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Peptidoglycan Synthesis in Cocci and Rods of a pH-Dependent, Morphologically Conditional Mutant of *Klebsiella pneumoniae*

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Mir M7 is a spontaneous morphologically conditional mutant of *Klebsiella pneumoniae* which grows as round cells (cocci) at pH 7 and as normal rods at pH 5.8. We studied the rates of peptidoglycan synthesis of cocci and rods growing at pH values of 7 and 5.8, respectively. It was found that exponentially growing cocci produced a reduced amount of peptidoglycan per cell, compared with rods. Moreover, a shift of cocci to the permissive pH (5.8) caused an increase in the rate of peptidoglycan synthesis, whereas the reverse shift of rods to pH 7 determined a twofold reduction in the rate of [^{3}H]diaminopimelic acid incorporation. During synchronous growth at pH 7, the rate of peptidoglycan synthesis after cell division decreased with time and rose before and during the first division. The susceptibilities of rods and cocci to β-lactam antibiotics were also studied. It was found that cocci were more sensitive both to penicillin G and to cephalexin than were rods, but they showed a high level of resistance to mecillinam. The peculiar behavior of this mutant was interpreted as supporting the existence in bacterial rods of two different sites for peptidoglycan synthesis: one responsible for lateral wall elongation and one responsible for septum formation. In Mir M7, shape damage is described as dependent on the specific inhibition, at the nonpermissive pH, of the site for lateral wall extension.

Determination of bacterial rod shape can be regarded as one of the simplest morphogenetic events in nature. However, little is known about the mechanism which regulates it.

Beginning with the *Klebsiella pneumoniae* Mir M7, originally described by Meloni and Monti-Bragadin in 1962 (25), several other morphologically conditional and constitutive mutants have been isolated and used as tools to confront this problem (2, 3, 11, 15, 20, 24, 29, 30, 32-35).

Some authors have proposed that peptidoglycan plays a critical role in shape determination (6, 20, 30). Others have suggested outer membrane proteins (in gram-negative bacteria) (5, 14, 16), accessory polymers (in gram-positive bacteria) (3, 35), and the inner membrane (15) as critical factors in this process. However, the process of rod morphogenesis is complex and involves the coordinated interaction of several different cell structures. Studies should therefore focus also on the identification of possible regulatory mechanisms which may cause the shape damages in morphology mutants. Interesting proposals have been recently made concerning shape regulation in bacteria. Higgins and Shockman (17) and Daneo-Moore and Shockman (7) have suggested that in cocci the increase in surface area can only be achieved by cross-wall construction, whereas in rods a separation of functions may exist: one for lateral wall formation and one for septal wall formation. This theory gained support recently through the finding by Schwarz et al. that labeled diaminopimelic acid (DAP) incorporated at two discrete zones: one corresponding to the septum and one corresponding to the lateral wall (40). More recently, Mirelman et al. (26, 27) have shown that D-alanine carboxypeptidase is activated during septum formation, whereas it is partially inhibited when cell division is prevented and cells elongate continuously. They have suggested that septation depends on the relative activities of D-alanine carboxypeptidase and transpeptidase.

K. pneumoniae Mir M7 is a spontaneous, pH-dependent, morphologically conditional mutant which grows as round cells (cocci) at pH 7 and as rods at pH 5.8 (36-39).

Less information is available on *K. pneumoniae* than on other bacteria, such as *Escherichia coli*. However, we decided to study strain Mir M7 because, among all the morphological mutants so far described, it is the only one demonstrating pH-conditional properties.

In addition, a linkage map has been recently described in *K. pneumoniae* (23) which is similar to those of both *E. coli* and *Salmonella typhimurium*. We have also developed systems of generalized transduction and conjugation in our strains (38).

In this paper we study the rates of peptidoglycan synthesis during the two reverse shape transitions and during the various phases of the cell cycles of rods and cocci. We describe also the susceptibilities of rods and cocci to both mecillinam and cephalexin, two β-lactam antibiotics which have been proposed to inhibit lateral wall extension and septum formation, respectively (19, 22, 41, 42).

Our results are consistent with the existence, in the rods, of two sites for peptidoglycan synthesis. One, responsible for cell elongation, is particularly sensitive to mecillinam and relatively resistant to cephalexin. The other site, responsible for cell septation, is resistant to mecillinam and particularly sensitive to cephalexin. In strain Mir M7 the site responsible for cell elongation is specifically inhibited at pH 7. Such specific inhibition is responsible for determination of the round shape at the nonpermissive pH. Owing to these properties, Mir M7 can be described more precisely as a mutant conditional for lateral wall extension.

MATERIALS AND METHODS

Bacterial strains. The strains used in this work were threonine- and lysine-requiring derivatives from the pH-dependent, morphologically conditional mutant *K. pneumoniae* Mir M7 and its normal-shaped revertant Mir A12, which have been described elsewhere (9, 10, 36, 37).

Media and bacterial growth. Bacteria were grown in M9 minimal medium, each liter containing: NH_4Cl, 5 g; NH_4NO_3, 1 g; Na_2SO_4, 2 g; K_2HPO_4, 3 g; KH_2PO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; glucose, 2 g. To the base components additions were made: either 6.3 g of $Na_2HPO_4 \cdot 2H_2O$ and 1.8 g of NaH_2PO_4 to obtain a medium of pH 7 or 0.7 g of Na_2HPO_4 and 6.9 g of NaH_2PO_4 to obtain a medium of pH 5.8. Minimal medium was supplemented with 20 μg of threonine and 20 μg of lysine per ml.

