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Alicia Cepaitis, MS
Chief Creative Nerd
Science Prof Online
Online Education Resources, LLC
alicia@scienceprofonline.com

Tami Port, MS
Creator of Science Prof Online
Chief Executive Nerd
Science Prof Online
Online Education Resources, LLC
info@scienceprofonline.com

Bacterial Cell Wall

&

Differential Staining

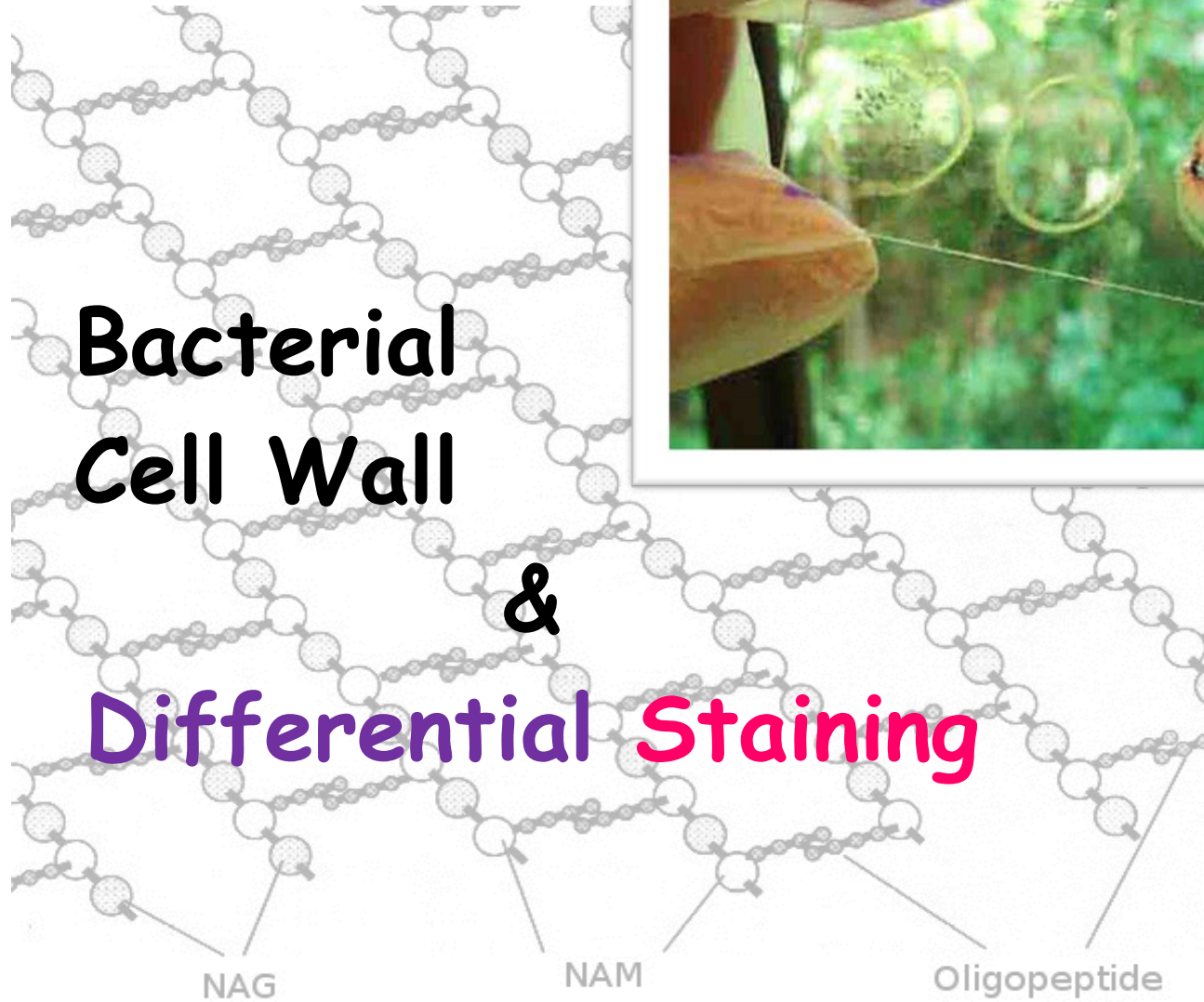


Image: [Bonding structure peptidoglycan](#),
Mouagip; Gram stained slide, T. Port

