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Deletion of the Penicillin-Binding Protein 5 Gene of *Escherichia coli*

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A strain of *Escherichia coli* that has a deletion of the entire *dacA* gene has been constructed. The complete lack of penicillin-binding protein 5 in this strain establishes that the activity of this protein is not essential for the growth of *E. coli*.

The lethal effects of β-lactam antibiotics in *Escherichia coli* have been demonstrated to be due to the inactivation of penicillin-binding proteins (PBPs) 1A/B, 2, and 3 (8). Interaction of β-lactams with PBP 4, 5, or 6 is not believed to be of major significance for the killing action of the antibiotics, and these PBPs do not appear to be essential for bacterial growth (2, 5, 6, 8, 11). PBPs 4, 5, and 6 have been shown to be identical to the two major D-alanine carboxypeptidases (CPases) of *E. coli* cell envelopes (12). CPase 1A is identical to PBPs 5 and 6 (5, 7, 10) and CPase 1B is identical to PBP 4 (2, 6, 8). Together these enzymes contribute at least 90% of the CPase activity detected in *E. coli* cell envelopes (5, 8).

CPase activity has been proposed to regulate the extent of cross-linking in peptidoglycan by removing the terminal D-alanine from a proportion of the pentapeptide side chains of nascent peptidoglycan, thus preventing their involvement in cross-links (3). The importance of this reaction for bacterial growth and the enzyme(s) that catalyzes the removal of the terminal D-alanine of peptidoglycan side chains in vivo are unknown.

Mutants that lack the activity of CPase 1B (PBP 4) show no obvious growth defects (2, 6, 11). Two mutants that lack the activity of CPase 1A have also been isolated, and these show defects in PBP 5 (7; Y. Nishimura, H. Suzuki, Y. Hirota, and J. T. Park, personal communication). The mutations in the latter strains map close to *leuS* at 14 min on the linkage map (1) in gene *dacA* (7, 11). The CPase 1A mutants, like the CPase 1B mutants, grow well, and double mutants that lack the activity of both enzymes grow without serious growth abnormalities (5). The double mutants appear to retain only a small percentage of the CPase activity of normal cells, and this residual activity is probably due to the activity of PBP 6 with possible contributions from PBPs 1 through 3. No mutants with defects in PBP 6 have been described, and the importance of this protein for peptidoglycan synthesis is therefore unknown.

A critical demonstration that PBPs 4, 5, and 6 are not essential for peptidoglycan synthesis requires the isolation of mutants that completely lack these activities, since the mutants so far obtained may grow apparently normally because they retain some residual activity in vivo, although none can be detected in vitro. I describe here the construction of an *E. coli* strain that completely lacks PBP 5 due to a genetic deletion of the entire *dacA* gene.

The gene order around the *dacA* gene has been shown, by mapping with a series of specialized λ-transducing phages, to be *lip-dacA-rodA-pbpA-leuS* (9). The strategy used to delete *dacA* was to lysogenize *E. coli* ED3184 with λpBS102 (Table 1), which must integrate by homologous recombination into the *lip-leuS* region of the chromosome. ED3184 (λpBS102) was made *recA* by P1 cotransduction with Tn*10*, using P1 *vir* grown on NK5304 (Table 1), and was plated at 42°C on minimal agar (9) supplemented with lipoic acid (1 μg/ml). Approximately $1:10^7$ formed colonies under these conditions, and many will have survived due to spontaneous loss of the temperature-inducible λpBS102 prophage. Since the bacterial strain was *recA* and the prophage was Δ(*att-gam*), the loss of the prophage must occur by illegitimate excision, which will in many cases delete both λpBS102 and flanking chromosomal genes.

To ensure the complete deletion of the *dacA* gene, the survivors were screened for the simultaneous loss of genes that are known to map on either side of *dacA*. These were recognized as lipoic acid-requiring (*lip*) strains that grew as spherical cells (*rodA or rodA pbpA*; inactivation of either, or both, of these cell shape genes results in the growth of *E. coli* as spherical cells;

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TABLE 1. *E. coli strains and λ phage*

Bacteria/phage	Relevant properties	Reference/source
Bacteria		
ED3184	*his supF*	W. J. Brammar
NK5304	*ilv thr thi srl*::Tn*10 recA56*	N. Kleckner (4)
SP5000	ED3184 *srl*::Tn*10 recA56* Δ(*lip-dacA-rodA*)	This paper
λ Phages[a]		
λpBS102	λ p*leuS pbpA rodA* Δ(*att-int-xis-red-gam*) c*I857 Sam7*	9
λ d*dacA*51	λ d*dacA rodA pbpA leuS*	9
λ d*rodA*1	λ d*rodA pbpA leuS*	9
λ d*pbpA*108	λ d*pbpA leuS*	9

[a] The orientation of chromosomal genes in the above genotypes corresponds to the physical orientation of the genes in the transducing phages.

