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STRUCTURE AND BIOSYNTHESIS OF THE BACTERIAL WALL PEPTIDOGLYCAN

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1. Introduction

The location of the peptidoglycan polymer among the bacterial extraprotoplasmic structures has been clearly established. Electron microscopy of thin sections of Gram-positive bacteria show the wall to be a, 200 to 800 Å thick, rather amorphous layer which completely surround the cell. The wall covers an alternating electron-dense-electron-transparent layering of about 75 Å of thickness which is the plasma membrane. Sonic or mechanical disruption of the Gram-positive bacteria, followed by differential centrifugation, yields homogenous preparations of walls. In the electron microscope, these appear as empty bags which retain the shape and the size of the original cells. The rigid matrix of the walls, a peptidoglycan polymer, represents about 50% of the dry weight of these layers. It is essentially composed of six different compounds: two acetamidosugars, 2-acetamido-2-deoxy-D-glucose or *N*-acetylglucosamine, and 2-acetamido-2-deoxy-3-*O*-(D-1-carboxethyl)-D-glucose or *N*-acetylmuramic acid, and four amino acids, L-alanine, D-alanine, D-glutamic acid and a diamino acid, L-lysine or *meso*-diaminopimelic acid. Glycine, L-serine, L-threonine, D-aspartic acid, additional L-alanine residues and amide ammonia are, in some Gram-positive bacteria, also constituents of the peptidoglycan polymer. The rigid peptidoglycan layer in the envelopes of the Gram-negative bacteria is a thin layer, 20 to 30 Å thick, sandwiched between the underlying plasma membrane and an outer multiple-track layer composed of lipopolysaccharides, lipoproteins etc. It represents as little as 5 to 10% of the dry weight of the isolated envelopes, from which it can be isolated by means of techniques involving treatments with phenol and detergents. As far as known, the peptidoglycans in the Gram-negative bacteria are always composed of *N*-acetylglucosamine, *N*-acetylmuramic acid, L-alanine, D-alanine, D-glutamic acid and *meso*-diaminopimelic acid.

2. Structure of the peptidoglycan

The bacterial peptidoglycan is a network [1-3], composed of three constituents: glycan strands, peptide subunits and crosslinking bridges. A monolayer representative of such a network is given in fig. 1. Rigidity and insolubility are properties only of the intact network. A loss of integrity resulting from the breakdown of either the glycan or the peptide moieties brings about the solubilization of the whole complex and, as a result, the death of the bacterium.

2.1. *The glycan*

In all bacteria so far examined, the glycan strands consist of alternating β-1,4 linked *N*-acetylglucosamine and *N*-acetylmuramic acid residues, both in the pyranose ring form, i.e., a chitin-like structure in which every other sugar is substituted by a 3-*O*-D-lactyl group. Analyses revealed native glycan chains averaging from 25 to 100 *N*-acetylhexosamine residues in length. Fig. 2 shows a glycan fragment with the established structure *N*-acetylglucosaminyl-β-1,4-*N*-acetylmuramyl-β-1,4-*N*-acetylglucosaminyl-β-1,4-*N*-acetylmuramic acid.

