Modified peptidoglycan chemical composition in shape-altered *Escherichia coli*

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Peptidoglycan synthesis and its fine chemical composition were studied in dividing cocci of *Escherichia coli* carrying the *lov-1* mutation and in which the coccal shape was obtained either by mecillinam treatment or by transferring a *pbpA* mutation (penicillin-binding protein 2⁻ phenotype), as compared to normal rods and non-dividing cocci. Synchronously dividing cocci showed peptidoglycan synthesis only in the cell cycle phase corresponding to cell septation. During the phase corresponding to lateral wall elongation, peptidoglycan synthesis was strongly reduced. This type of synthesis suggests that the dividing cocci consisted only of the two poles. Analysis of the muropeptide composition revealed a specific fourfold increase in the tetra-tetra trimer in dividing cocci as compared to non-dividing cocci or parental rods. We postulate that, in *E. coli*, the chemical composition of septal peptidoglycan differs from that of lateral wall peptidoglycan.

Keywords: *Escherichia coli*, peptidoglycan, bacterial shape, cell division

INTRODUCTION

Bacterial rods have two distinct machineries for peptidoglycan (PG) assembly which lead alternatively and in a mutually exclusive way to lateral wall elongation and to septation (for a recent review and the relevant literature see Satta *et al.*, 1994). For this reason rods are regarded as more evolved than cocci (Higgins & Shockman, 1971; Daneo-Moore & Shockman, 1977; Lleò *et al.*, 1990; Satta *et al.*, 1994) as a result of the insertion of a cylinder between the two poles of a coccus. It has also been claimed that a control mechanism exists which blocks septum formation when lateral wall elongation is not expressed in a normal rod, thus preventing the regression to a coccus (Satta *et al.*, 1980). An open question is whether the two machineries involved in PG synthesis lead to the formation of two biochemically distinguishable polymers (Driehuis & Wouters, 1987; Kraus & Høltje, 1987; Tuomanen & Cozens, 1987; Schwarz & Glauner, 1988; de Jonge *et al.*, 1989; Leduc *et al.*, 1989; Canepari *et al.*, 1993; Obermann & Høltje, 1994). In recent years Schwarz and co-workers (Glauner, 1988; Glauner *et al.*, 1988; Glauner & Høltje, 1990) have described a sophisticated HPLC technique for the determination of the fine chemical composition of Gram-negative PG. According

to this analysis a more complicated PG architecture is postulated. Alongside the classic monomers and dimers, trimers and tetramers have also been identified, thus suggesting PG organization in more than one plane, as in the case of Gram-positive bacteria (Glauner & Høltje, 1990). The availability of true coccal-shaped mutants, i.e. due to loss of the cylindrical portion of the cell, may shed light on this aspect.

We were the first to describe a morphology mutant of *Klebsiella pneumoniae* (the Mir M7 strain) which, when grown as cocci, has lost the cylindrical portion and whose cells consist only of poles. Cells divide regularly in the coccal shape due to the loss of the above-mentioned control mechanism that blocks cell division when lateral wall elongation is prevented (Satta *et al.*, 1980). As regards the analysis of the fine chemical composition of PG, we showed that the Mir M7 strain dividing as cocci displays a modified muropeptide composition consisting in a 50-fold increase in the tetramer family. This alteration is not seen in the cocci that do not divide as such (Canepari *et al.*, 1993).

More recently, D'Ari and co-workers (Boulloc *et al.*, 1988, 1989) have described the *lov-1* mutation in *Escherichia coli* which produces a phenotype apparently similar to that of the Mir M7 strain. The strain carrying the *lov-1* mutation grows as rods, but in the presence of mecillinam [a β -lactam antibiotic that specifically binds penicillin binding protein 2 (PBP2) of *E. coli* and inhibits lateral wall

Abbreviations: PG, peptidoglycan; DAP, diaminopimelic acid; PBP, penicillin-binding protein.

formation (Spratt, 1975; Satta *et al.*, 1981)], cells become cocci which then continue to divide. The *lov-1* mutation (also known as *argS201*) is located in the *argS* gene; it affects tRNA synthetase and confers mecillinam resistance (Boulloc *et al.*, 1989). Evidence has been presented that the *lov* gene product interacts with the ribosomes, suggesting that the Lov protein may be capable of measuring the cytoplasmic concentration of ribosomes and of transmitting the information to PBPs so as to trigger cell division (a switch from elongation to septation) when the appropriate cell mass is achieved (Boulloc *et al.*, 1989).

