1 Introduction

The cell wall represents the outermost boundary of bacterial cells. Because of its exposed location it possesses manifold functions, some of which are apparently contradictory such as:
- the separatory functions: the cell wall separates the interior of the bacteria from the environment, while protecting the cell from harmful influences exerted by the surrounding milieu;
- the connecting functions: the cell wall enables transport of substances and information from outside to inside and vice versa, and enables the bacteria to contact (communicate with) the environment.

Furthermore, the cell wall provides the bacteria with sufficient solidity (e.g. shape stability, insensitivity to osmotic shock) and enables metabolism, growth and multiplication of the cells. However, it must be elastic enough to withstand considerable expansion.

The structures of bacterial cell walls are adapted to these functions, but solving the problem is absolutely different in individual bacteria. Thus, the cell walls appear to be rather complex and may vary greatly in details. However, on comparing the cell walls of different bacteria, one also can find structural principles common to many or most of them.

More than 100 years ago (1884), Gram succeeded in differentiating into two large groups all bacteria known at that time, using a simple staining method that was later named after him. After heat fixation, the bacteria are stained successively by solutions of carbolgentiana violet and of iodine (Lugol solution). Then ethanol is added until no further dye is eluted from the layer. Subsequently, the bacteria are restained with a diluted ethanolic fuchsine solution. Bacteria appearing darkblue to violet under the microscope are designated Gram-positive, red ones Gram-negative. The difference in stainability between typical representatives of both groups depends on the structure of their cell walls. Both types of cell walls contain the so-called peptidoglycan (murein, see below) as rigidity-causing component; however, that of the Gram-positive bacteria is much thicker than that of the Gram-negative. In addition, both groups contain different accessory cell wall components. Gram-positive cell walls can differ much more from each other in composition and structure than the Gram-negative ones, which follow a more general structural format.

Later, two additional groups of microorganisms were described: mycoplasma, containing no cell wall and no peptidoglycan; and archaea, possessing a differently composed rigid layer instead of peptidoglycan in or on the cell wall, e.g. a pseudomurein or an S-layer, respectively.

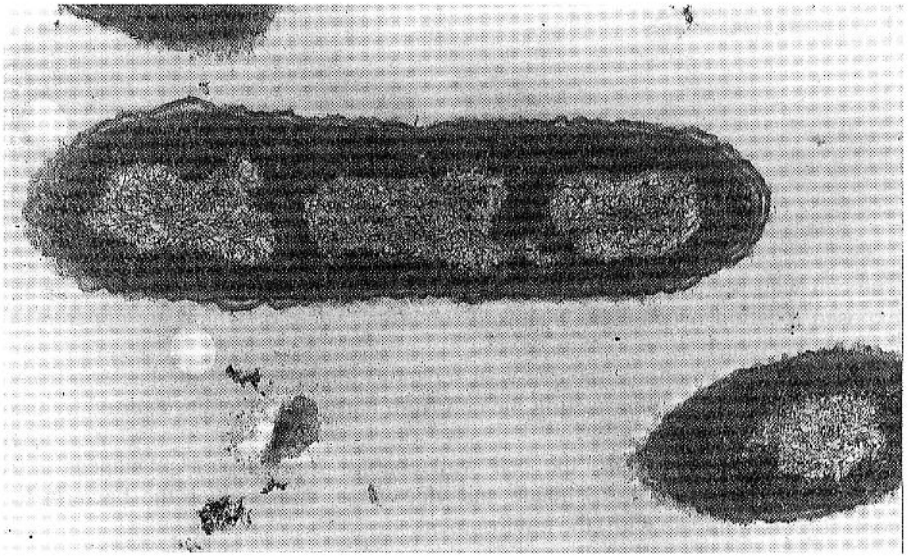

Fig. 1.1. Electron micrograph of an ultrathin section of a Gram-negative bacterium (*Pseudomonas aeruginosa*, F. Mach)

Not in all cases does the Gram stain yield clear and unambiguous results. Several bacteria, e.g. *Clostridium tetani*, may react in a Gram-positive or Gram-negative way depending on cultivation conditions; these are called Gram-variable bacteria. In addition, untypical Gram-negative and Gram-positive bacteria have been described: the Gram-negative representative of cyanobacteria containing a thick peptidoglycan and the Gram-positive mycolata, the cell wall of which resemble that of Gram-negative bacteria. Likewise, in the case of archaea the results are equivocal and therefore without taxonomic importance. However, because of its simplicity and high reliability, the Gram stain is still commonly used.

In general, microorganisms of the four groups mentioned above differ fundamentally from each other in many details of cell wall composition and structure. In Fig. 1.1 the electron micrograph of an ultrathin section from a Gram-negative bacterium is shown. Such pictures may look different depending on the cultivation conditions and the fixation procedure. However, close to the cell surface three layers are clearly visible. The innermost layer represents the inner or cytoplasmic membrane. Although most authors do not regard this membrane as a cell wall component, it represents the structural unit in which many components of the cell wall are biosynthesised. To point out this relationship the term cell envelope is defined, which comprises both the cell wall and the cytoplasmic membrane.