To obtain synchronous cultures, the sucrose gradient procedure described by Mitchison and Vincent (28) was used. Cultures were incubated at 37°C with shaking to provide aeration. Cell number was determined with a Coulter Counter.

Strain selection. To measure peptidoglycan synthesis conveniently, we tried to isolate DAP- and lysine (Lys)-dependent mutants from Mir M7 and Mir A12 strains by nitrosoguanidine mutagenesis (1). Lys^- mutants were promptly obtained, but the isolation of DAP-requiring derivatives was unsuccessful. Therefore, it was decided to assay different Lys mutants, selecting one which was able to incorporate all the exogenous DAP into the peptidoglycan when grown in a medium supplemented with 20 μg of lysine per ml. For this purpose, cultures of 100 ml of Lys strains labeled with [^{3}H]DAP (300 mCi/mmol; Radiochemical Centre, Amersham, England) were centrifuged, washed, and suspended for 20 min in cold 5% trichloroacetic acid to remove the intracellular pool of free radioactive compounds. The acid-insoluble precipitate was washed with water, and the peptidoglycan was purified as described (9, 10). The radioactively pure peptidoglycan was then suspended in 1 ml of 4 N HCl and hydrolyzed for 14 h at 104°C. The hydrolysate was evaporated to dryness in vacuo, and the residue was dissolved in 0.5 ml of water. Fifty microliters of this solution was chromatographed on MN 2214 Macherey-Nagel paper and developed in butanol-water-acetic acid (50:50:10) for 48 h at 37°C. Parallel runs were made with unlabeled marker DAP and lysine as controls. After chromatography, the paper was cut into 1-cm strips and assayed for radioactivity in toluene scintillation fluid. The controls were sprayed with 0.5% ninhydrin in acetone and developed at 100°C for 10 min. The remainder of the hydrolyzed peptidoglycan was analyzed in a Beckman amino acid analyzer to check peptidoglycan purity. Among the Lys^- mutants submitted to this analysis, two strains, one from Mir M7 and one from Mir A12, were selected, which were shown to incorporate 90 and 91%, respectively, of the cellular trichloroacetic acid-precipitable radioactivity into the peptidoglycan layer (Table 1). After hydrolysis of the pure peptidoglycan, the total incorporated radioactivity moved as DAP in a paper chromatogram.

Estimation of specific activity of DAP. Two milligrams of cells (dry wt) labeled with [^{3}H]DAP was suspended in 2 ml of 4 N HCl and hydrolyzed for 14 h at 104°C. The hydrolysate was evaporated to dryness in vacuo, and the residue was dissolved in 1 ml of water. A 0.5-ml amount was analyzed for amino acid content as described above, and 0.5 ml was assayed for radioactivity.

Estimation of peptidoglycan and protein synthesis. Cells exponentially growing in minimal medium containing 20 μg of threonine and 20 μg of lysine per ml were harvested by centrifugation and inoculated into 50 ml of a fresh medium at a density of about 10^7 cells per ml or centrifuged onto a sucrose gradient to obtain synchronized cells (28).

Every 10 min, two samples of 1 ml, each one in duplicate, were taken from the exponential or synchronized culture. To these samples either 50 μCi of [^{3}H]DAP or 5 μCi of [^{3}H]leucine (52 Ci/mmol; Radiochemical Centre) per ml was added, and then all samples were incubated at 37°C with shaking for 5 min.

Incorporation of the label was stopped by adding 5 ml of cold trichloroacetic acid (5%) containing 100 μg of cold DAP and 100 μg of leucine per ml. After 30 min on ice, each sample was filtered onto Whatman glass fiber filters (Whatman GS, 1.8 cm), washed, and counted as described below.

Evaluation of effect of pH on [^{14}C]DAP uptake. Cells growing exponentially in M9 glucose minimal medium at pH 7 or 5.8 were harvested by centrifugation, washed twice, and then resuspended in the same M9 medium, to which chloramphenicol (150 μg/ml) was added. [^{14}C]DAP (44 mCi/mmol; Radiochemical Centre) was added to these cell suspensions. At inter-

TABLE 1. *Recovery of trichloroacetic acid-precipitable radioactivity in various cellular fractions of K. pneumoniae strains Mir M7 and Mir A12 grown in minimal medium at pH 7*[a]

Fraction[b]	Mir M7		Mir A12	
	Total cpm	Recovery (%)	Total cpm	Recovery (%)
Acid insoluble	4.5×10^5	100	7.8×10^5	100
SDS-solubilized material from acid-insoluble fraction	1.8×10^4	4	1.5×10^4	2
Pronase-solubilized material from SDS-insoluble residue	2.7×10^4	6	5.4×10^4	7
Peptidoglycan	4.0×10^5	90	7.1×10^5	91

[a] Similar percentages of recovery were obtained when the strains were grown in minimal medium at pH 5.8.
[b] Cells labeled with [^{3}H]DAP were treated with 5% trichloroacetic acid to remove the intracellular pool of free radioactive compounds. The acid-insoluble precipitate was then treated with boiling 4% sodium dodecyl sulfate (SDS), and the SDS-insoluble residue was treated with Pronase (9).

vals 1-ml samples were withdrawn, immediately filtered onto membrane filters (0.45-μm pore size; Millipore Corp., Bedford, Mass.), and washed twice with 10 ml of a solution containing 0.01 M Tris-hydrochloride (pH 7.3), 0.15 M NaCl, and 5×10^{-4} M $MgCl_2$. The filters were dried and counted in a liquid scintillation counter as described below.