In this paper we describe the characterization of strains carrying the *lov* mutation in terms of PG synthesis and fine chemical composition. The results indicate that mutants growing with a coccal shape consist mainly of poles, and analysis of the fine chemical composition of PG shows a dramatic increase in the trimer family, thus indicating that a difference may exist in biochemical composition of lateral and septal wall PG.

METHODS

Bacterial strains and growth conditions. Bacterial strains used in this study are listed in Table 1 together with their main characteristics. Strains were grown in LB broth (Miller, 1972) at 37 °C with vigorous shaking in a gyratory bath. Synchronously growing cells were obtained by the sucrose gradient method as previously described (Satta *et al.*, 1981). Cell number was monitored with a Coulter counter model ZBI equipped with a probe with a 50 µm orifice. OD₆₄₀ was monitored with an LKB Ultraspec II spectrophotometer.

Peptidoglycan synthesis evaluation. To evaluate PG synthesis in synchronous or exponentially growing cultures, 1 ml samples were taken in duplicate from the culture at the desired time and immediately transferred into a tube containing 100 µl 2,6-*meso*-diamino[³H]pimelic acid ([³H]DAP) (50 µCi ml⁻¹; 1.85 MBq ml⁻¹) and 5 µg ml⁻¹ of unlabelled DL-2,6-diaminopimelic acid (DAP, Serva). The tubes were incubated at 37 °C for 5 min, then 5 ml cold 5% (w/v) trichloroacetic acid (TCA) was added. After 30 min in ice, the suspension was filtered on a 0.22 µm Millipore filter, washed twice with 5 ml cold 5% TCA and with 5 ml ethanol. After drying at room temperature (30 min), the radioactivity of the filters was measured in a Beckman LS7000 scintillator.

Analysis of peptidoglycan by HPLC. One litre of exponentially growing cells (OD₆₄₀ 0.8) was collected by centrifugation at

4 °C and PG was prepared by the method proposed by Glauner *et al.* (1988). PG was completely digested by *Chalaropsis* muramidase (Hash & Rothlauf, 1967), and the mucopeptides released were reduced with borohydride and subjected to HPLC (Waters) under conditions established by Glauner *et al.* (1988) on a reverse-phase column (250 × 4 mm) filled with Lichrosorb RP18 (Merck). For temperature control a 655A-52 column oven (Hitachi) was used. The column was eluted at a flow rate of 0.5 ml min⁻¹ with a linear gradient (130 min run time) from 50 mM sodium phosphate buffer pH 4.31 to 75 mM sodium phosphate buffer pH 4.95 containing 15% (v/v) methanol. A temperature of 35 °C was used instead of the suggested temperature of 55 °C (Glauner *et al.*, 1988). This lower temperature was chosen for the purpose of enlarging the chromatogram for better calculation of the relative amount of each peak. The eluted compounds were detected at 202 nm. The relative molar amounts of various compounds were calculated from the integration of the UV response and by applying the formula described by Glauner (1988). The various mucopeptides were grouped in families as follows: (1) disaccharide peptides (di-, tri-, tetra-, penta-peptides) or monomers; (2) bis-disaccharide peptides or dimers (two cross-linked monomers); (3) tri-disaccharide peptides or trimers; (4) tetra-disaccharide peptides or tetramers; (5) bis- and tri-disaccharide peptides with DL-2,6-diaminopimelic acid interpeptide bonds (DAP-DAP mucopeptides); (6) mono-, bis- and tri-disaccharide peptides with Lys-Arg residues of lipoprotein (lipoprotein mucopeptides); (7) mono-, bis- and tri-disaccharide peptides with 1,6-anhydromuramic acid at the end of the glycan strand (anhydro mucopeptides) (Glauner *et al.*, 1988).

Antibiotics, chemicals and radiochemicals. Mecillinam was a generous gift from Leo Pharmaceutical Products, Ballerup, Denmark. [³H]DAP (250 µCi mmol⁻¹; 9.25 MBq mmol⁻¹) was from Amersham. All the reagents used were commercially purchased reagent grade (Merck) and the chemicals used for HPLC were HPLC grade from J. T. Baker.