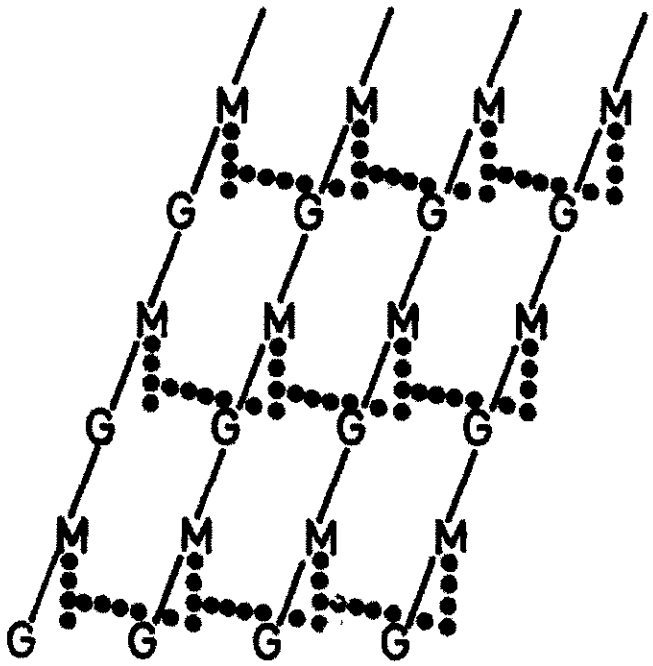

Fig. 1. Schematic representation of a peptidoglycan sheet. The glycan chains are composed of *N*-acetylglucosamine (G) and *N*-acetylmuramic acid (M). The vertical dots from M represent the peptide subunits. The horizontal dots represent the crosslinking bridges.

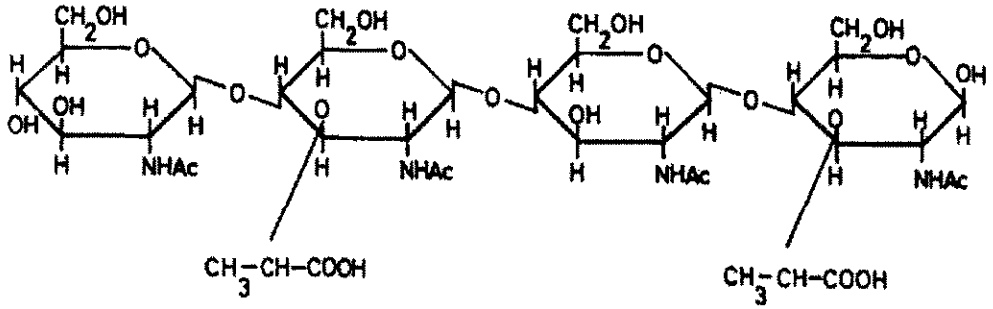

Fig. 2. Tetrasaccharide *N*-acetylglucosaminyl-β-1,4-*N*-acetylmuramyl-β-1,4-*N*-acetylglucosaminyl-β-1,4-*N*-acetylmuramic acid.

2.2. *The peptide subunits*

The peptide subunits substitute through their *N*-termini the lactic acid moiety of some of the *N*-acetylmuramic acid residues in the glycan. They usually have one of the following sequences: (1) N^{α}-(L-alanyl-D-isoglutaminyl)-L-lysyl-D-alanine (found in the *Lactobacteriaceae* and in many *Micrococcaceae*); (2) N^{α}[L-alanyl-γ(α-D-glutamyl-glycine)] - L-lysyl-D-alanine (found in *Micrococcus lysodeikticus* and other related *Micrococcaceae*); (3) L-alanyl-γ(?) D-glutamyl-*meso*-diaminopimelyl-D-alanine (found in *Propionobacteriaceae, Corynebacteriaceae,* and *Bacillaceae* and in all Gram-negative bacteria). The structure of these meso-DAP containing peptide subunits has not yet been completely elucidated. However, in the cases of *E. coli* [4] and of *B. megaterium* [5], it is known that the amino group of this amino acid engaged in peptide linkage to glutamic acid is located on the L-carbon of *meso*-DAP. Diamino acids other than L-lysine or *meso*-diaminopimelic acid are occasionally found in some bacterial peptidoglycans. LL-DAP, DD-DAP, hydroxyllysine, 3-hydroxy-2,6-diaminopimelic acid, ornithine, 2,4-diaminobutyric acid have been encountered. Finally, three peptide subunits in which the *N*-terminal amino acid, that is the one which is joined to the glycan, is not an L-alanine residue, have been characterized. These subunits have the following sequences: N^{α}(glycyl-threo-3-hydroxyglutamyl)-L-lysyl-D-alanine (in *Microbacterium lacticum*) [6], N^{α}(L-seryl-γ-D-glutamyl)-L-ornithyl-D-alanine (in *Butyribacterium rettgeri*) [7] and N^{α}(glycyl-γ-D-glutamyl)-homoseryl-D-alanine (in some plant-pathogenic *Corynebacteria*) [8].

