochemistry* **114**, 193–197.
- JOYCE, C. M. & GRINDLEY, N. D. F. (1984). Method for determining whether a gene of *Escherichia coli* is essential: application to the *polA* gene. *Journal of Bacteriology* **158**, 636–643.
- KATO, J.-I., SUZUKI, H. & HIROTA, Y. (1984). Overlapping of the coding regions for α and γ components of penicillin-binding protein 1B in *Escherichia coli*. *Molecular and General Genetics* **196**, 449–457.
- KECK, W., GLAUNER, B., SCHWARZ, U., BROOME-SMITH, J. K. & SPRATT, B. G. (1985). Sequences of the active site peptides of three of the high molecular weight penicillin-binding proteins of *Escherichia coli* K12. *Proceedings of the National Academy of Sciences of the United States of America* **82**, 1999–2003.
- LENNOX, E. S. (1955). Transduction of linked genetic characters of the host by bacteriophage P1. *Virology* **1**, 190–206.
- MATSUHASHI, M., NAKAGAWA, J., TOMIOKA, S., ISHINO, F. & TAMAKI, S. (1982). Mechanism of peptidoglycan synthesis by penicillin-binding proteins in bacteria and effects of antibiotics. In *Drug Resistance in Bacteria – Genetics, Biochemistry and Molecular Biology*, pp. 297–310. Edited by S. Mitsuhashi. Tokyo: Japanese Scientific Societies Press.
- MONK, M. & KINROSS, J. (1972). Conditional lethality of *recA* and *recB* derivatives of a strain of *E. coli* with a temperature-sensitive DNA polymerase I. *Journal of Bacteriology* **109**, 971–978.
- PRENTKI, P. & KRISCH, H. M. (1984). *In vitro* insertional mutagenesis with a selectable DNA fragment. *Gene* **29**, 303–313.
- SOBERON, X., COVARRUBIAS, L. & BOLIVAR, F. (1980). Construction and characterization of new cloning vehicles. IV. Deletion derivatives of pBR322 and pBR325. *Gene* **9**, 287–305.
- SPRATT, B. G. (1975). Distinct penicillin-binding proteins involved in the division, elongation and shape of *Escherichia coli* K12. *Proceedings of the National Academy of Sciences of the United States of America* **72**, 2999–3003.
- SPRATT, B. G. (1983). Penicillin-binding proteins and the future of β -lactam antibiotics. *Journal of General Microbiology* **129**, 1247–1260.
- SPRATT, B. G., JOBANPUTRA, V. & SCHWARZ, U. (1977). Mutants of *Escherichia coli* which lack a component of penicillin-binding protein 1 are viable. *FEBS Letters* **79**, 374–378.
- SPRATT, B. G., BOYD, A., & STOKER, N. (1980). Defective and plaque-forming lambda transducing bacteriophage carrying penicillin-binding protein-cell shape genes. *Journal of Bacteriology* **143**, 569–581.
- STOKER, N. G., FAIRWEATHER, N. F. & SPRATT, B. G. (1982). Versatile low-copy-number plasmid vectors for cloning in *Escherichia coli*. *Gene* **18**, 335–341.
- SUZUKI, H., NISHIMURA, Y. & HIROTA, Y. (1978). On the process of cellular division in *Escherichia coli*: a series of mutants of *E. coli* altered in the penicillin-binding proteins. *Proceedings of the National Academy of Sciences of the United States of America* **75**, 664–668.
- TAMAKI, S., NAKAJIMA, S. & MATSUHASHI, M. (1977). Thermosensitive mutation in *Escherichia coli* simultaneously causing defects in penicillin-binding protein 1Bs and in enzyme activity for peptidoglycan synthesis *in vitro*. *Proceedings of the National Academy of Sciences of the United States of America* **74**, 5472–5476.
- VIEIRA, J. & MESSING, J. (1982). The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. *Gene* **19**, 259–268.