Bacterial Cell Wall

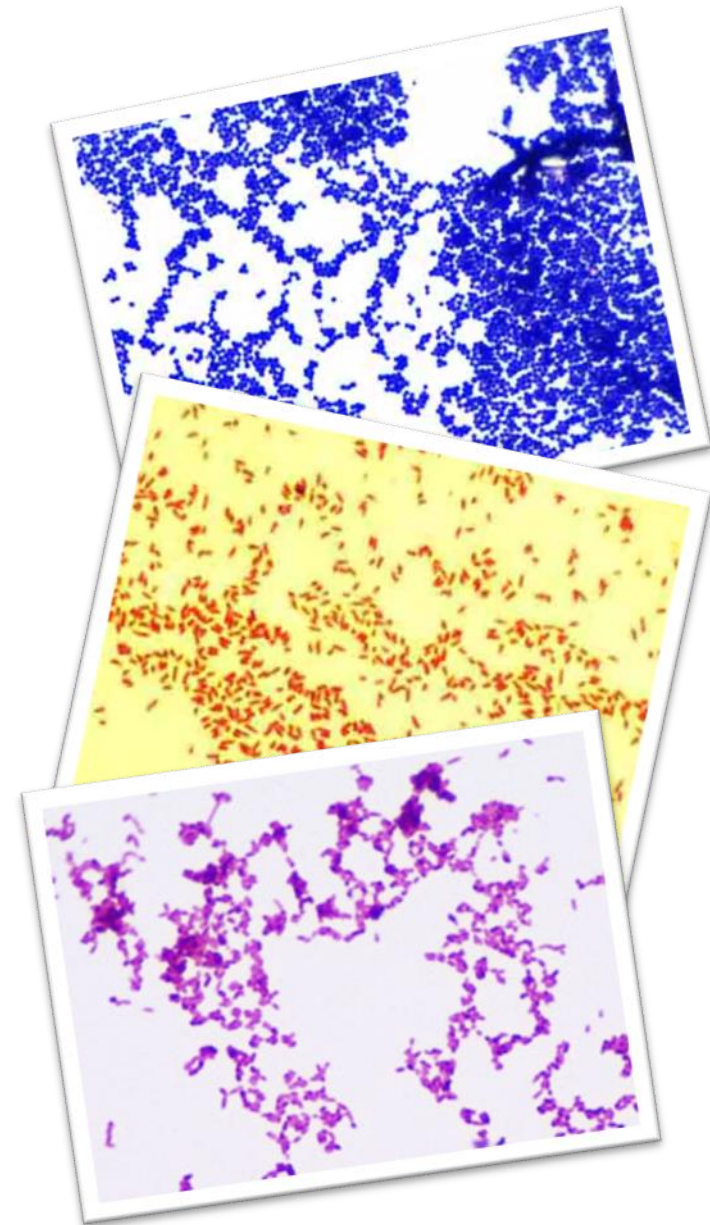
Function: Shape and protection

Structure: Distinguishes groups of bacteria

➤ Cells that Gram stain

- Gram positive and Gram negative

- Cells that resist Gram staining
 - Genus *Mycobacterium* and *Nocardia*
 - Stained using Acid-fast staining techniques
- Cells that lack cell walls
 - Will retain counterstain (second color applied during differential staining).



Images: Gram positive bacteria , Gram-negative bacteria & Acid fast bacteria, all under oil immersion @1000XTM, T. Port

Bacterial Cell Wall

- **Peptidoglycan** is a huge polymer of interlocking chains of alternating monomers.
- Provides rigid support while freely permeable to solutes.
- Backbone of peptidoglycan molecule composed of two amino sugar derivatives of glucose. The "glycan" part of peptidoglycan:
 - N-acetylglucosamine (NAG)
 - N-acetylmuramic acid (NAM)
- NAG / NAM strands are connected by interlocking peptide bridges. The "peptid" part of peptidoglycan.