Determination of amounts of peptidoglycan in rods and cocci. The percentages of peptidoglycan in the whole cells were determined by calculating the amounts of pure peptidoglycan directly isolated from known numbers of cells grown at pH values of 7 and 5.8 as described by Fontana (8) and Fontana et al. (10).

Determination of turnover. Overnight cultures grown in minimal medium supplemented with 1 μCi of [^{3}H]DAP per ml were transferred into fresh medium and incubated until they began exponential growth. Cells were then harvested, synchronized, transferred into fresh medium containing 200 μg of cold DAP per ml, and incubated at 37°C with shaking. At intervals, samples of 1 ml were taken in duplicate and treated exactly as described above to determine the trichloroacetic acid-insoluble radioactivity.

Measurement of radioactivity. Aqueous samples were measured in Triton-toluene scintillator (0.5-ml sample in 5 ml of scintillator [10 g of 2,5-diphenyloxazole in a mixture of 1 liter of toluene and 0.5 liter of Triton X-100]). Radioactive samples on paper were counted in 5 ml of toluene scintillator (5 g of PPO per liter of toluene). A Beckman LS-133 liquid scintillation counter was used.

Determination of 50% lethal dose. Cells growing exponentially at pH 7 or 5.8 were suitably diluted, and 0.1-ml samples were spread on Diagnostic Sensitivity Test agar (Oxoid Ltd., Basingstoke, England) plates containing different antibiotic (penicillin G, cephalexin, mecillinam) concentrations. Diagnostic Sensitivity Test agar medium was acidified by adding HCl. After 18 h of incubation, colonies were counted. The concentration of antibiotic inhibiting colony formation to half that in the control plates was reported as the 50% lethal dose.

RESULTS

Rates of peptidoglycan synthesis under permissive and nonpermissive conditions. Figure 1 shows the rates of [^{3}H]leucine and [^{3}H]DAP incorporation by strain Mir M7 (the pH-dependent, morphologically conditional mutant) and Mir A12 (the revertant with normal rod shape) both at permissive (pH 5.8) and at nonpermissive (pH 7) conditions. It is evident that at pH 7 the rate of peptidoglycan synthesis was significantly reduced. The fact that DNA, RNA (data not shown), and protein syntheses were not influenced at pH 7 indicates that this reduction of peptidoglycan synthesis was specific and not the result of a general effect on cell growth. Moreover, the revertant Mir A12 showed similar rates of [^{3}H]DAP incorporation both at pH 5.8 and at pH 7. To exclude alteration in the efficiency of incorporation of the precursor depending on growth conditions, we evaluated both the rates of [^{14}C]DAP uptake by Mir M7 cells at pH 5.8 and pH 7 and the specific activities of [^{3}H]DAP after growth of the mutant at pH 5.8 and pH 7. [^{14}C]DAP uptake was not significantly influenced by the pH apart from a slight increase at pH 7 (Fig. 2). Furthermore, the specific activities of [^{3}H]DAP were similar in both conditions of growth (Table 2).

Rate of peptidoglycan synthesis during shape transitions. Figure 3 shows the effects of pH shift on the rates of [^{3}H]DAP incorporation of Mir M7 and Mir A12.

It appears that soon after the shift from pH 5.8 to pH 7, the mutant Mir M7 underwent a marked reduction in the rate of peptidoglycan synthesis. The opposite effect was evident when cocci were transferred to a medium of pH 5.8. These shifts did not cause any significant variation either in the rate of leucine incorporation or in the rates of both viable count and dry weight increases (data not shown). No gross variation was seen in the rate of peptidoglycan or protein synthesis when strain Mir A12 was shifted from pH 5.8 to pH 7 or vice versa (data not shown).