RESULTS

Effect of mecillinam on *E. coli* strains carrying the *lov* mutation

Mecillinam is a β-lactam antibiotic which, by binding specifically to PBP2 and by specific inhibition of lateral wall elongation (Spratt, 1975; Satta *et al.*, 1981), transforms Gram-negative rods into cocci, which are incapable of further division [see also Fig. 1b, parent strain GC2700]. When 1 µg mecillinam ml⁻¹ was added to an exponentially growing culture of strain GC2702 (*lov-1*), as

Table 1. *E. coli* strains used and their relevant characteristics

Strain	Genotype	Relevant characteristics	Source/reference
GC2700	F ⁻ (λ ⁻) prototroph	Parent, rod-shaped, sensitive to mecillinam	Boulloc <i>et al.</i> (1989)
GC2702	As GC2700 but <i>argS201</i> (<i>lov-1</i>)	Rod shaped, sensitive to mecillinam as for morphological effect and resistant as for cell division inhibition	Boulloc <i>et al.</i> (1989)
GC5391	<i>argS201</i> (<i>lov-1</i>) <i>pbpA</i>	Coccal shaped, PBP 2 ⁻ and mecillinam resistant	R. D'Ari, Université Paris 7, France

Fig. 1. Effect of mecillinam ($1 \mu\text{g ml}^{-1}$) on cell division and cell mass increase in exponentially growing *E. coli* strain GC2702 (*lov-1*) (a) and its parent GC2700 (b). ●, Untreated control; ○, mecillinam added at time zero. Results of a representative experiment.

expected, after 60–90 min incubation cells were converted to cocci which continued to divide regularly (Fig. 1a), in contrast to the parental strain GC2700 which, as soon as the cells became cocci, immediately stopped dividing (Fig. 1b); the cell mass continued to increase in both strains for a few hours. In strain GC5391 (carrying *lov-1* and *phpA* mutations), which constitutively shows a coccal morphology, mecillinam had no effect on cell shape or cell division (data not shown).

Effect of mecillinam on PG synthesis of synchronous cells carrying the *lov* mutation

Before evaluating PG synthesis as incorporation of [^3H]DAP, and since we did not have a DAP⁻ Lys⁻ mutant, we defined the fate of this radioactive precursor when incorporated into the macromolecules. In a representative experiment with strain GC2700, of 37 560 c.p.m. TCA-precipitable [^3H]DAP radioactivity, 79% was recovered from PG. This result is consistent with the observation of Leive & Davis (1965), who demonstrated, in a prototrophic *E. coli* strain, that 'exogenous DAP regularly contributed a larger fraction of wall DAP than of protein lysine,' on the basis of a gradient as compared to the relative contribution from an exogenous and endogenous source, in such a way that, if the enzymes responsible for DAP incorporation into wall peptide are more peripherally located than DAP decarboxylase, wall DAP would be derived preferentially from the peripheral part of the intracellular DAP pool. In the above experiment with strain GC2700, only 3% of [^3H]DAP was incorporated into proteins and into PG cytoplasmic precursors. This value is probably underestimated, since we evaluated the residual radioactivity simply by measuring it in a small volume of the supernatant of cells

Fig. 2. Effect of mecillinam ($1 \mu\text{g ml}^{-1}$) on peptidoglycan synthesis and number of cells of *E. coli* GC2700 (parent) in a synchronously growing culture. ●, Untreated control; ○, mecillinam added at time zero; △, mecillinam added at 20 min. Results of a representative experiment.

boiled with SDS and multiplying this value by the total volume. The low count and the quenching due to the aqueous suspension may have enlarged the error to such an extent as to underestimate the amount of the PG precursors, which should be anything but negligible in these conditions.

Fig. 2 shows, by way of a control, that in the parent strain GC2700 inhibition of peptidoglycan synthesis was caused by mecillinam only when the antibiotic was added to cells at the very beginning of the cell cycle. When the antibiotic was added later (at 20 min, when lateral wall elongation had already started) no effect was observed until the beginning of the next cell cycle. Under conditions causing inhibition of PG, the inhibition reached about 60–70% within the first 20 min. After this the synthesis of the macromolecule continued at a constant rate. As regards cell division, Fig. 2 also reveals that addition of mecillinam always allowed additional cell division, thus clearly showing that the shape change in normal rods was accompanied by an approximately 50–60% reduction in the rate of PG synthesis. When a similar experiment was performed with strain GC2702 (*lov-1*), mecillinam immediately inhibited PG synthesis not only when added at the very beginning of the cell cycle, but also during the

Fig. 3. Effect of mecillinam ($1 \mu\text{g ml}^{-1}$) on peptidoglycan synthesis and number of cells of *E. coli* GC2702 (*lov-1*) in a synchronously growing culture. ●, Untreated control; ○, mecillinam added at time zero; △, mecillinam added at 20 min; □, mecillinam added at 40 min. Results of a representative experiment.