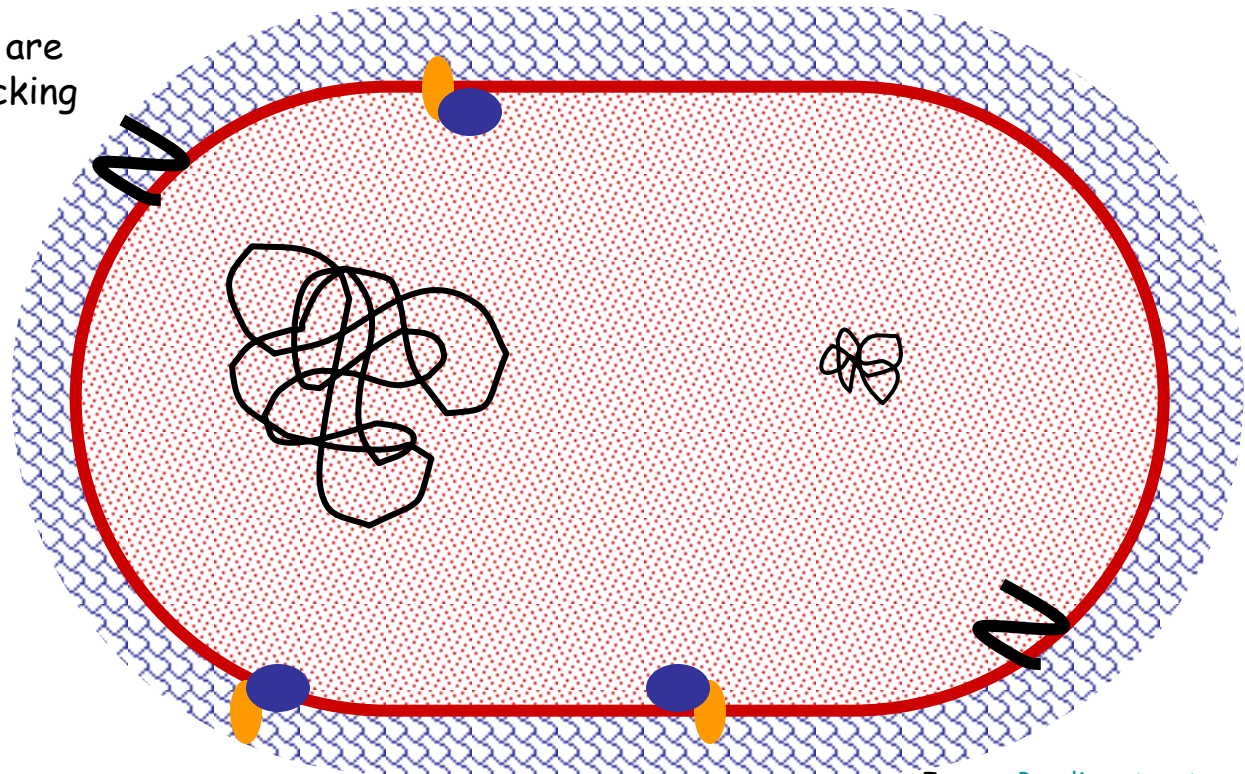
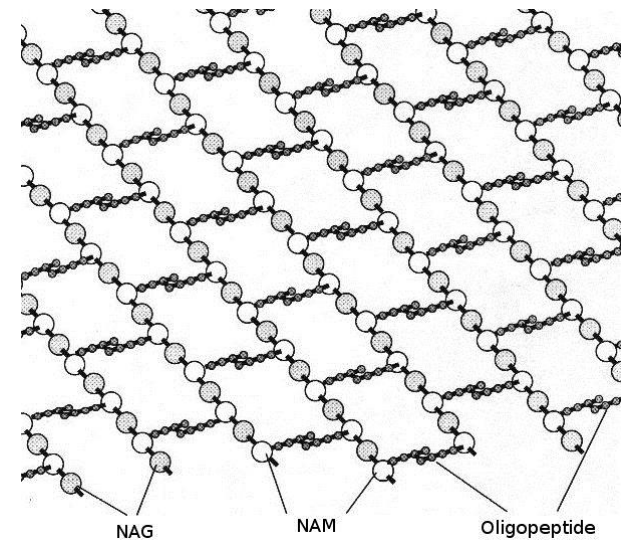
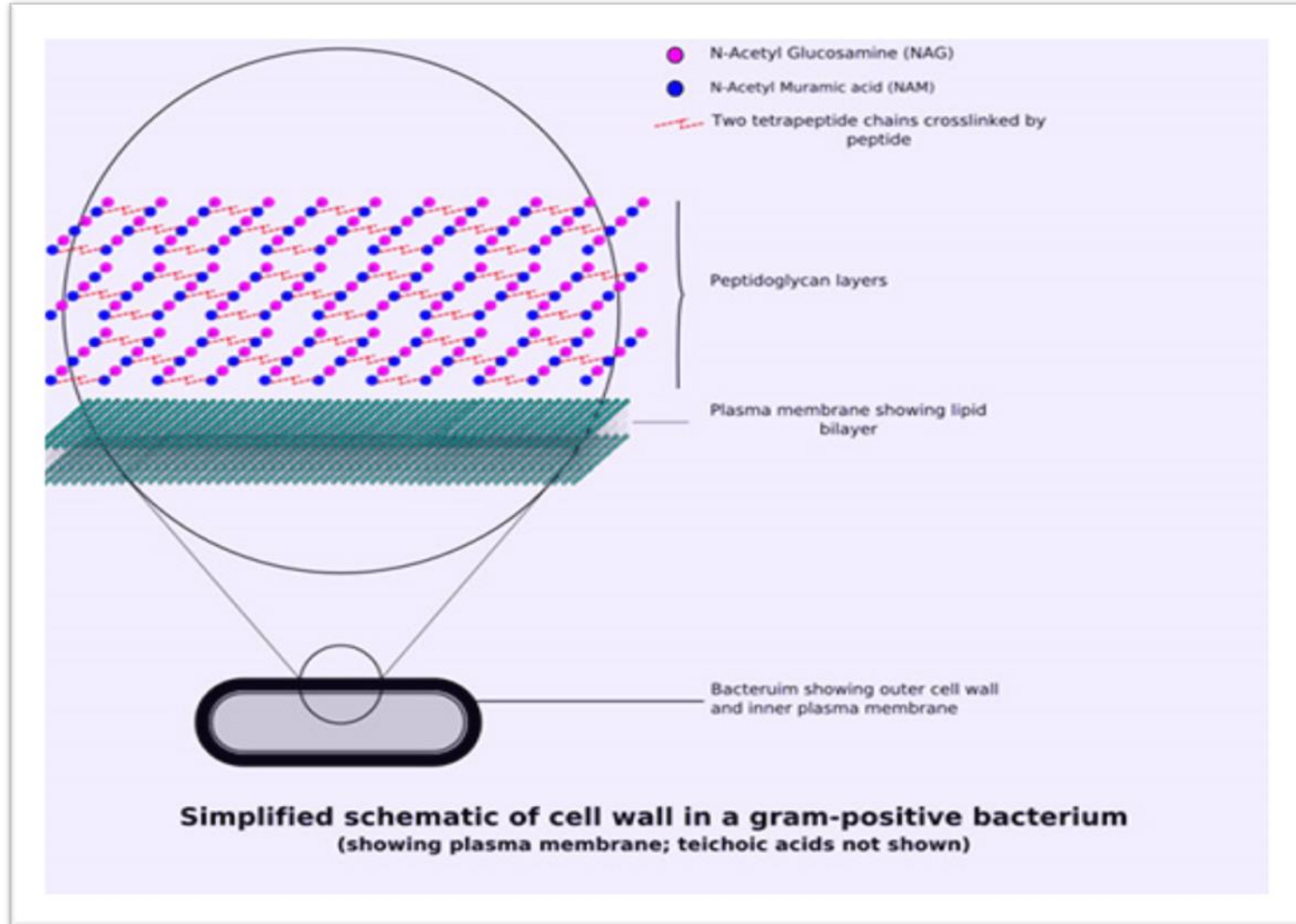


