

Bacterial Cell Walls

I. Introduction

- Characteristic envelope of prokaryotes
- Present in all bacterial species Except for **Mycoplasma**.
- Surrounds the bacterium: exoskeleton
- Forms the outermost constant structure.

Depending on composition and structure, two types of cell walls can be distinguished:

1. Thick and dense walls (Gram-positive)
2. Thin and loose walls (Gram-negative)

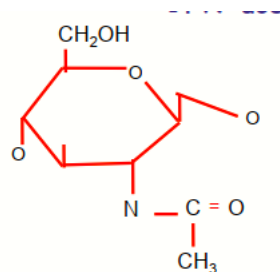
II. Chemical Composition

The elements of the cell wall:

1. Osamines
2. Amino acids
3. Teichoic acids
4. Sugars
5. Lipids

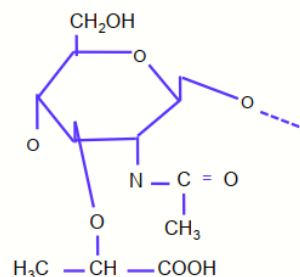
1. Osamines (amino sugar)

N-acetylglucosamine (NAG)



and

N-acetylmuramic acid (NAM), (Lactic acid



3. Sometimes galactosamine is present.

2. Amino Acids

Four common amino acids:

1. D-Alanine
2. L-Alanine
3. D-Glutamic acid

4. L-Lysine

Or

Diaminopimelic acid (a structure similar to lysine but with a -COOH functional group on the last carbon).

3. Teichoic Acids:

- Found only in Gram positive bacteria.
- They are incorporated into the peptidoglycan via carbon No. 6 of NAM.

4. Sugars:

- Many sugars can be identified, including glucose, galactose, mannose, fucose, etc.

5. Lipids:

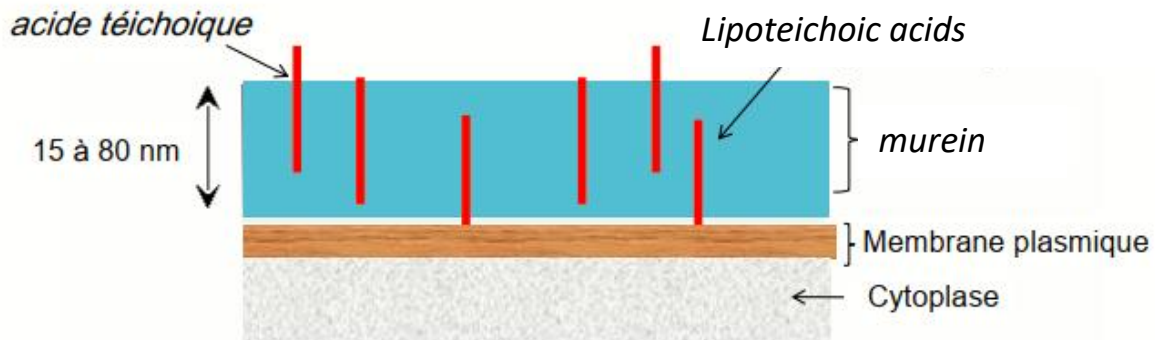
- Present in small quantities and almost absent in Gram-positive bacteria.

Comparison of the global chemical composition of the wall in Gram+ and Gram- bacteria

Éléments de la paroi	Gram +	Gram –
1. Osamines	++	+
2. Acides aminés	24 – 35 %	≈ 50 %
Nombre	4 à 10	16 à 17
Acide diamino-pimélique	+++ Exclut la lysine	– N'exclut pas la Lysine
3. Acides teichoïques	+++	–
4. Oses	20-60 %	20-60 %
5. Lipides	1-2.5 %	10 – 22 %