2.3. *The peptide bridges*

The bridges which crosslink the peptide subunits have identical locations in almost all bacterial peptidoglycans. They extend from one of the amino groups of the diamino acid of one peptide subunit to the C-terminal D-alanine

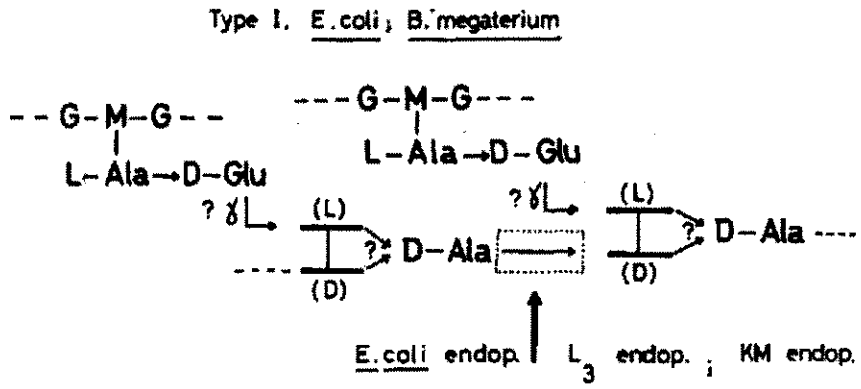

Fig. 3. Peptide subunits and bridging linkages in peptidoglycans of *Escherichia coli* and *Bacillus megaterium* KM. Arrows indicate the sites of action of peptidases.
G: *N*-acetylglucosamine M: *N*-acetylmuramic acid.

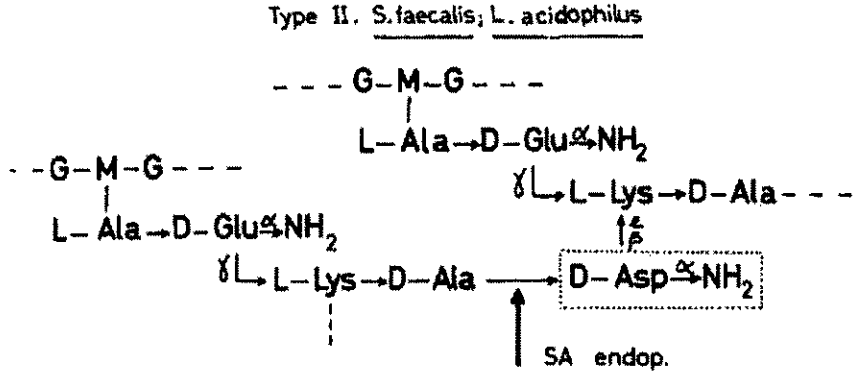

Fig. 4. Peptide subunits and peptide bridges in peptidoglycans of *Streptomyces faecalis* ATCC 9790 and other *Lactobacillaceae*. Arrows; G; M: see fig. 3.

carboxyl group of another peptide subunit. With all the L-lysine or *meso*-DAP-containing peptidoglycans so far studied, this is the ϵ-amino group of L-lysine or the amino group of the D-carbon of meso-DAP which are engaged in these crosslinkages. According to the species, the bridges present great variations in the chemical composition. Type I (fig. 3): The bridges result from direct peptide bonds between peptide subunits. This type of binding seems to be frequent among *meso*-DAP-containing peptidoglycans. In *Escherichia coli* [4] and *Bacillus megaterium* [5], the bridges have been characterized as D-Ala-(D)-meso-DAP linkages. Type II (fig. 4): The bridges are composed of one D-amino acid residue, namely D-isoasparagine [9]. Such a bridge is characteristic of *Lactobacilli* and of the lactic group of *Streptococci* type III [10] (fig. 5): The bridges are built up from several glycine and/or L-amino acid residues. These bridges are typical of many *Micrococcaceae* [11-13]. Type