first 30 min (Fig. 3). Nevertheless, in contrast to the parental strain, the inhibition of PG synthesis was followed by a rapid increase in the rate of synthesis of this polymer for another 20 min until just before the next

division. After this division, a rapid decrease in PG synthesis was observed. It should be recalled that cells of strain GC2702 became cocci after the first synchronous division and then continued to divide with this shape. This type of PG synthesis was reminiscent of that described for the morphology mutant *K. pneumoniae* Mir M7 (Satta *et al.*, 1979; Canepari *et al.*, 1993). When PG synthesis was evaluated in the constitutive cocci of strain GC5391 an up and down rate of PG synthesis was observed like that of strain GC2702 after rod to coccus transition caused by mecillinam (data not shown). The experiments described above were repeated three to five times with identical results.

Chemical composition of PG in strains carrying the *lov* mutation

HPLC analysis of mucopeptides obtained by muramidase digestion of purified PG was performed in all strains used in this study. Fig. 4 shows a typical chromatogram for mucopeptides of the parental strain GC2700. Data for all three strains investigated are tabulated as the relative amount of each PG component in Table 2, and collected in families of mucopeptides in Table 3. From Fig. 4 and Table 2 we see that a representative *E. coli* mucopeptide chromatogram is very similar to that obtained by Glauner *et al.* (1988). As regards the mecillinam effect, a slight increase was observed in the tetra-tetra dimer and in the tetra-tetra-tetra trimer, such as in DAP-DAP mucopeptides, lipoprotein-carrying mucopeptides and anhydro mucopeptides, as previously indicated by others (Blasco *et al.*, 1988). When strain GC2702 (*lov-1*) was compared to the parental strain (it should be recalled that both strains are rod shaped), no significant differences were observed. However, when strain GC 2702 (*lov-1*) was converted to cocci by mecillinam treatment, a 3.8-fold increase in the tetra-tetra-tetra trimer was observed, which caused a

Fig. 4. HPLC profile of *E. coli* GC2700 mucopeptides. Mucopeptide peak numbers are identified in Table 2.

Table 2. Composition of PG in various *E. coli* strains treated or not with mecillinam

Mecillinam (Mec, 1 µg ml⁻¹) was added in the early exponential growth phase (OD₆₄₀ 0.2) and treatment was performed for 2 h. Data are from a representative experiment. anh, anhydro; ND, not detected.

Muropeptide	Composition of muropeptides (molar fraction × 100) in strain:				
	GC2700	GC2700 + Mec	GC2702	GC2702 + Mec	GC5391
1. Tri	4.7	3.9	4.4	4.5	3.8
2. Tetra (Gly)	1.6	1.4	1.6	1.5	1.4
3. Tetra	51.8	42.0	50.7	35.8	36.0
4. Penta (Gly)	0.2	0.2	0.2	0.1	0.2
5. Di	1.4	1.4	1.2	1.5	1.6
6. Penta	0.1	0.1	0.1	0.1	0.1
7. Tri-Lys-Arg	5.1	7.1	5.3	7.8	7.9
8. Tri (anh)	0.1	0.1	ND	0.2	0.1
9. Tetra-tri (Gly)	0.1	0.1	0.1	0.2	0.2
10. Tri-tri (DAP-DAP)	ND	0.3	0.1	0.6	0.5
11. Tetra-tri (DAP-DAP)	2.1	3.1	2.3	3.8	3.7
12. Tetra-tri	3.9	4.2	3.8	4.3	4.4
13. Tetra-penta (Gly)	0.1	0.1	ND	0.3	0.2
14. Tetra-tetra	19.4	22.6	20.1	17.5	18.3
15. Tetra-penta	0.4	0.7	0.4	0.7	0.6
16. Tri-tri (DAP-DAP)-Lys-Arg	0.5	1.3	0.6	1.7	1.5
17. Tetra (anh)	0.6	0.6	0.8	0.5	0.6
18. Tetra-tetra-tri (DAP-DAP)	0.5	0.7	0.5	0.7	0.9
19. Tetra-tetra-tetra	2.9	4.1	3.0	11.4	9.5
20. Tetra-tri-Lys-Arg	2.1	2.2	2.1	2.3	2.1
21. Tri-tri (DAP-DAP) (anh)	0.1	0.2	0.1	0.2	0.2
22. Tetra-tri-tri (DAP-DAP)-Lys-Arg	0.1	0.2	0.1	0.2	0.4
23. Tetra-tri (anh)	0.2	0.2	0.2	0.1	0.2
24. Tetra-tri (DAP-DAP) (anh)	0.5	0.7	0.6	0.7	0.9
25. Tetra-tetra (anh)	1.3	1.9	1.4	1.9	1.8
26. Tetra-tetra-tri (anh)	ND	0.2	0.1	0.1	0.2
27. Tetra-tetra-tri (DAP-DAP) (anh)	ND	ND	ND	ND	0.4
28. Tetra-tetra-tetra (anh)	0.6	0.7	0.5	0.7	0.9
29. Tetra-tri-Lys-Arg (anh I)	0.1	0.2	0.1	0.4	0.8
30. Tetra-tri-Lys-Arg (anh II)	0.1	0.2	0.1	0.2	0.6
31. Tetra-tetra-tri-Lys-Arg (anh)	ND	ND	ND	ND	0.2