Further outwards is situated the periplasmic space, in which a rigid layer, the peptidoglycan, is located. This layer is present in all vegetative Gram-positive and

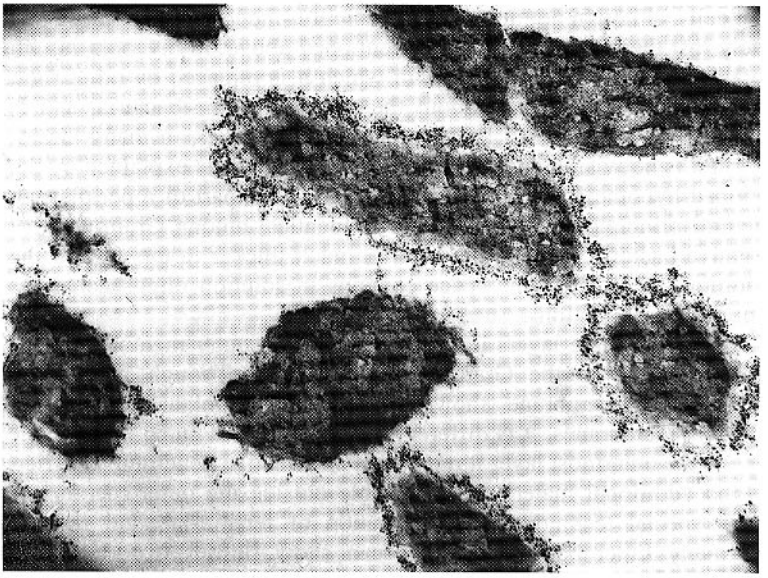

Fig. 1.2. Electron micrograph of an ultrathin section of Gram-negative bacteria after reaction with ferritin-labelled antibodies directed against surface O-antigens (*Shigella sonnei*, B. and M. Wagner)

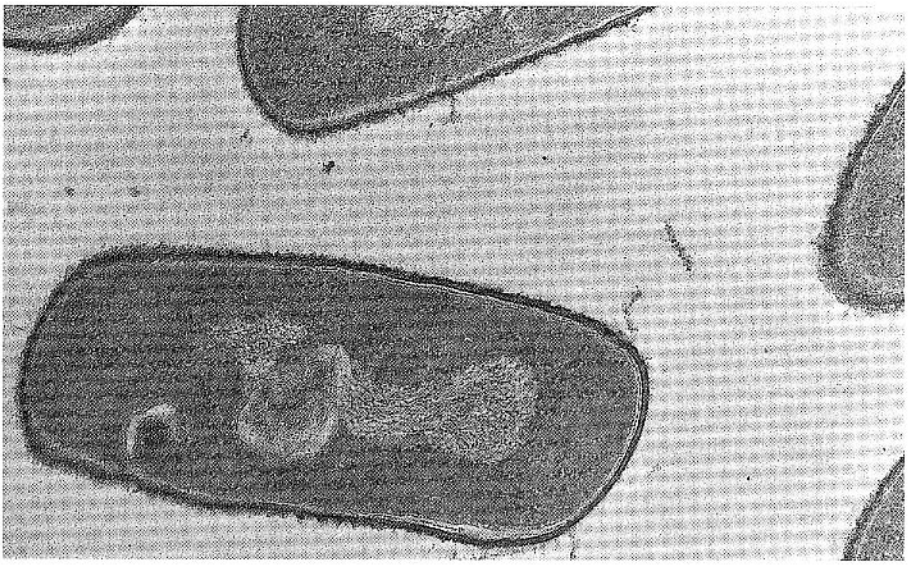

Fig. 1.3. Electron micrograph of an ultrathin section of a Gram-positive bacterium (*Bacillus subtilis*, F. Mach)

Gram-negative cells, although not in L-forms (penicillin-induced stable forms that have completely lost their cell wall) and, as mentioned above, in mycoplasma, in which it is totally missimg, and in archaea, in which it is replaced by other rigid structures. The peptidoglycan brings about the stability of the bacteria, and is therefore common to practically all bacterial species. However, in many Gram-negative bacteria it is not visible in electron micrographs. This is explained by the recently developed hypothesis that the peptidoglycan forms part of a periplasmic gel. The structural principle of the rigid layer is identical among Gram-positive and Gram-negative bacteria, though this layer is much thicker in the case of the former. In addition, the periplasmic space contains in solution oligosaccharides and proteins of different functions.

The outermost of the three layers is represented by the outer membrane. This structural unit is unique to the Gram-negative bacteria. Though in electron micrographs the outer membrane shows the typical trilaminar structure of common biological membranes, it is differently composed and much more stable than the latter. As the outermost layer it represents an effective penetration barrier.