Image: [Bonding structure peptidoglycan](#),
Mouagip; Other Image Source Unknown

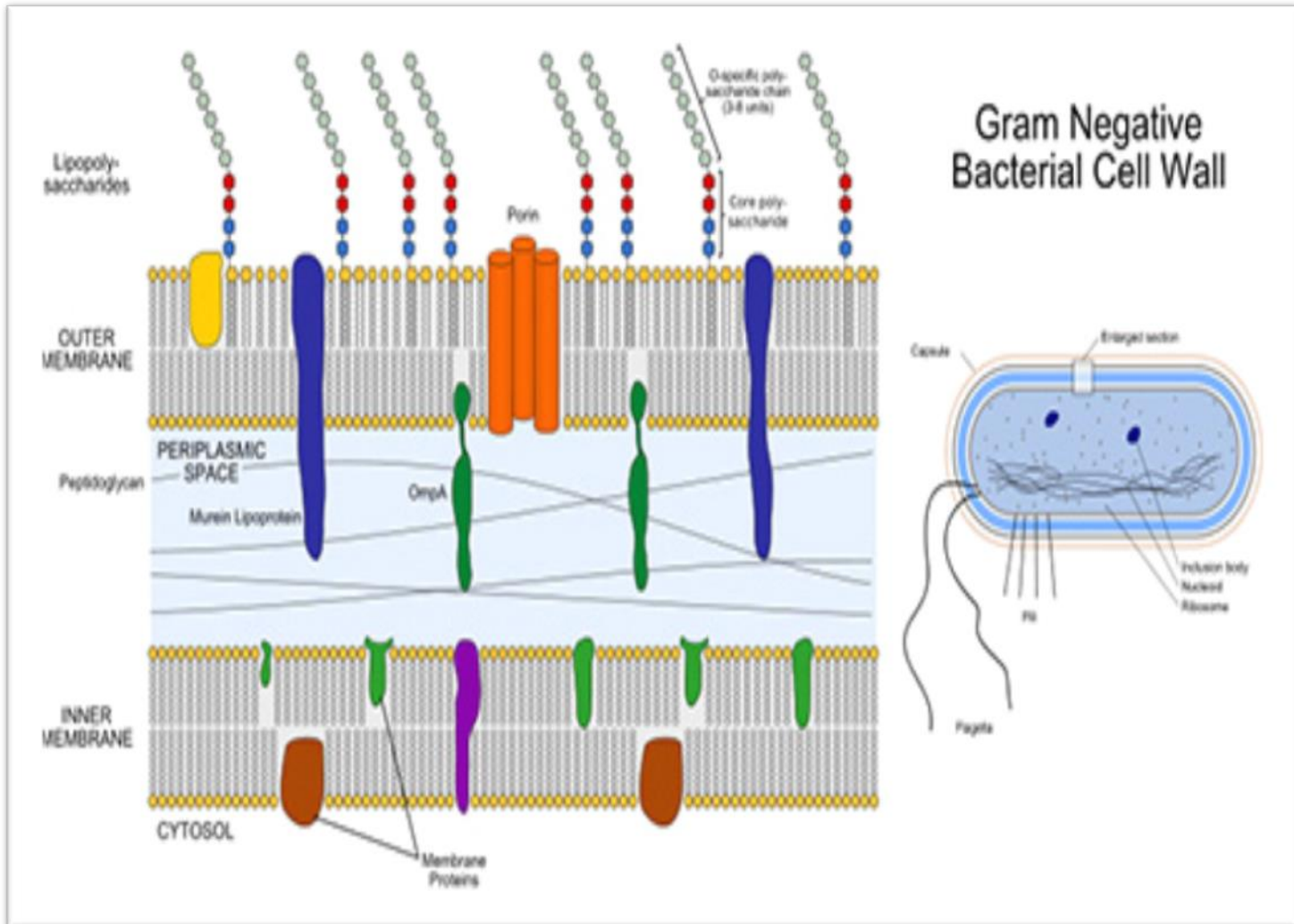
Bacterial Cell Wall

Gram-Positive



Bacterial Cell Wall

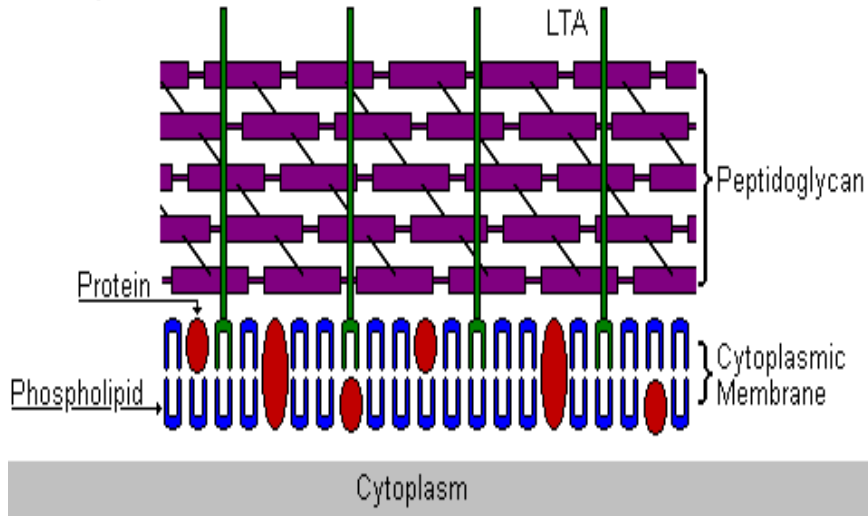
Gram-Negative



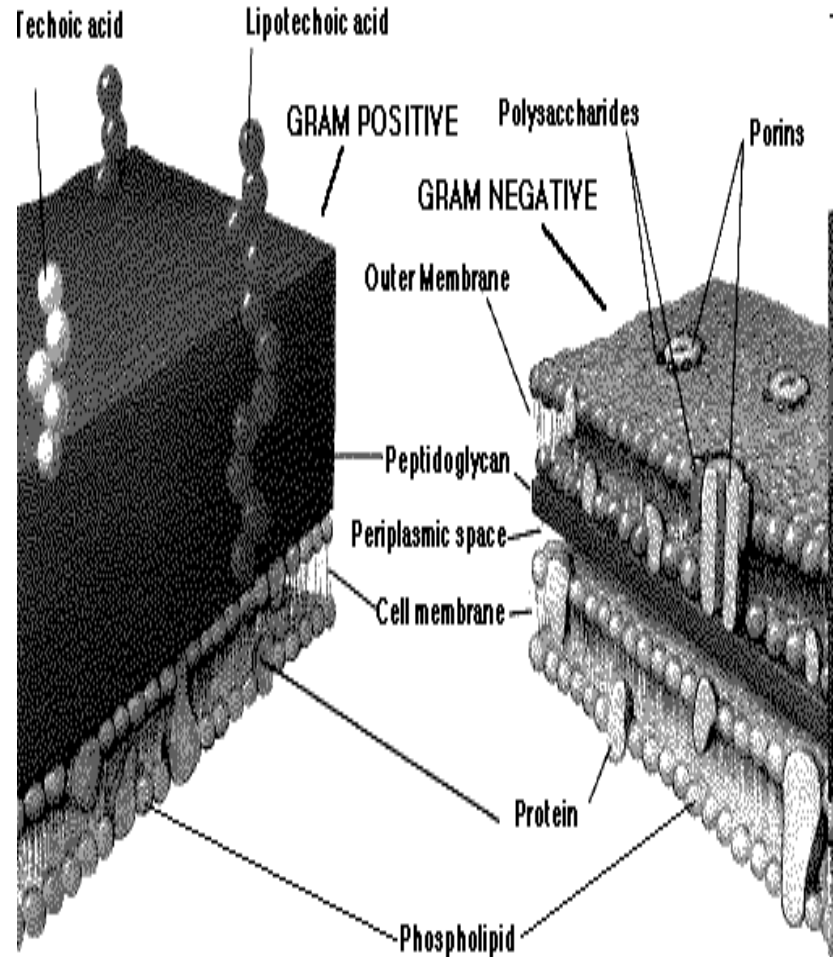
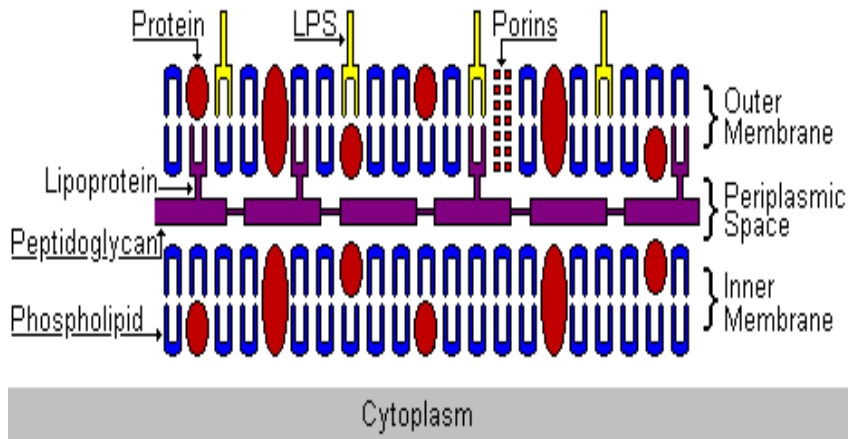
Prokaryotes - Cell Wall