III. Structure

❖ Gram-positive wall



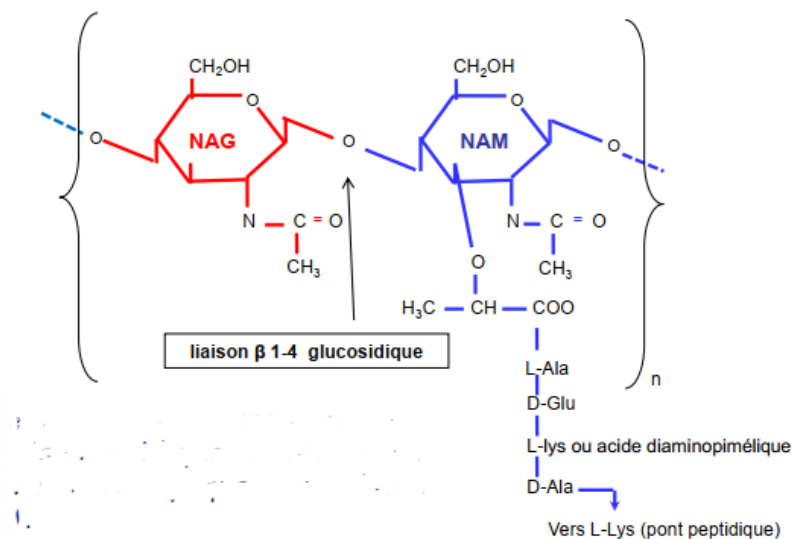
- Thick

- Does not let ethanol pass through.

Peptidoglycan: Murein, Mucocomplex, or Mucopeptide

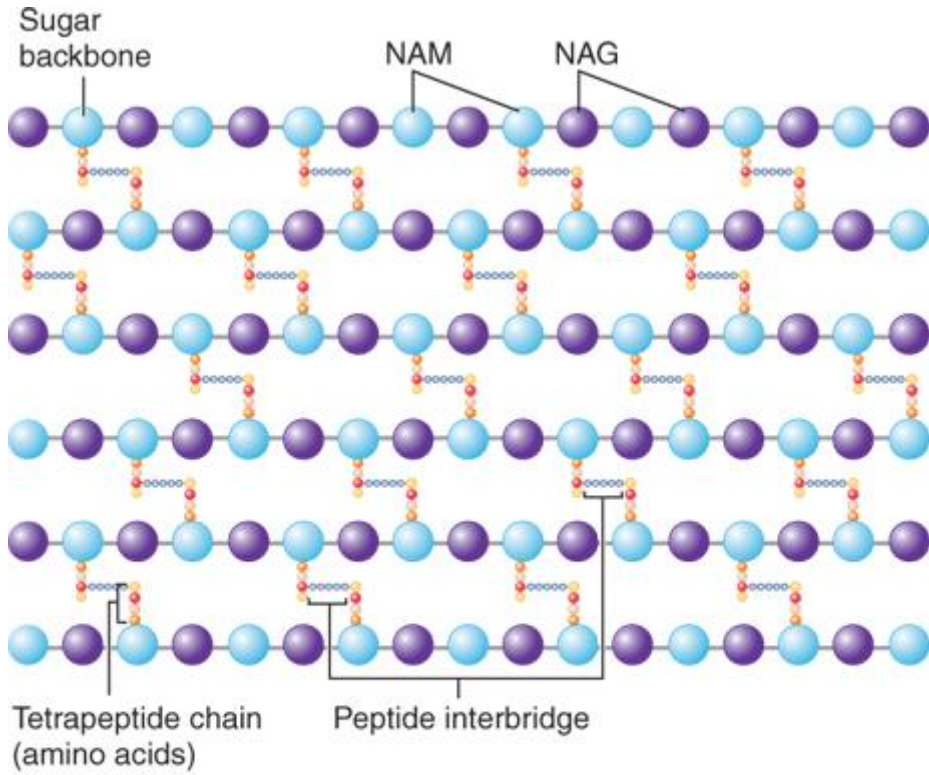
It consists of:

1. **Glycan part:** Alternating NAG (N-acetylglucosamine) and NAM (N-acetylmuramic acid) units linked by β 1-4 glycosidic bonds.