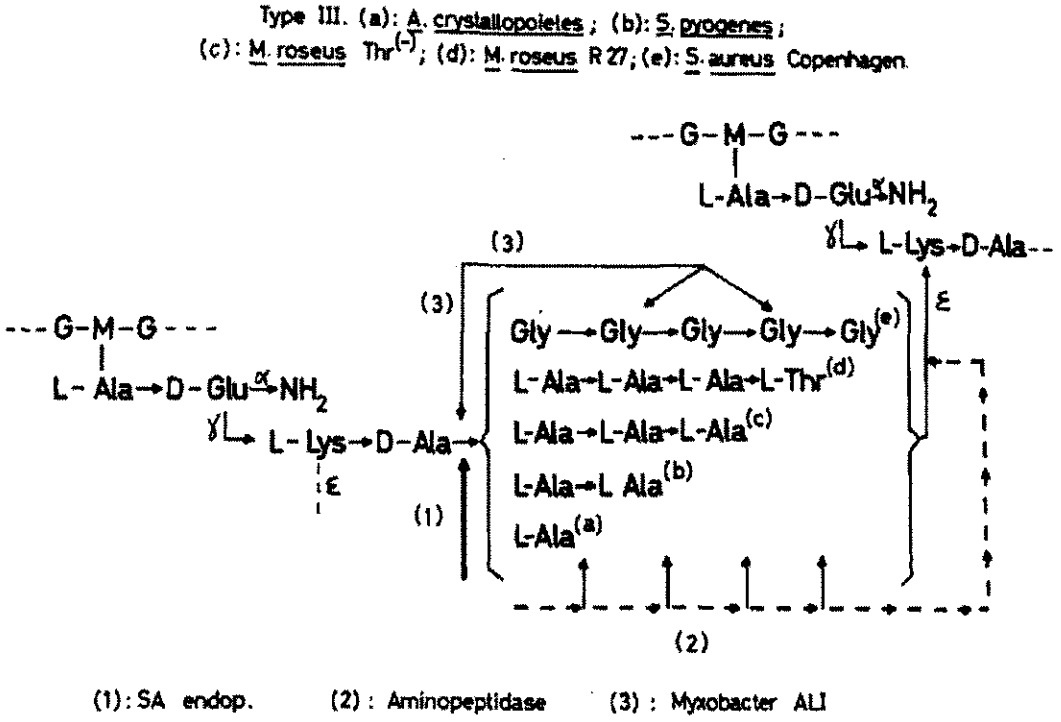

Fig. 5. Peptide subunits and peptide bridges in peptidoglycans of several *Micrococcaceae*. Arrows; G; M: see fig. 3.