Gram-Positive & Gram-Negative

Gram-positive Cell Wall



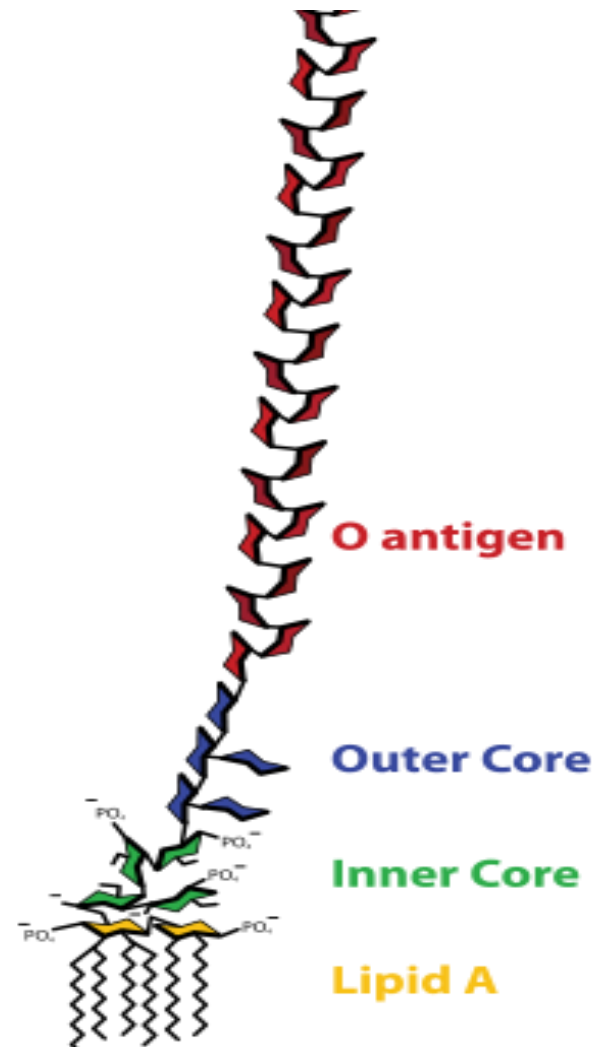
Gram-negative Cell Wall



Lipopolysaccharide (LPS)

- LPS is a lipid-sugar.
- Lipid portion is known as **lipid-A**.
- Dead **Gram-negative** bacteria release lipid-A when this outer membrane disintegrates.
- In animals with a Gram-negative bacterial infection, free lipid-A may trigger fever, vasodilation, inflammation, shock and blood clotting.
- Killing large numbers of Gram-negative bacteria with antimicrobial drugs releases lots of lipid-A, which can threaten the patient more than the presence of live Gram-negative bacteria.

Q: Why are these differences in cell wall structure so important?



CHEMICAL WARFARE

Between Species

&

Selective Toxicity of
Antimicrobials

Q: Why are these differences in cell wall structure so important?



DANGER
HAZARDOUS
CHEMICALS

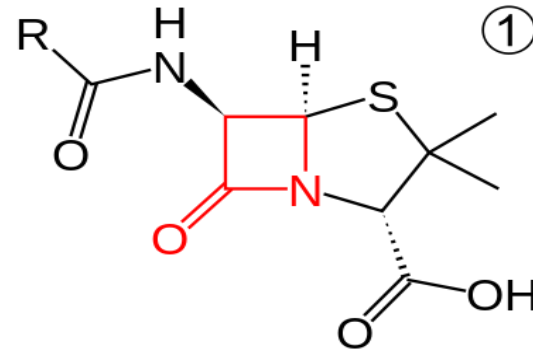
Beta-lactam Antibiotics

β -Lactams are a broad class of antibiotics that all contain a β -lactam ring in their molecular structures.

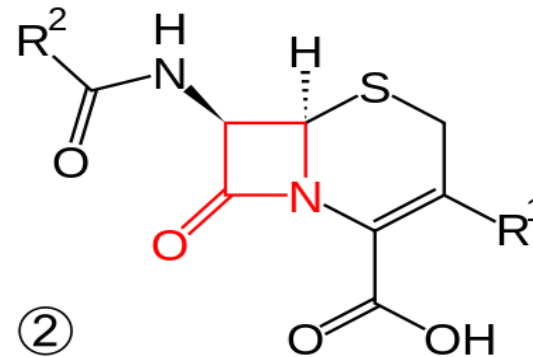
Beta-lactam drugs include penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems.