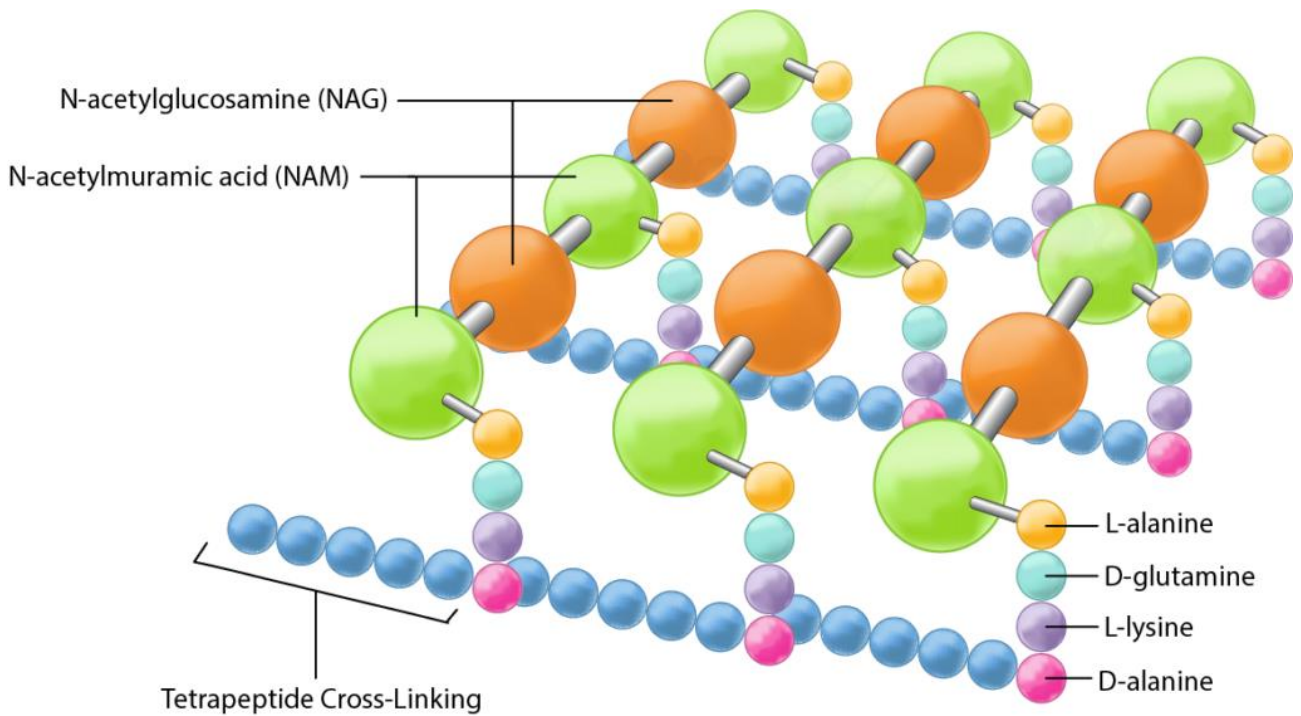
2. **Peptide part:** Four amino acids connected by an amide bond at the carboxyl function of the pyruvate residue of NAM.

- Peptidoglycan is composed of long repetitive chains alternating NAG and NAM.
- These macromolecules are linked at the tetrapeptides to form a solid structure.

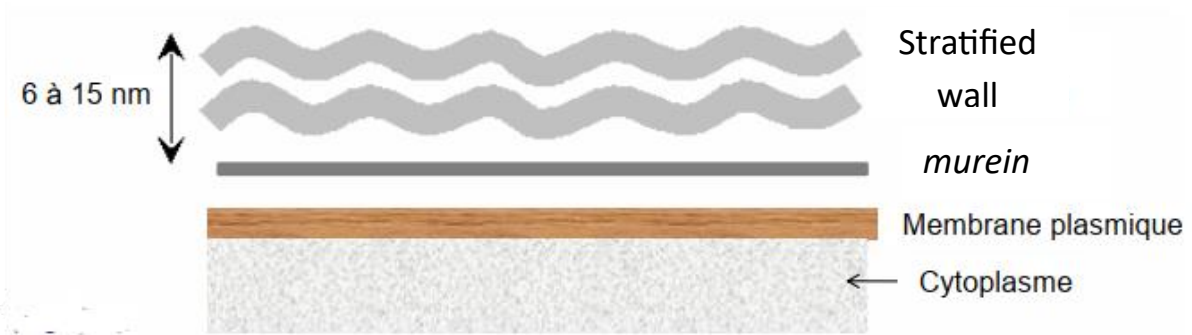


B

Source: Warren Levinson: Review of Medical Microbiology and Immunology, 14th Edition, www.accessmedicine.com Copyright © McGraw-Hill Education. All rights reserved.



❖ Gram negative wall



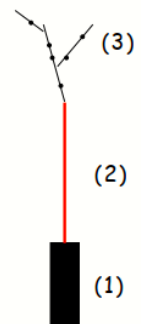
- Thinner
- More complex stratified structure.
- Includes three polymeric structures:
 1. Peptidoglycan
 2. Phospholipid layer: “outer membrane” (which explains why alcohol, which is fat-soluble, passes through this type of wall).
 3. Lipopolysaccharide (LPS) representing enterobacteria's O antigen and toxin.

Lipopolysaccharides

Macromolecular complexes

LPS consists of three parts:

1. **Lipid A:** A glycophospholipid and the most stable part.
2. **Core region:** Common to all Gram-negative bacteria.
3. **Outer region:** Known as the O antigen.



IV. Gram Staining

1. Definition :

- Gram staining was developed by a Danish physician (Christian Gram) in 1884.