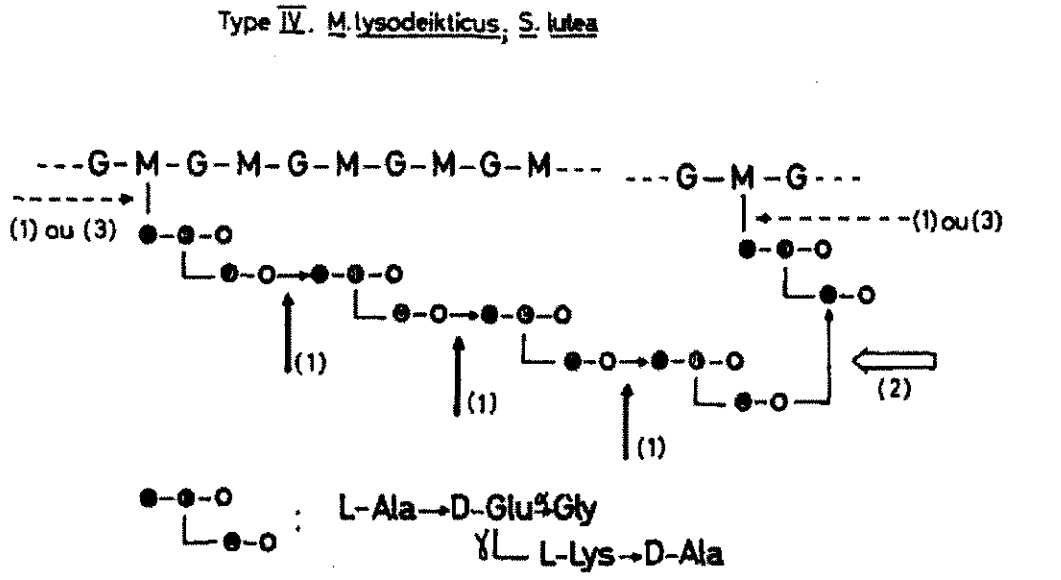

Fig. 6. Peptide subunits and peptide bridges in peptidoglycans of several *Micrococcaceae*. Arrows; G; M: see fig. 3.

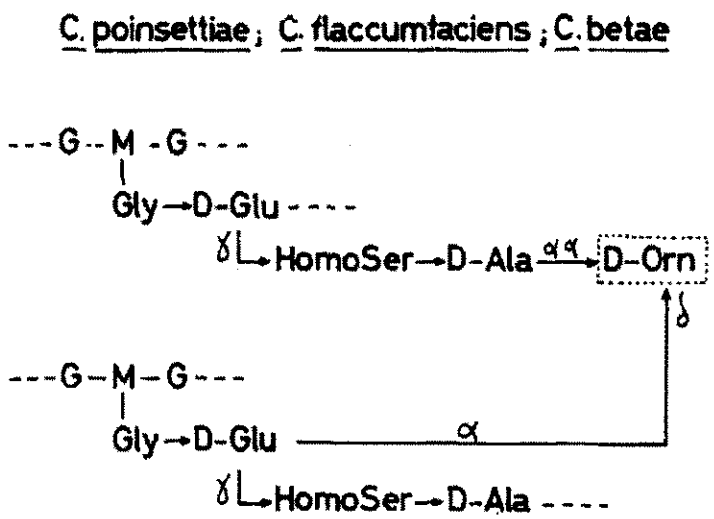

Fig. 7. Peptide subunits and peptide bridges in peptidoglycans of several plant pathogens *Corynebacteria* [8].

IV (fig. 6): The bridges are formed by a "head to tail" assembly of several peptides, each having that sequence of the peptide subunit, interlinked by D-alanyl-L-alanine linkages. This structure is typical of *Micrococcus lysodeikticus* and related *Micrococcaceae* [14-16]. Finally, attention should be called to the very unusual bridging which occurs in *Corynebacterium poinsettiae* (fig. 7) [8]. As pointed out above, the peptide subunits in this organism contain no diamino acid residue. They are bridged through D-ornithine residues which link, by their δ-amino group, the α-carboxyl group of the glutamic acid of one peptide subunit and, by their α-amino group, the carboxyl terminal D-alanine group of another peptide subunit.

3. Bacteriolytic enzymes [1-3]

The establishment of the structure of the bacterial peptidoglycans has resulted from the discovery of a series of hydrolytic agents of high specificity. These agents are enzymes which hydrolyze linkages at important connecting points within the peptidoglycans. Essentially three classes of enzymes have been used [1-3].

3.1. *Glycosidases*

Glycosidases are known which hydrolyze linkages either between *N*-acetylmuramic acid and *N*-acetylglucosamine (i.e., endo-*N*-acetylmuramidases) or between *N*-acetylglucosamine and *N*-acetylmuramic acid (i.e., endo-*N*-acetyl-

glucosaminidases). In most cases, the glycan chains are broken into small disaccharide fragments which remain substituted by the peptide moiety.