As for cell shape (Fig. 4), the expected shape

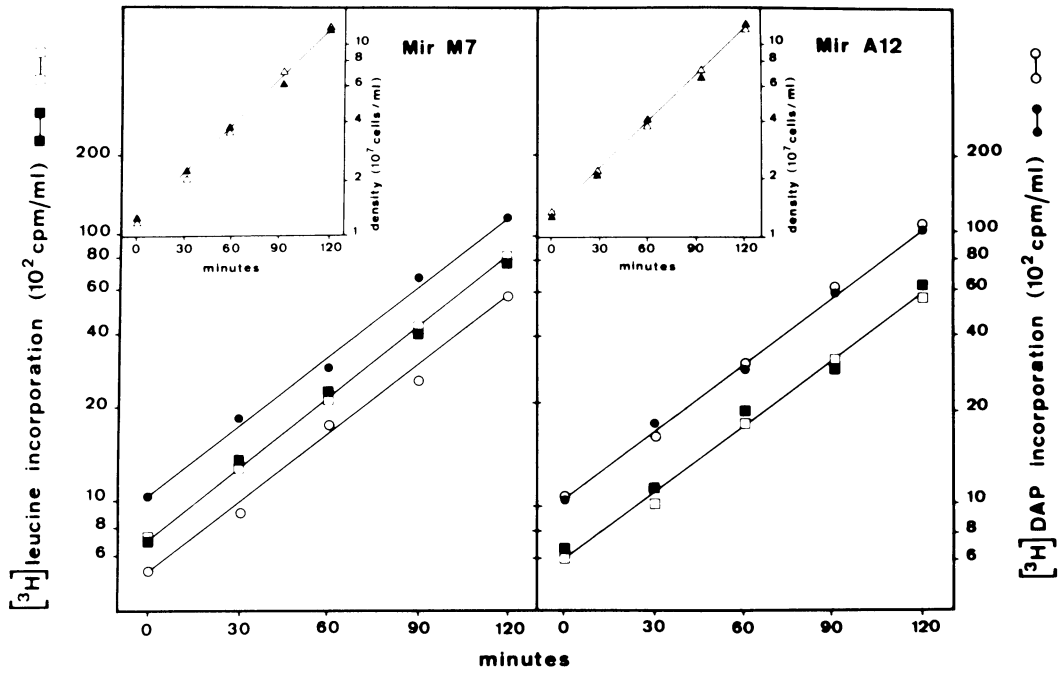

FIG. 1. *Incorporation of [^{3}H]leucine and [^{3}H]DAP into K. pneumoniae strains Mir M7 and Mir A12 grown both at pH 7 (open symbols) and at pH 5.8 (closed symbols). Cells growing exponentially were suspended in minimal medium at pH 7 or 5.8. At intervals duplicate samples (1 ml) were pulse-labeled for 5 min with either [^{3}H]leucine or [^{3}H]DAP as described in the text.*

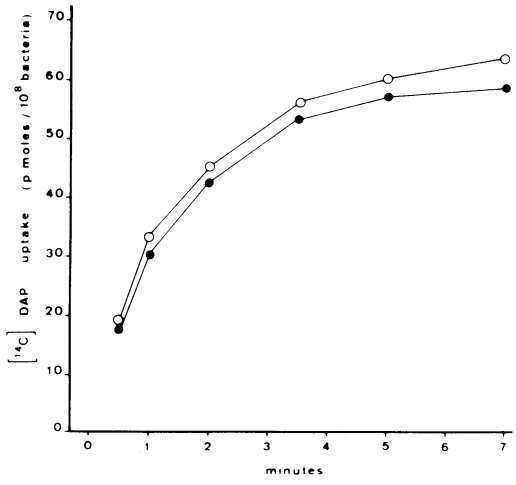

FIG. 2. *Kinetics of [^{14}C]DAP uptake by K. pneumoniae Mir M7 cells at pH 7 (○) and pH 5.8 (●). The experimental procedure is described in the text.*

TABLE 2. *Peptidoglycan amount and [^{3}H]DAP specific activity in K. pneumoniae strains Mir M7 and Mir A12 grown at pH 7 and pH 5.8*

Strain	pH	Peptidoglycan in whole cells (%, dry wt)[a]	DAP	
			Total (nmol/mg, dry wt)	Sp act (cpm/nmol)[b]
Mir M7	7	0.82	21	1,300
	5.8	2.1	54	1,470
Mir A12	7	1.75	48	1,380
	5.8	1.8	50	1,420

[a] Evaluation of whole cell peptidoglycan content was made by calculating the amount of polymer isolated from a known weight of cells. The results are expressed as percentages of the salt-free dry weights of the cells.

[b] Two milligrams of cells (dry weight) labeled with [^{3}H]DAP were suspended in 2 ml of 4 N HCl and hydrolyzed for 14 h at 104°C. The hydrolysate was dried and dissolved in 1 ml of water. A 0.5-ml amount was analyzed for amino acid content, using a Beckman amino acid analyzer, and 0.5 ml was analyzed for radioactivity.

transition was observed in all the described experiments approximately 60 min after the pH shift.

It is evident, therefore, that the shift to pH 7, causing a transition from rod to coccal shape, also determines a twofold decrease in the rate of peptidoglycan synthesis, whereas the shift to pH 5.8, causing transition from coccal to rod shape, causes an approximate twofold increase in the same rate.

Determination of amounts of peptidogly-

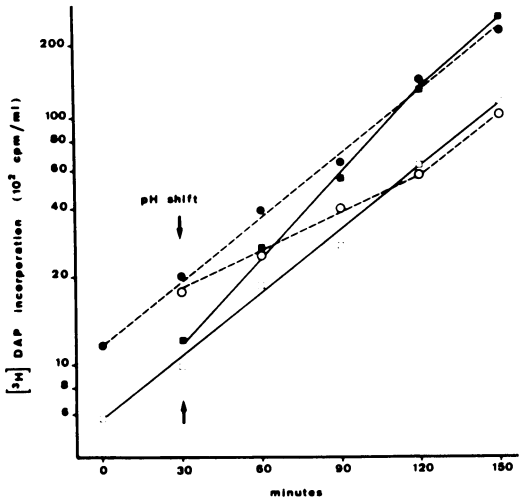

FIG. 3. *Effect of pH shift on peptidoglycan synthesis of K. pneumoniae Mir M7. Cells growing exponentially at pH 5.8 (●) were transferred to pH 7. (○). Cells growing exponentially at pH 7 (□) were transferred to pH 5.8 (■). At intervals duplicate samples (1 ml) were pulse-labeled for 5 min with [³H]DAP as described in the text.*