In Fig. 1.1 no additional material is detectable outside the outer membrane. However, using special techniques, i.e. utilisation of ferritin-labelled specific antibodies, in many Enterobacteriaceae one can show fibrous material being firmly connected with the outer membrane and protruding into the surrounding medium (Fig. 1.2). From results of chemical analyses it can be concluded that in most cases this material consists of the polysaccharide chains of the lipopolysaccharides (LPS, see Sect. 2.2), a substance specific to the Gram-negative cell wall.

The cell wall of Gram-positive bacteria (Fig. 1.3) is distinctly different. It is much thicker than that of the Gram-negative bacteria and appears in many cases relatively structureless. However, it also contains a thick electron-dense rigid layer (20-80 nm compared with 10-15 nm in Gram-negative bacteria). In some Gram-positive bacteria, e.g. *Bacillus polymyxa*, distinct cell wall structures have been found electronmicroscopically. Likewise, using special techniques one can show that, for instance, staphylococcal cell walls also reveal a highly sophisticated architecture. Although an outer membrane is not detectable, the existence of a region comparable with the periplasmic space of the Gram-negative bacteria is postulated by some authors (Dijkstra and Keck 1996). Because of the missing outer membrane the Gram-positive cell wall is much more penetrable than the Gram-negative.

The Gram-positive cell walls, like the Gram-negative ones contain substantial amounts of polysaccharides, mainly the (lipo-)teichoic acids (see Sect. 4.1.1). Without previous specific treatment of the cells they cannot be detected in electron micrographs; however, they become visible after reacting with ferritin-labelled specific antisera.

Mycolata are classified as Gram-positive bacteria, although their staining is rather Gram-variable. Their cell wall structure is highly complex and unique (see Sect. 4.3) and, thus, differs from that of other Gram-positive species. A peptidoglycan layer is present, on top of which a thick layer composed of various lipoglycans, glyco(peptido)lipids, and proteins is present. Evidence for the presence of a

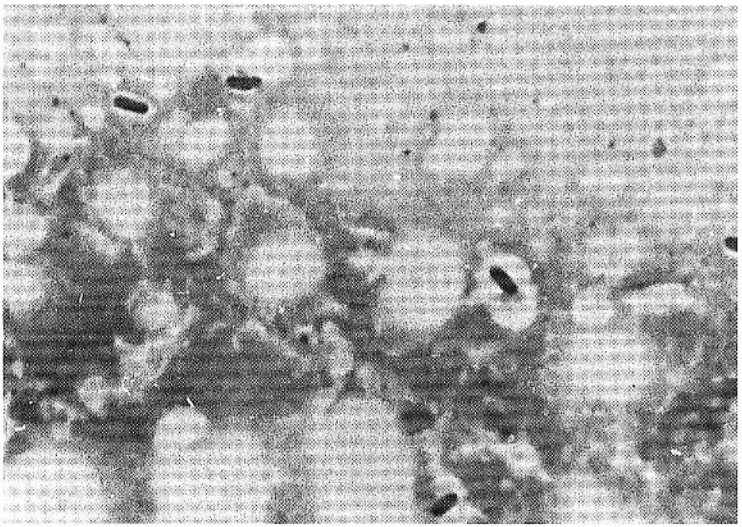

Fig. 1.4. Microscopical picture of a capsule-carrying bacterium (*Clostridium perfringens*, E. Dinger)

capsule as outer surface layer that is composed of proteins and polysaccharides has been obtained in recent years.

Finally, using specific techniques, one can frequently demonstrate additional layers above the cell wall, of which the longest known are the so-called capsules. An appropriate procedure for their demonstration is to treat bacteria spread out on a slide with Indian ink and then stain with fuchsin. Under the microscope the colourless capsules are readily identified between the reddish bacteria and the black background (Fig. 1.4).

Further examples are slime layers (slime walls), S-layers and sheaths. They are all not actual components of the cell walls, but situated at the cell surface, thus providing the bacteria with significant advantages in the struggle with the environment (see Chap. 6).

Finally, filamentous proteinaceous appendages of different length and diameter (flagellae, fimbriae, pili) must be mentioned. They are anchored with one end in the cell wall, while the other end extends into the surrounding medium.

The scientific investigation of the cell walls and the interpretation of the results are impaired by the fact that most investigations are carried out on laboratory cultures of bacteria. It is well known that bacteria grown in vitro are composed differently to those grown in natural habitats and are, therefore, not identical in behaviour and reactions. In addition, even under in vitro conditions, composition and structure of the bacteria are strongly dependent on the medium, the growth temperature, and the age of the culture. Thus, for instance, carbon-starved *E. coli* cells are more resistant to heat shock, osmotic challenge or oxidative stress than exponential-phase cells. On the other hand, under in vivo conditions the bacteria need not be stable in composition and structure. For instance, the different growth

situations during an infection cycle require fast adaptations to new situations by the expressions of different factors.

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