2. Objectives: dual interest

1. Morphology (shape, size, grouping...);
2. Cell wall type.

2. Technique : There are many variations.

We choose: the "*Minute Technique*":

3.1. Preparation of a smear

3.2. treatment of the preparation

After fixing the bacteria on a microscope slide, the preparation is treated as follows:

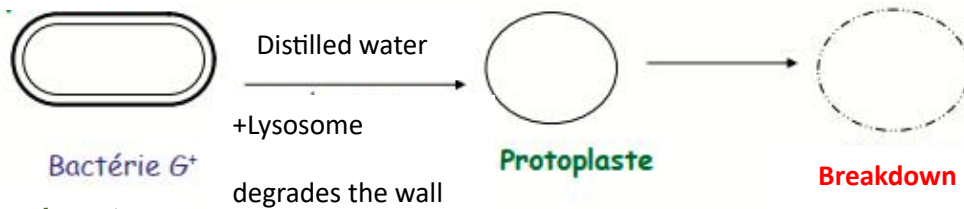
Step	Procedure	What happens?	State of the bacteria at this stage
Gentian Violet Staining	Cover the slide with gentian violet. Let it act for 1 minute.	Gentian violet stains both Gram positive and Gram negative bacteria.	All bacteria are stained purple.
Mordanting with Lugol	Cover the slide with Lugol. Let it act for 20 seconds, then repeat twice.	Lugol and gentian violet form a complex that enhances staining.	All bacteria remain purple.
Decolorization with Ethanol	Tilt the slide and allow 95% ethanol (95°GL) to flow over it for 4 seconds. Caution: this step is critical!	- The cell wall of Gram positive bacteria prevents ethanol from dissolving the gentian violet. - The cell wall of Gram negative bacteria allows alcohol to dissolve the gentian violet.	- Gram positive bacteria remain purple. - Gram negative bacteria lose their color.
Rinsing with distilled water			
Counterstaining with Fuchsin	Cover the slide with water and add a few drops of Ziehl's fuchsin. Let it act for 30 seconds to 1 minute, then rinse with distilled water.	Fuchsin stains the previously decolorized bacteria.	- Gram positive bacteria remain purple. - Gram negative bacteria are stained pink.

After Gram staining:

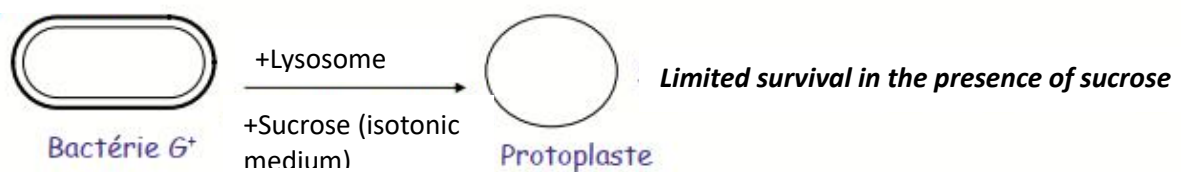
- Bacteria with thick cell walls appear **purple** and are classified as **Gram positive**.
- Bacteria with thin cell walls appear **pink** and are classified as **Gram negative**.

V. Action of lysozyme

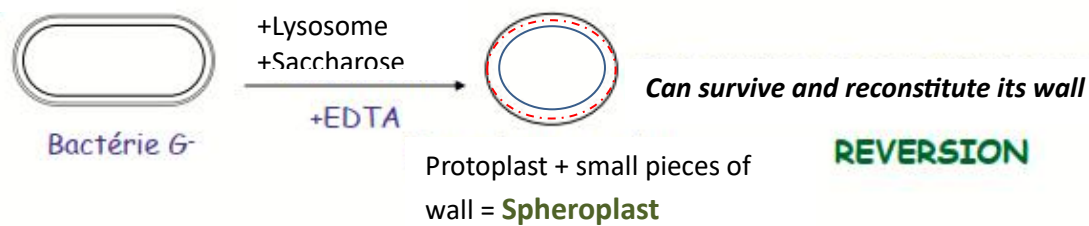
Experiment 1



Experiment 2



Experiment 3



Protoplast: bacterial cell freed from its mucopolysaccharide wall

Spheroplasts: Protoplast isolated from Gram-negative bacterial cells.

VI. Function :

- Against osmotic shocks
- Responsible for the structural integrity and shape of bacteria
- An essential element for identification
- The origin of the formation of the cell division septum in bacteria
- Significant role in the distribution of genetic material to the two daughter cells