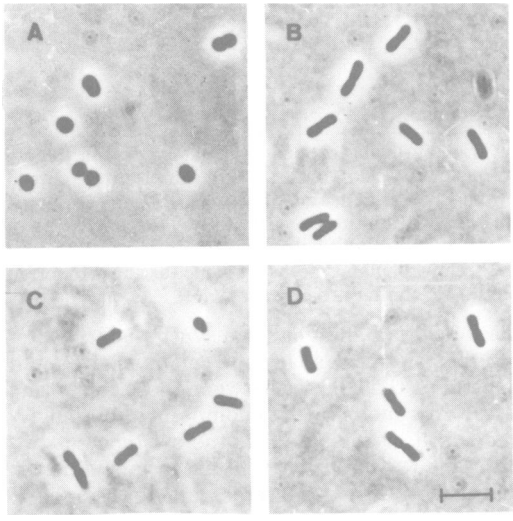

FIG. 4. *Morphologies of K. pneumoniae strains Mir M7 (A, B) and Mir A12 (C, D) grown at pH 7 (A, C) and pH 5.8 (B, D). Bar, 3 μm.*

can in rods and cocci. If the changes in the rates of [³H]DAP incorporation into trichloroacetic acid-precipitable material associated with the pH shift and change of shape were due to a reduced capability of synthesizing peptidoglycan, then Mir M7 rods would have contained approximately twice the amount of peptidoglycan contained in cocci. Although the peptidoglycan content of Mir A12 cells was not influenced by the pH, Mir M7 cocci contained half the amount of the peptidoglycan contained in rods (Table 2).

Rate of peptidoglycan synthesis during cell cycle in synchronously dividing cells. The time course of peptidoglycan synthesis of the mutant Mir M7 (Fig. 5) grown as synchronous rods at pH 5.8 was similar to that already described for other wild-type strains (12, 18). The rate of [³H]DAP incorporation remained constant for the first 20 min and then rose for the following 20 min, corresponding to the first synchronous cell division.

The time course of peptidoglycan synthesis of synchronous Mir M7 cells grown as cocci at pH 7 was significantly different. In no stage of the cell cycle did the rate of [³H]DAP incorporation appear to be constant. On the contrary, it continuously decreased during the first 20 min of incubation and then began to rise 20 min before the first synchronous division. During the second synchronous cell cycle, a similar time course of peptidoglycan synthesis was seen. As for protein synthesis, the rate of incorporation of leucine was exponential throughout the growth cycle, and no differences could be seen in the rates of [²H]leucine incorporation of Mir M7 synchronous cocci and rods (data not shown).

Analysis of peptidoglycan turnover in Mir M7 and Mir A12 cells. It has been recently shown that in *E. coli* autolysins are activated by suspending the cells in sucrose (13). Since the synchronization technique used included centrifugation through a 5 to 30% sucrose gradient, we checked the possibility that the apparent decrease in the rate of [³H]DAP incorporation shown by Mir M7 cocci was due to a stimulation of peptidoglycan turnover.

Peptidoglycan turnover of Mir M7 cocci was not activated during cell synchronization. Therefore, interference of peptidoglycan turnover in the apparent time course of peptidoglycan synthesis of synchronous Mir M7 cocci can be excluded (data not shown).

Different susceptibilities of rods and cocci to β-lactam antibiotics. It has been recently suggested that, among the penicillins and cephalosporins, there are some which inhibit lateral wall elongation, whereas others inhibit septum formation (19, 41, 42). It has been proposed in particular that cephalexin at low doses inhibits septum formation, whereas mecillinam inhibits lateral wall elongation. We determined the 50% lethal doses of the above antibiotics on Mir M7 rods and cocci. The susceptibilities to cephalexin of Mir A12 were almost identical at pH 7 and pH 5.8 (Table 3). On the contrary, cocci of the mutant Mir M7 grown at pH 7 turned out to be approximately sevenfold

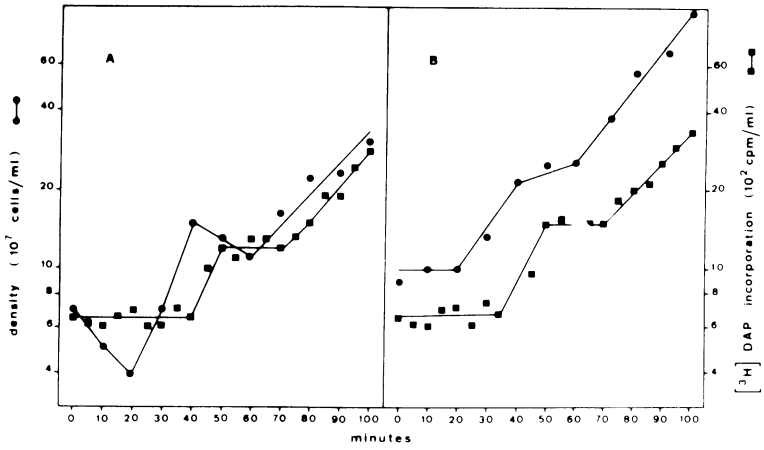

FIG. 5. *Incorporation of [^{3}H]DAP into synchronized K. pneumoniae Mir M7 cells grown at pH 7 (A) and pH 5.8 (B). Exponentially growing cells were synchronized as described in the text. Every 10 min, duplicate samples (1 ml) were pulse-labeled for 5 min with [^{3}H]DAP.*

more susceptible to cephalexin than were the rods of the same mutant tested at pH 5.8. Also, the susceptibilities to penicillin G of strain Mir A12 were identical at both pH values, whereas strain Mir M7 turned out to be over 3.5-fold more susceptible when grown as cocci at pH 7 than when grown as rods at pH 5.8. Cocci and rods of Mir M7 were resistant to 200 μg of mecillinam per ml when the 50% lethal dose was considered. However, we show in another paper (Satta et al., submitted for publication) that, whereas mecillinam has no effect on the shape of cocci, this same antibiotic causes the transition from rod to coccal shape in both Mir M7 and Mir A12. This indicates that Mir M7 rods (as opposed to cocci) are fully susceptible to mecillinam when the effect on cell shape is considered.