consequent 3.2-fold increase in the total trimer family (Table 3). This increase in the trimer family was accompanied by a decrease in the monomer family, especially in the tetrapeptide monomer (Table 3). To rule out the possibility that the altered PG chemical composition in strain GC2702 grown in the presence of mecillinam was due to the antibiotic treatment, we examined the muropeptide composition of strain GC5391, which carries *lov-1* and *pbpA* mutations and for this reason has a stable dividing round shape. Tables 2 and 3 clearly indicate that the above-described alterations of the *lov-1* mutant growing with a round shape in the presence of mecillinam were also observable when a mutation conferring absence of PBP2 and thus a stable round shape was present, suggesting that they were due to the shape

alteration rather than to the effect of the antibiotic treatment.

DISCUSSION

The data presented here show that the *E. coli* strain carrying the *lov-1* mutation, either when grown in the presence of mecillinam, which inhibits the lateral wall formation of a rod (Spratt, 1975; Satta *et al.*, 1981), or in association with a *pbpA* mutation (PBP2⁻ phenotype), has probably lost the cylindrical portion between the two poles of the cell. This is indicated by the fact that PG is synthesized predominantly in a specific phase of the cell cycle corresponding to the time of septum formation. During the phase of the cell cycle corresponding to lateral

Table 3. Composition of the PG purified from dividing and not dividing cocci and rods of *E. coli*

Data are from a representative experiment.

Strain	Mecillinam present*	Cell shape	Composition of mucopeptides (molar fraction $\times 100$)†					
			Monomers	Dimers	Trimers	DAP-DAP mucopeptides	Lipoprotein mucopeptides	Anhydro mucopeptides
GC2700	—	Rods	65.1	30.8	4.1	3.8	8.0	3.6
	+	Cocci (not dividing)	56.2	37.9	5.9	6.5	11.2	5.0
GC2702	—	Rods	63.8	32.0	4.2	4.3	8.3	3.9
	+	Cocci (dividing)	52.0	34.9	13.1	7.9	12.6	5.0
GC5391	—	Cocci (dividing)	51.6	35.9	12.5	8.5	13.5	6.9

* Where indicated (+), mecillinam ($1 \mu\text{g ml}^{-1}$) was added in the early exponential growth phase (OD_{640} 0.2) and treatment was performed for 2 h.

† Tetramers were undetectable in any of the strains, even after cell shape change induced by mecillinam.

wall elongation, a dramatic reduction in the synthesis of PG was observed in cocci. This type of PG synthesis is reminiscent of that of the *K. pneumoniae* strain Mir M7 (Satta *et al.*, 1979; Canepari *et al.*, 1993) when grown with a round shape. These data suggest that *E. coli* strains carrying the *lov-1* mutation and in which a round shape was obtained either by mecillinam treatment or by inserting a *pbpA* mutation consist mainly of the two poles. At present it is not possible to claim that PG of cocci is made exclusively of polar (or septal) PG; however, this conclusion may be possible, also bearing in mind that the synchronous growth in the experiments presented here was not ideal (see Figs 2 and 3) and therefore allowed partial overlapping of lateral and septal wall PG synthesis. This may account for the fact that PG synthesis during the phase of the cell cycle corresponding to cell elongation was not completely suppressed in cocci.