3.2. *N-acetylmuramyl-L-alanine amidases*

These enzymes hydrolyze the D-lactic acid-L-alanine bonds between the glycan strands (or the glycan debris) and the peptide subunits, i.e., at the *N*-terminus of the peptide subunits (fig. 6).

3.3. *Peptidases*

Several endopeptidases hydrolyze linkages which involve the C-terminal D-alanine of the peptide subunits. The following endopeptidases have been particularly useful. The *Streptomyces* KM endopeptidase [5] and the *E. coli* endopeptidase hydrolyze the cross-peptide linkages which serve as bridges between DAP-containing peptide subunits (fig. 3). The *Streptomyces* SA endopeptidase hydrolyzes the linkages at the *N*-termini of the peptide bridges of type II and III (figs. 4 and 5). The sensitive linkages are thus, for example, D-alanyl-glycine, D-alanine-L-alanyl, D-alanyl-D-isoasparaginyl. When opened at their *N*-termini, the type III peptide bridges can then be degraded by an aminopeptidase such as that secreted by *Streptomyces* strains (fig. 5). The amino acids are sequentially liberated until the ϵ-amino group of L-lysine in the peptide subunits, to which the peptide bridges are linked at their C-termini, are exposed. Quantitation of the number of free amino acids liberated, per lysine residue, at the end of the process, allowed the establishment of the stoichiometry of the bridges. Moreover, kinetics of the degradation permitted the determination of the bridge sequences. The *Streptomyces* ML endopeptidase acts on L-lysine containing peptidoglycans with peptide bridges of type IV in which it specifically hydrolyzes the N^{ϵ}-(D-alanyl)-L-lysine linkages (fig. 6). *Myxobacter* AL 1 enzyme has several sites of action in the wall peptide. Particularly, it is able to split D-alanyl-L-alanine linkages irrespective of their location, that is in bridges type III as does the SA endopeptidase (fig. 5) [11] and, in addition, the "head to tail" D-alanyl-L-alanine linkages in the type IV bridges (fig. 6) [16]. One notes that, in this latter case, the linkages hydrolyzed by *Myxobacter* AL 1 enzyme involve both C- and *N*-termini of the peptide subunits.

Several peptide subunits, isolated after sequential degradation of the isolated cell walls by means of these enzymes, have been completely characterized. With *Streptococcus faecalis* and *Lactobacillus acidophilus*, however, the D-isoasparaginyl bridges, after they had been opened by the SA endopeptidase, could not be liberated as free amino acids by means of the aminopeptidase (because of their D-configuration) (fig. 4). Consequently, the penta-

peptide N^{α}(L-alanyl-D-isoglutaminyl)-N^{ϵ}-(D-isoasparaginyl)-L-lysyl-D-alanine was isolated and characterized [9]. The structure of the *C. poinsettiae* peptidoglycan (fig. 7) has been elegantly established by Perkins [8]. His demonstration involved the isolation and characterization of the peptidoglycan precursor (*vide infra*) as well as the isolation of the peptide moiety after endo-*N*-acetylmuramidase degradation of the walls, followed by β-elimination of the peptide. Degraded peptides, arising from partial acid hydrolysis, were isolated and the structure was established using, among other procedures, the photolability of the dinitrophenyl derivatives.

4. Biosynthesis of the peptidoglycan [3]

In spite of the extreme variations in chemical composition and in structural details, all bacterial peptidoglycans possess essentially the same network type of structure and seem to be synthesized by means of analogous biosynthetic mechanisms. The major steps involved in the peptidoglycan synthesis by *S. aureus* and *E. coli* have been elucidated. The following is a generalization of these findings.