It has been shown recently that the susceptibilities of some gram-positive microorganisms to lysis by penicillins are influenced by the pH of the medium (21). However, the different susceptibilities shown by Mir M7 cocci and rods cannot directly depend on the pH of growth, because the parental strain shows almost the same susceptibility to both antibiotics at pH 5.8 and 7. Therefore, Mir M7 cocci appear particularly susceptible to the β-lactam antibiotic that inhibits septum formation, whereas Mir M7 rods are particularly susceptible to the β-lactam which is known to inhibit lateral wall extension.

TABLE 3. *Effect of pH of the medium on the susceptibilities of strains Mir M7 and Mir A12 to some β-lactam antibiotics*

Antibiotic	50% lethal dose (μg/ml)[a]			
	Mir M7		Mir A12	
	pH 7	pH 5.8	pH 7	pH 5.8
Penicillin G	10	35	35	35
Cephalexin	0.3	2	1.5	2
Mecillinam	>200	>200	0.7	0.7

[a] Cells growing exponentially at pH 7 and pH 5.8 were suitably diluted, and 0.1-ml samples were spread on Diagnostic Sensitivity Test agar plates containing different antibiotic concentrations. The concentration of antibiotic inhibiting colony formation to half that in the control plates after 18 h of incubation is reported as the 50% lethal dose.

DISCUSSION

The two different cell wall functions (see introduction) suggested by others (7, 17, 40) may well correspond to two specific enzyme reactions topologically located (sites) in the envelopes of rod-shaped bacteria: one responsible for lateral peptidoglycan synthesis and the other responsible for septal peptidoglycan synthesis. In bacterial rods, both cell shape and cell division should depend on the balance between these competing sites. This model of two competing peptidoglycan synthesis sites implies the existence of two different types of cell wall extension mutants: one defective in the lateral wall extension site, which would grow as cocci, and one defective in the site responsible for septation, which would make filaments. Many mutants of this last class have already been described and characterized as cell division mutants. Mir M7 is the first among the strains described as cell shape mutants which can be characterized as a mutant conditional for lateral wall extension.

We have shown here that in Mir M7 cocci the rate of incorporation of [^{3}H]DAP into trichloroacetic acid-precipitable material is 50% lower than that in Mir M7 rods (Fig. 1). Furthermore, direct isolation of the peptidoglycan layer from a known amount of cells has yielded 50% less

peptidoglycan in cells grown at pH 7 than it has in cells grown at pH 5.8. Such a reduced amount of peptidoglycan could be explained either by partial inhibition of both sites or by complete blockage of only one. Support for the second possibility comes from the studies on susceptibility to the antibiotics. Mir M7 cocci were found to be sevenfold more susceptible than rods to cephalexin, an antibiotic already described as a specific inhibitor of septum formation (41, 42), Mecillinam, which has been described as a specific inhibitor of cell elongation, showed no evident effects on Mir M7 cocci but was able to cause a transition of rods to cocci (Satta et al., submitted for publication). Other authors have recently shown that mecillinam causes a 50% reduction in the rate of peptidoglycan synthesis of susceptible gram-negative rods (4, 31). These findings are consistent with mecillinam being a specific inhibitor of the site responsible for lateral peptidoglycan synthesis and indicate that in the cocci this specific site is missing.

The labeled DAP incorporations of synchronous cells have shown that in the Mir M7 cocci peptidoglycan synthesis during septation is equal to that in the rods. The cocci and rods differ in their incorporation patterns after septum synthesis is terminated, during the intervals between septations. Whereas in the cocci there is practically no synthesis during this period, the rods still incorporate at a constant rate. This finding indicates that the reduction in the rate of peptidoglycan synthesis evidenced in Mir M7 cocci with respect to Mir M7 rods is linked to the specific inhibition of peptidoglycan synthesis at the phase of the cell cycle during which cell elongation occurs in the rods. This therefore strongly supports the proposal that in Mir M7 incubated at pH 7 one of the two sites, the one for lateral peptidoglycan synthesis, is inhibited.

Other characteristics of strain Mir M7 can be explained by this model of two competing peptidoglycan synthesis sites. In fact, though pH 7 and 5.8 have been generally used to grow Mir M7 cocci and rods, respectively, this strain can grow as cells of widely variable length. In general, cells appear longer the lower the pH (36). Extreme conditions in peptone-lactose medium (36) are pH 5, which causes formation of filaments, and pH 7, which determines the formation of cocci. When Mir M7 rods are transferred to pH 7 in the presence of a minimal dose (8 μg/ml) of penicillin G sufficient to block cell division, very long filaments are formed (37).