These antibiotics work by inhibiting cell wall synthesis in bacteria and are the most widely used group of antibiotics.

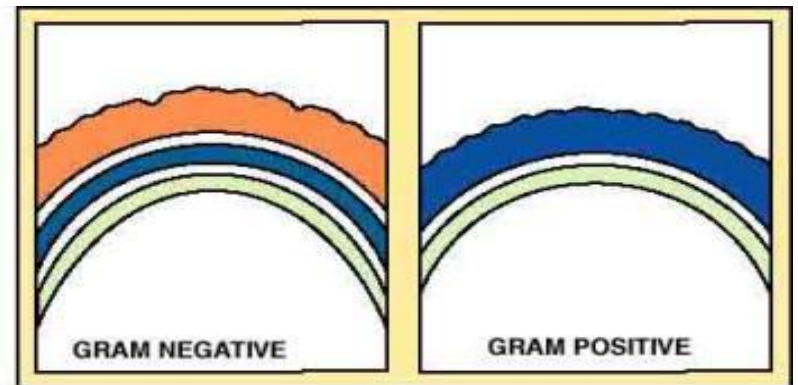
Q: *Penicillin needs to come directly into contact with peptidoglycan to cause cell wall damage. So what type of cell wall do you think is more vulnerable to damage by penicillin?*