9). One λ⁻, lipoic acid-requiring, spherical strain was found among the first 100 survivors screened. Figure 1B shows that this strain, SP5000, completely lacked PBP 5 activity. The deletion in SP5000 extended from *lip* through *dacA* and into the *rodA* gene, but left *pbpA* intact since the strain retained (Fig. 1E) activity of PBP 2 (the *pbpA* gene product). This was confirmed by showing that SP5000 was transduced (in the presence of λ⁺ helper) to normal cell shape by λ phage that carried the *rodA-pbpA-leuS* region (e.g., λ d*dacA*51 or λd*rodA*1; Table 1) but not by the phage that only carried the *pbpA-leuS* region (e.g., λd*pbpA*108; Table 1).

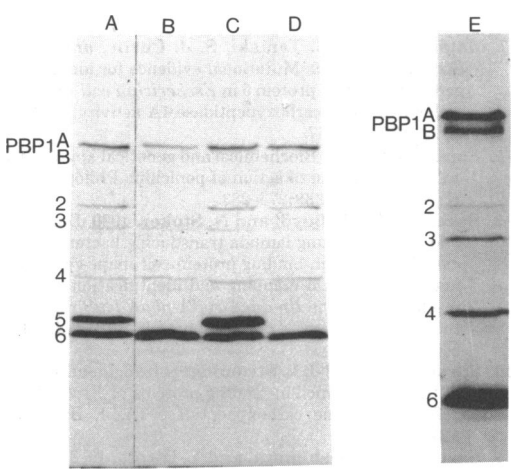

FIG. 1. *PBPs of SP5000 and derivatives. The PBPs of (A) ED3184 recA (λpBS102), (B) SP5000, (C) SP5000 (λddacA51), and (D) SP5000 (λdrodA1) were labeled with [¹⁴C]benzylpenicillin, separated on a sodium dodecylsulfate-polyacrylamide gel, and detected by fluorography as described previously (9). The levels of PBP 2 in slots A, C, and D are higher than in B since in the former cases the strains were diploid for the pbpA gene. The fluorograph (A-D) has been exposed to show PBPs 5 and 6 clearly, and slot E shows a separate assay of the PBPs of SP5000 exposed to show clearly the presence of the minor PBPs.*

Figure 1C and D shows the PBPs of SP5000 lysogenized by λd*dacA*51 and λd*rodA*1. As expected, SP5000 (λd*dacA*51) regained PBP 5 activity, whereas SP5000 (λd*rodA*1) did not. This result confirms that the *dacA* gene is the structural gene for PBP 5 and confirms the previous mapping of the gene (9) between *lip* and *rodA*.

SP5000 grew as spherical cells on minimal agar but plated with very low efficiency on nutrient agar (a feature of all freshly isolated spontaneous spherical mutants that we have isolated), and a spontaneous derivative, SP5001, that grew well on nutrient agar (plus lipoic acid) was isolated. Figure 2B shows the spherical morphology of SP5001.

SP5000 (λd*rodA*1), which completely lacks the *dacA* gene, grew as osmotically stable rod-shaped cells (Fig. 2C). These cells were slightly irregular compared with their parent strain (Fig. 2A), but this effect may be due to deletion of some gene other than *dacA* since SP5000 (λd*dacA*51) showed a similar irregular morphology (data not shown). In any case the ease with which a strain completely deleted for *dacA* was isolated, and its relatively normal morphology, shows conclusively that PBP 5 activity is not essential for the growth of *E. coli*.

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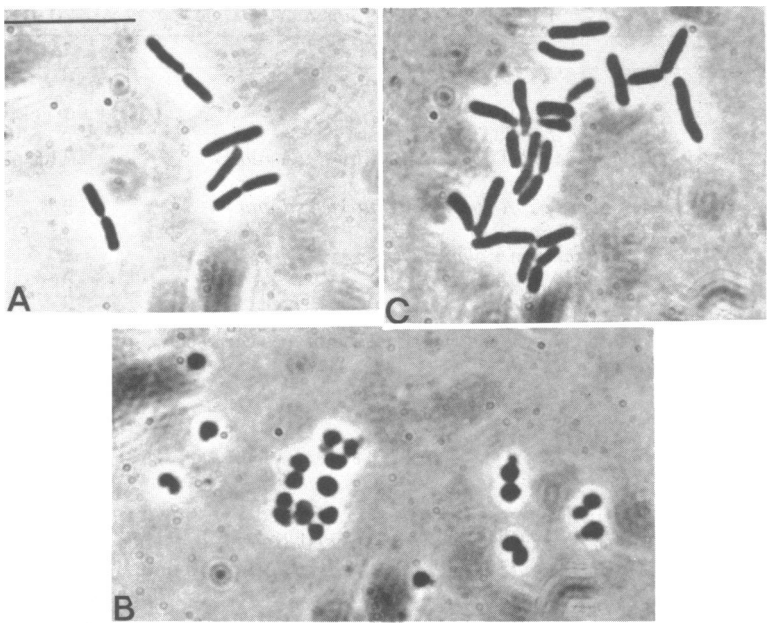

FIG. 2. *Morphology of strains lacking dacA. (A) ED3184 recA (λpBS102), (B) SP5001, and (C) SP5000 (λdrodA1) were grown to the midexponential growth phase at 30°C in nutrient broth (Difco) supplemented with lipoic acid (1 μg/ml) and were photographed under phase-contrast illumination. The magnification is the same for all three strains. Bar, 10 μm.*

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