These phenomena indicate that in strain Mir M7 the balance between elongation and septation is influenced by the pH of the medium in such a way that, under the extreme conditions of pH 5, the site for elongation prevails and no septa can be formed. At pH 7, the site for lateral wall extension is inactive and cells can increase surface area only by septation. Under the intermediate conditions, at pH values between 5.2 and 6.8, both sites are active and a balance is reached between them, for which the lower the pH, the more elongation will occur.

Filament formation by Mir M7 rods in the presence of 8 μg of penicillin G per ml at pH 7 can also be explained by suggesting that penicillin G can correct the inhibitory effect of pH on elongation and stimulate the site for lateral wall extension, allowing it to prevail over the septation site. An interesting consequence of this suggestion is that the well-known specific inhibition of cell division by 8 μg of penicillin G per ml could also be achieved by stimulation of the site for lateral wall extension. The finding by Mirelman et al. (27) that a low dose of ampicillin (0.5 μg/ml) raises the rate of peptidoglycan synthesis in ether-treated cells supports this possibility.

The properties of strain Mir M7 here described are such as to make this mutant a valuable tool for identifying and possibly isolating enzymes and regulatory proteins responsible for the balance between lateral wall extension and cell septation, which regulates both cell shape and cell division.

The shape-altering mutation of strain Mir M7 has been preliminarily mapped by conjugation and will be described after we have determined a more precise location by coliphage P1 transduction.

LITERATURE CITED

1. **Adelberg, E. A., M. Mandel, and G. Chen.** 1965. Optimal conditions for mutagenesis by N-methyl-N'-nitrosoguanidine in *Escherichia coli* K12. Biochem. Biophys. Res. Commun. **18**:788-795.
2. **Boylan, R. J., and N. H. Mendelson.** 1969. Initial characterization of a temperature-sensitive rod$^-$ mutant of *Bacillus subtilis*. J. Bacteriol. **100**:1316-1321.
3. **Boylan, R. J., N. H. Mendelson, D. Brooks, and F. E. Young.** 1972. Regulation of the bacterial cell wall: analysis of a mutant of *Bacillus subtilis* defective in biosynthesis of teichoic acid. J. Bacteriol. **110**:281-290.
4. **Braun, V., and H. Wolff.** 1975. Attachment of lipoprotein to murein (peptidoglycan) of *Escherichia coli* in the presence and absence of penicillin FL 1060. J. Bacteriol. **123**:888-897.
5. **Chai, T., and J. Foulds.** 1974. Demonstration of a missing outer membrane protein in *tol*G mutants of *Escherichia coli*. J. Mol. Biol. **85**:465-474.
6. **Chatterjee, A. N., and F. E. Young.** 1972. Regulation of the bacterial cell wall: isolation and characterization of peptidoglycan mutants of *Staphylococcus aureus*. J. Bacteriol. **111**:220-230.
7. **Daneo-Moore, L., and G. D. Shockman.** 1977. The bacterial cell surface in growth and division, p. 597-715. *In* G. D. Poste and G. Nicolson (ed.), Cell surface reviews, vol. 4. Elsevier/North Holland Publishing Co., Amsterdam.
8. **Fontana, R.** 1978. The chemical composition of the peptidoglycan of *Klebsiella pneumoniae* strain Mir A12 and ATCC 13883. FEMS Microbiol. Lett. **3**:77-80.