Analysis of the fine chemical composition of PG indicated that specific alterations can be observed that may be directly attributable to the altered shape rather than to the effect of mecillinam. Most significantly, a dramatic increase in the tetra-tetra-tetra trimer was observed. This may also be related to cell division ability in the round shape, given the fact that a considerable increase in this mucopeptide family was observed only in dividing cocci of the mutant GC2702 (*lov-1*) treated with mecillinam, but not in cocci of the parent GC2700 which were treated with mecillinam but were unable to divide. Other alterations in PG composition such as the increase in dimers (especially those containing the DAP-DAP linkage) or in lipoprotein-carrying mucopeptides, as previously suggested by others (Blasco *et al.*, 1988; Obermann & Hölte, 1994) were also observed, but may have been related to a mecillinam effect on cell shape rather than to the ability to divide in the altered shape. The dramatic and specific increase in the tetra-tetra-tetra trimer in dividing cocci was also strongly supported by the finding of a similar

increase in the trimer family when a round shape was obtained in the *lov-1* mutant not only by mecillinam treatment but also by inserting a *pbpA* mutation which mimics the mecillinam effect as far as shape is concerned. Consequently, it clearly emerges from this study that the increase in the trimer family is observable in dividing cocci, thus indicating that this may be a mechanism whereby cell division is allowed in *E. coli* cocci.

For some time it has been a debatable issue whether or not there are any real differences in the biochemical composition of septal and lateral wall PG. Many authors have suggested the existence of two distinct machineries involved in PG assembly (Spratt, 1975; Daneo-Moore & Shockman, 1977; Satta *et al.*, 1979; de Jonge *et al.*, 1989; Lleó *et al.*, 1990), but some have denied the existence of any such biochemical differences (Driehuis & Wouters, 1987; de Jonge *et al.*, 1989). Recently, Obermann & Hölte (1994) compared *E. coli* minicells (which hypothetically lack cylindrical PG and therefore may consist of regular polar cap PG only) with normal whole cells (rods) and demonstrated alterations in PG structure consisting in an increase in DAP-DAP cross-bridged mucopeptides, a reduction in the monomer family and a shortening of the length of glycan strands; but these authors were unable to present conclusive evidence of a specific correlation between the shape of the PG and its chemistry. It should be recalled here that minicells are DNA-less cells and are incapable of further division. For this reason they may be similar to non-dividing cocci obtained by mecillinam treatment of normal rods. Recently, we also described an increase in the tetramer family in dividing cocci of the *K. pneumoniae* strain Mir M7 (Canepari *et al.*, 1993).

Since, as claimed by Glauner *et al.* (1988), trimers and tetramers are involved in generating a PG with a multilayer structure, we suggest here that this may be the structure of septal (or polar) PG. Since trimers and

tetramers are greatly reduced in normal dividing rods as compared to dividing cocci (consisting of poles), we suggest, speculatively, a monolayer organization for lateral wall PG. This conclusion is not at variance with the report of Driehuis & Wouters (1987), denying the existence of biochemical alterations in *E. coli* cocci (short rods, enriched with poles, as the authors suggest), as compared to normal rods. This can be explained quite simply by the fact that in those experiments polar PG was contaminated by an excess of lateral wall. That structural (and possibly biochemical) differences may exist in PG has already been proven by analysis of PG by neutron small-angle scattering, which suggests a partially multilayered PG in *E. coli*, with 20–25% of the total PG organized in a triple layer (Labischinski *et al.*, 1991). However, these authors were unable to establish whether the distribution of single- and triple layered structures occurring in two clearly distinct regions of the sacculus was due to (i) the existence of a single-layered PG in the cylindrical wall region and a triple-layered PG at the polar caps or (ii) the existence of multilayered regions across some areas of the cylindrical surface representing the growth zones in an inside-to-outside growth model, as proposed by Glauner *et al.* (1988). Recently, Park (1993) analysed turnover and recycling of the PG sacculus and concluded that the PG organization of *E. coli* was essentially monolayered. However, on comparing and integrating his results with those of Labischinski *et al.* (1991), Park (1993) also suggested speculatively that, while lateral wall PG may be monolayered, septal wall PG (and poles) may be triple-layered. Now, for the first time, biochemical evidence supporting this conclusion has been found in *E. coli*. As compared to our previous results in which we demonstrated an excess of tetramers in dividing cocci of *K. pneumoniae* Mir M7 (Canepari *et al.*, 1993), we can speculate that, in these two related micro-organisms, differences in PG chemical composition of septal and lateral wall PG do exist; the septa are multilayered, but this goal is reached via two different routes, i.e. preferential synthesis of trimers in *E. coli*, and tetramer production in *K. pneumoniae*.

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