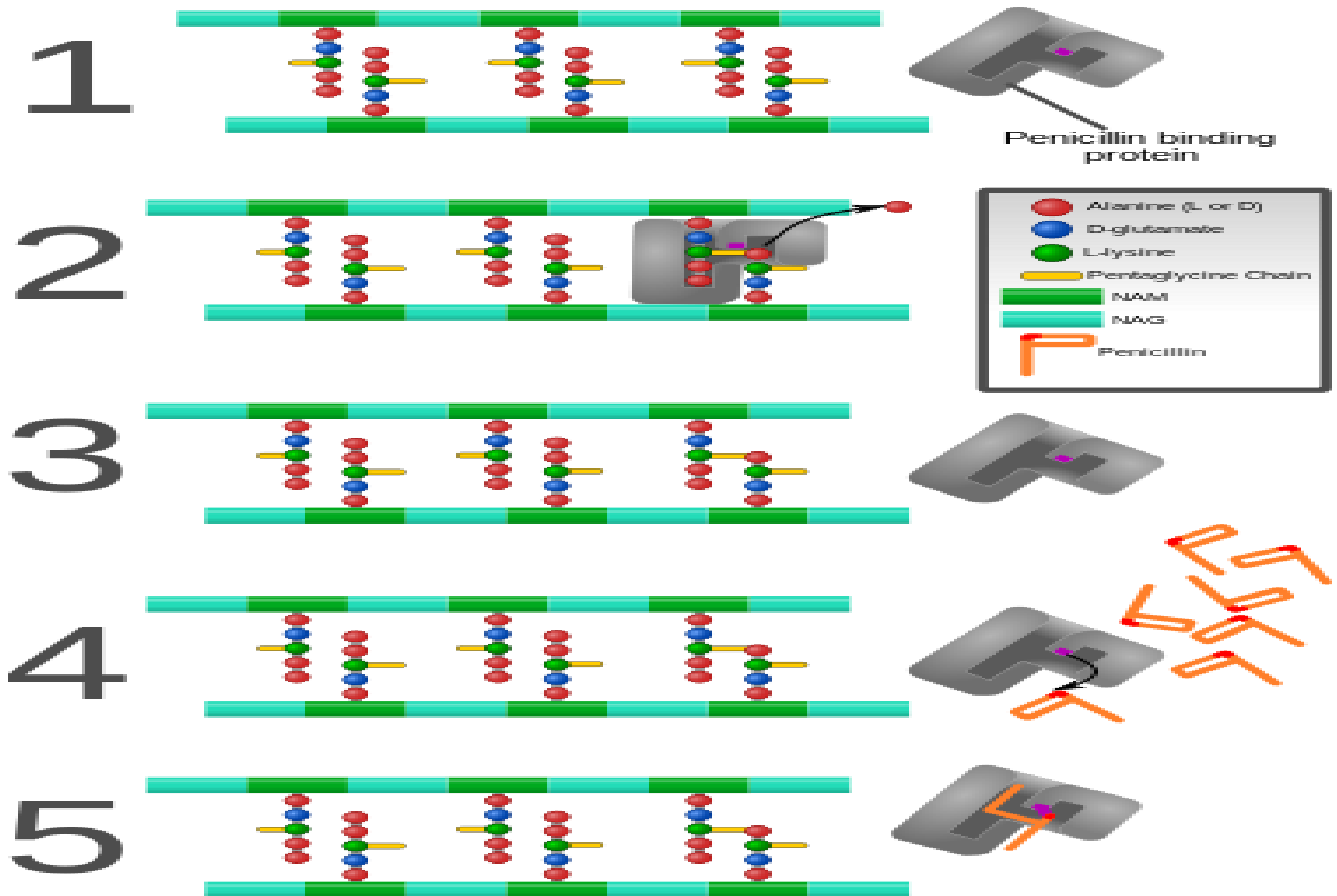


1. Penicillin



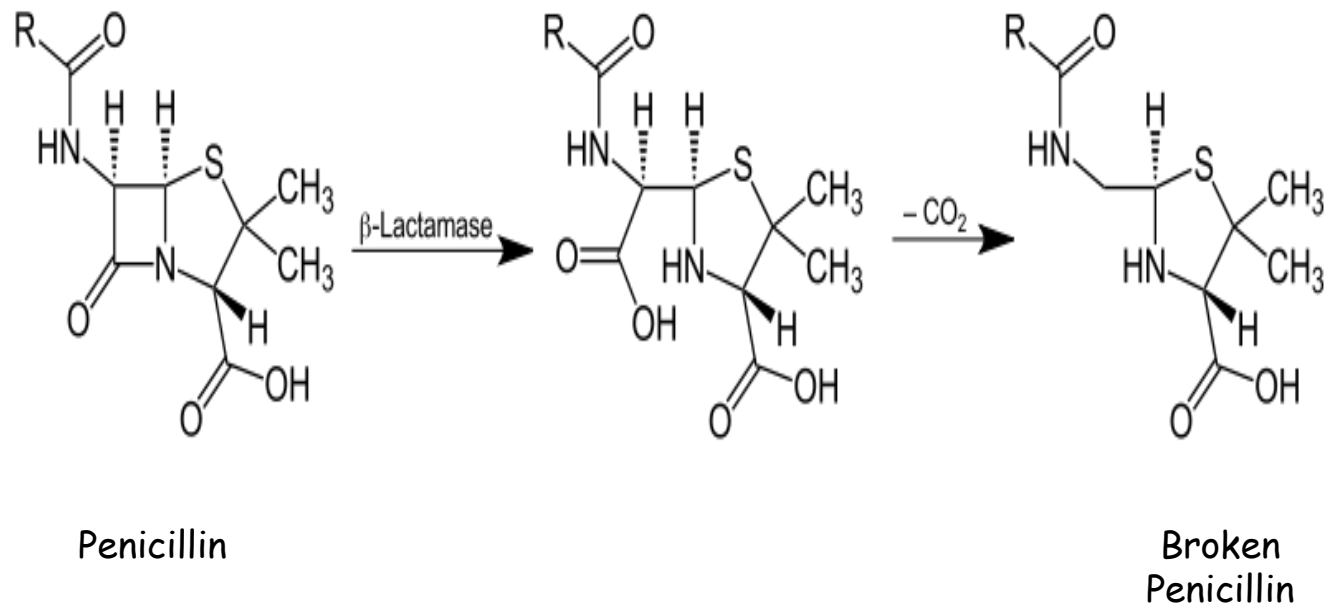
2. Cephalosporin





Beta-lactam Antibiotic **Resistance**

Some bacteria have developed resistance to β -lactam antibiotics and are able to synthesize an enzyme called β -lactamase, that attacks the β -lactam ring, inactivating the antibiotic.



Before selection



After selection



Final population



Resistance level



REVIEW!

- Animated video:
[Antimicrobial Resistance](#)
(You'll need to download the first video listed on the page "Animation of Antimicrobial Resistance".)

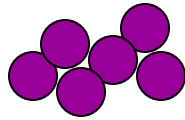
- Animated video:
["Beta-Lactams: Mechanisms of Action & Resistance"](#).

Meet the Microbes: *Staphylococcus* sp.

GRAM-POSITIVE

Facultative anaerobe

coccus-shaped

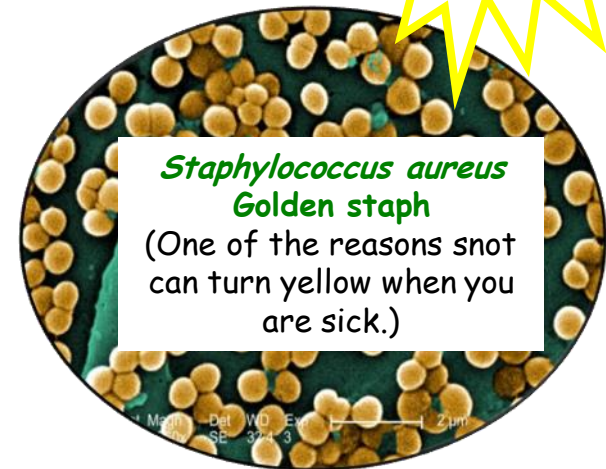


Coccus-shaped bacteria, which divides in a way that results in grape-like clusters.

- *Staphylococcus aureus* (golden staph), most common cause of staph infections.
- Approximately 20-30% of general population "Staph (*aureus*) carriers."
- *S. aureus* can cause illnesses ranging from minor skin infections to life-threatening diseases, such as meningitis, toxic shock syndrome (TSS) & septicemia.
- **MRSA** = **M**ethicillin-**r**esistant *Staphylococcus aureus*
- One of the four most common causes of **nosocomial infections**, often causing postsurgical wound infections.
- *S. epidermidis* is normal flora which inhabits the skin of healthy humans.



Mannitol Salt



Staphylococcus aureus
Golden staph
(One of the reasons snot can turn yellow when you are sick.)



Our lab friend
Staphylococcus epidermidis.

Gram Stain

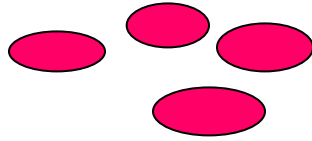
Image: Mannitol salt plates, T. Port; *S. aureus*, Janice Haney Carr, [PHIL](#) #10046; [Gram stain](#) *Staph*, T. Port

Meet the Microbe: *Escherichia coli*

GRAM-NEGATIVE

Facultative anaerobe

bacillus-shaped



Some strains of *E. coli* inhabit gastrointestinal tracts of warm-blooded animals as normal flora and provide a portion of the microbially-derived vitamin K for their host.

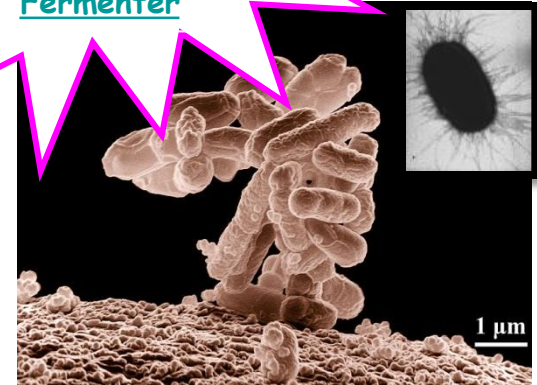
While many strains of *E. coli* are harmless commensals, some are human pathogens.

Common cause of bacterial food poisoning and urinary tract infections.

Bacteria must be able to "stick" to cause infection (otherwise, in case of UTI, bacteria would just get peed out).

Bladder lined with proteins, to prevent this. *E. coli* has fimbriae to help it stick.