9. **Fontana, R., G. Satta, and L. Calegari.** 1974. The role of divalent cations in the rigid layer of morphological and conditional mutant of *Klebsiella pneumoniae* Mir M7. Ann Microbiol. (Paris) **125B:**275-292.
10. **Fontana, R., G. Satta, L. Calegari, and C. A. Romanzi.** 1974. The murein-lipoprotein of *Klebsiella pneumoniae* Mir A12 and its morphology mutant Mir M7, p. 486-490. Proceedings of the First Intersectorial Congress of the IAMS. Science Council of Japan, Tokyo.
11. **Fujiwara, T., and S. Fukui.** 1972. Isolation of morphological mutants of *Agrobacterium tumefaciens*. J. Bacteriol. **110:**743-476.
12. **Hakenbeck, R., and W. Messer.** 1977. Oscillations in the synthesis of cell wall components in synchronized cultures of *Escherichia coli*. J. Bacteriol. **129:**1234-1238.
13. **Hartmann, R., S. B. Bock-Hennig, and U. Schwarz.** 1974. Murein hydrolases in the envelope of *Escherichia coli*. Properties *in situ* and solubilization from the envelope. Eur. J. Biochem. **41:**203-208.
14. **Henning, U.** 1975. Determination of cell shape in bacteria. Annu. Rev. Microbiol. **29:**45-60.
15. **Henning, U., K. Rehn, V. Braun, B. Hoehn, and U. Schwarz.** 1972. Cell envelope and shape of *Escherichia coli* K 12. Properties of a temperature-sensitive rod-mutant. Eur. J. Biochem. **26:**570-586.
16. **Henning, U., K. Rehn, and B. Hoehn.** 1973. Cell envelope and shape in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **70:**2033-2036.
17. **Higgins, M. L., and G. D. Shockman.** 1971. Procaryotic cell division with respect to wall and membranes. Crit. Rev. Microbiol. **1:**29-72.
18. **Hoffmann, B., W. Messer, and U. Schwarz.** 1972. Regulation of polar cap formation in the life cycle of *Escherichia coli*. J. Supramol. Struct. **1:**29-37.
19. **James, R., J. Y. Haga, and A. B. Pardee.** 1975. Inhibition of an early event in the cell division cycle of *Escherichia coli* by FL 1060, an amidinopenicillanic acid. J. Bacteriol. **122:**1283-1292.
20. **Lazdunski, C., and B. M. Shapiro.** 1972. Relationship between permeability, cell division, and murein metabolism in a mutant of *Escherichia coli*. J. Bacteriol. **111:**499-509.
21. **Lopez, R., C. Ronda-Lain, A. Tapia, S. B. Waks, and A. Tomasz.** 1976. Suppression of the lytic and bactericidal effects of cell wall-inhibitory antibiotics. Antimicrob. Agents Chemother. **10:**697-706.
22. **Lund, F., and L. Tybring.** 1972. 6β-Amidinopenicillanic acids: a new group of antibiotics. Nature (London) New Biol. **236:**135-136.
23. **Matsumoto, H., and T. Tazaki.** 1971. Genetic mapping of *aro, pyr,* and *pur* markers in *Klebsiella pneumoniae*. Jpn. J. Microbiol. **15:**11-20.
24. **Matsuzawa, H., K. Hayakawa, T. Sato, and K. Imahori.** 1973. Characterization and genetic analysis of a mutant of *Escherichia coli* K-12 with rounded morphology. J. Bacteriol. **115:**436-442.
25. **Meloni, G. A., and C. Monti-Bragadin.** 1962. Osservazioni sull'isolamento dall'uomo di sferoplasti "spontanei" derivati da un ceppo di *Klebsiella*. Ann. Sclavo **4:**143-152.
26. **Mirelman, D., Y. Yashouv-Gan, and U. Schwarz.** 1976. Peptidoglycan biosynthesis in a thermosensitive division mutant of *Escherichia coli*. Biochemistry **15:**1781-1790.
27. **Mirelman, D., Y. Yashouv-Gan, and U. Schwarz.** 1977. Regulation of murein biosynthesis and septum formation in filamentous cells of *Escherichia coli* PAT 84. J. Bacteriol. **129:**1593-1600.
28. **Mitchison, J. M., and W. S. Vincent.** 1965. Preparation of synchronous cell cultures by sedimentation. Nature (London) **205:**987-989.
29. **Normark, S.** 1969. Mutation in *Escherichia coli* K-12 mediating spherelike envelopes and changed tolerance to ultraviolet irradiation and some antibiotics. J. Bacteriol. **98:**1274-1277.
30. **Olden, K., S. Ito, and T. H. Wilson.** 1975. D-Alanine-requiring cell wall mutant of *Escherichia coli*. J. Bacteriol. **122:**1310-1321.
31. **Park, T. J., and L. Burman.** 1973. FL 1060: a new penicillin with a unique mode of action. Biochem. Biophys. Res. Commun. **51:**863-868.
32. **Rodolakis, A., P. Thomas, and J. Starka.** 1973. Morphological mutants of *Escherichia coli*. Isolation and ultrastructure of a chain-forming *envC* mutant. J. Gen. Microbiol. **75:**409-416.
33. **Rogers, H. J., M. McConnell, and I. D. J. Burdett.** 1970. The isolation and characterization of mutants of *Bacillus subtilis* and *Bacillus licheniformis* with disturbed morphology and cell division. J. Gen. Microbiol. **61:**155-171.
34. **Rogers, H. J., M. McConnell, and J. R. C. Hughes.** 1971. The chemistry of the cell walls of rod mutants of *Bacillus subtilis*. J. Gen. Microbiol. **66:**297-308.
35. **Rogers, H. J., P. F. Thurman, C. Taylor, and J. N. Reeve.** 1974. Mucopeptide synthesis by rod mutants of *Bacillus subtilis*. J. Gen. Microbiol. **85:**335-350.
36. **Satta, G., and R. Fontana.** 1974. Characterization of a conditional mutant with altered envelope showing pH-dependent morphology and temperature-dependent division. J. Gen. Microbiol. **80:**51-63.
37. **Satta, G., and R. Fontana.** 1974. Cell division, macromolecular synthesis and morphology dependent on the state of the envelope in a mutant of *Klebsiella pneumoniae*. J. Gen. Microbiol. **80:**65-75.
38. **Satta, G., C. Pruzzo, E. Debbia, and R. Fontana.** 1978. Close association between shape alteration and loss of immunity to superinfection in a wild-type *Klebsiella pneumoniae* stable lysogen which can be both immune and nonimmune to superinfection. J. Virol. **28:**772-785
39. **Satta, G., G. C. Schito, and G. A. Meloni.** 1969. Transizione bastoncino-sfera in un ceppo di *Klebsiella pneumoniae*, p. 247-253. Ultrastruttura delle forme coccoidi tipiche ed abnormi. XV Congresso Nazionale Microbiologia. Torino-Saint Vincent.
40. **Schwarz, U., A. Ryter, A. Rambach, R. Hellio, and Y. Hirota.** 1975. Process of cellular division in *Escherichia coli*: differentiation of growth zones in the sacculus. J. Mol. Biol. **98:**749-759.
41. **Spratt, B. G.** 1975. Distinct penicillin-binding proteins involved in the division, elongation, and shape of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **72:**2999-3003.
42. **Spratt, B. G.** 1977. Temperature-sensitive cell division mutants of *Escherichia coli* with thermolabile penicillin-binding proteins. J. Bacteriol. **131:**293-305.