Firstly, all bacterial peptidoglycans would be synthesized from the two nucleotide precursors, the UDP *N*-acetylglucosamine and an UDP *N*-acetylmuramyl-pentapeptide with the composition of the peptide depending upon the bacterial species. In all cases, however, the pentapeptide would terminate at the C-terminus by a D-alanyl-D-alanine moiety. Examples of pentapeptide precursors are: L-Ala-D-Glu-L-Lys-D-Ala-D-Ala (*S. aureus; M. lysodeikticus*), L-Ala-D-Glu-*meso*-DAP-D-Ala-D-Ala (*E. coli*), Gly-D-Glu- HomoSer-D-Ala-D-Ala (*C. poinsettiae*) [8], L-Ser-D-Glu-L-Orn-D-Ala-D-Ala (*B. rettgeri*) [17].

Secondly, the synthesis of the precursors is followed by a complex reaction cycle which leads to the formation of a polyisoprenoid-P-P-*N*-acetylglucosaminyl-*N*-acetylmuramyl-(pentapeptide). This polyisoprenoid compound [18]) intermediate may be visualized as a means of transporting the peptidoglycan unit through the plasma membrane. During this passage, the disaccharide-pentapeptide is transferred to an endogenous acceptor, probably an incomplete peptidoglycan and, simultaneously, the polyisoprenoid-P-P is released. This polyisoprenoid-P-P is, subsequently, dephosphorylated (a reaction specifically inhibited by Bacitracin [19]), yielding the original phosphoisoprenoid carrier which can then enter a new cycle. While in the form of phosphoisoprenoid intermediates, the peptide subunits undergo several modifica-

tions such as amidation of the α-carboxyl group of glutamic acid (in *S. aureus*, for example), substitution of the same caboxyl group by a glycine residue (in *M. lysodeikticus*, for example), addition of the pentaglycine bridges to the ε-amino group of the L-lysine residue (in *S. aureus*, for example). The glycine donor for the formation of these bridges has been shown to be glycyl sRNA but ribosomes are not involved in this reaction. This means that it differs from "normal" protein synthesis. Other types of possible modifications of the peptide precursors would be: amidation of the α-carboxyl group of D-aspartic acid and the attachment of its β-carboxyl group to the ε-amino group of L-lysine (in *S. faecalis* and *L. acidophilus*) or, the substitution of the α-carboxyl group of D-glutamic acid by the δ-amino group of D-ornithine (in *C. poinsettiae*).

Thirdly, the last step in the synthesis of the peptidoglycan net is a bridge-closing reaction, i.e., a transpeptidation in which the energy in the bond of the terminal dipeptide D-alanyl-D-alanine in one peptide subunit is utilized to effect the closure of the peptide crosslink with the concomitant release of the terminal D-alanine residue. The acceptor involved in this transpeptidation depends upon the bacterial species, for example: the NH_2-(D)-*meso*-DAP of another peptide subunit (*E. coli*), the amino terminus of the peptide bridge grouping such as glycine (*S. aureus*), L-alanine (*S. pyogenes*), D-isoasparagine (*S. faecalis; L. acodophilus*) or α-NH_2-D-ornithine (*C. poinsettiae*). Penicillins and Cephalosporins are specific inhibitors of these transpeptidations. It has been proposed that their activities depend upon a structural analogy with the D-alanyl-D-alanine dipeptide residu at the end of the peptide subunit. Finally, attention should be called to the biosynthesis of those peptide bridges found in *M. lysodeikticus* (fig. 6) and related *Micrococcaceae* in which several peptide subunits are linked in "head to tail" sequences through D-alanyl-L-alanine linkages, i.e., the bridge peptides are identical with the glycan peptide substituent. It has been hypothesized [15, 16] that the formation of such bridges from glycan strands substituted by uncrosslinked hexapaptide subunits N^{α}[L-alanyl-γ(α-D-glutamyl-glycine)]-L-lysyl-D-alanyl-D-alanine might occur through successive alternating transpeptidation and amidase activities (for more details, see ref. [16]).

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Reference to original articles is made only when the work is not cited in [1], [2], [3].

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