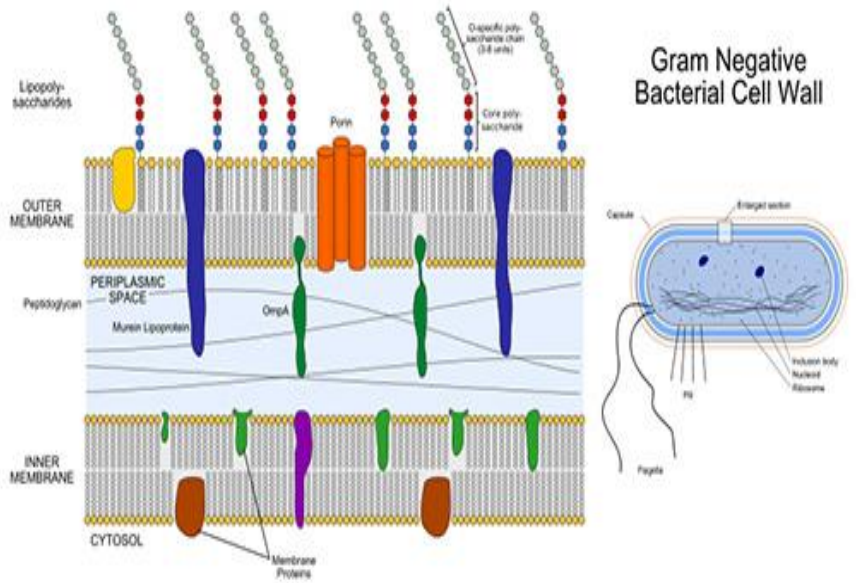
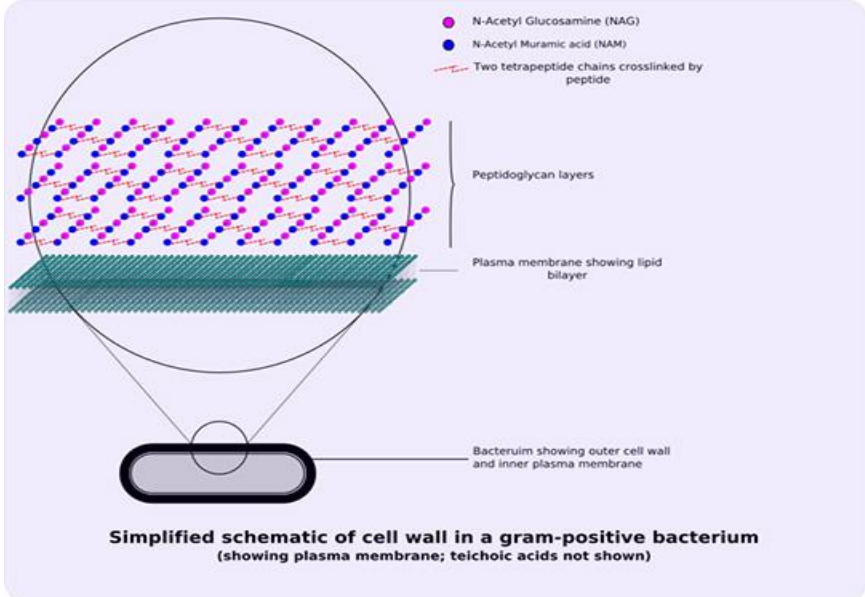
**MacConkey's
Lactose
Fermenter**



**Gram
Stain**

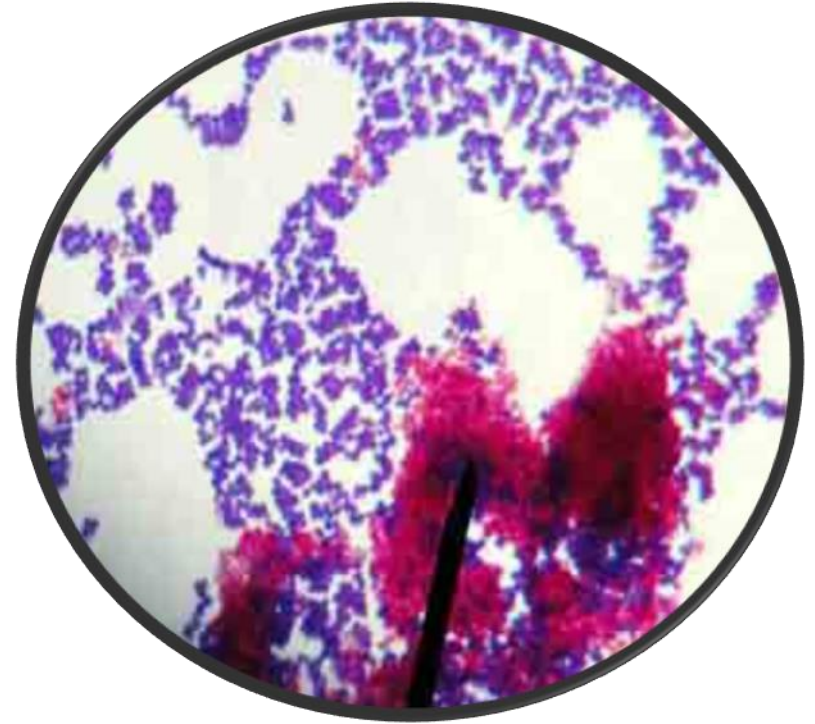
REVIEW!

Interactive lesson on Bacterial Cell Wall



Differential Stains

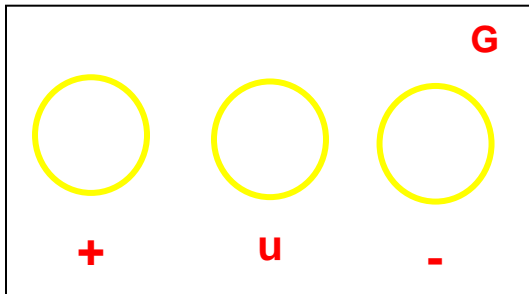
- Most stains used in microbiology are differential.
- Differential stains involve use of more than one dye, so that certain differences between cell type or structures can be distinguished.

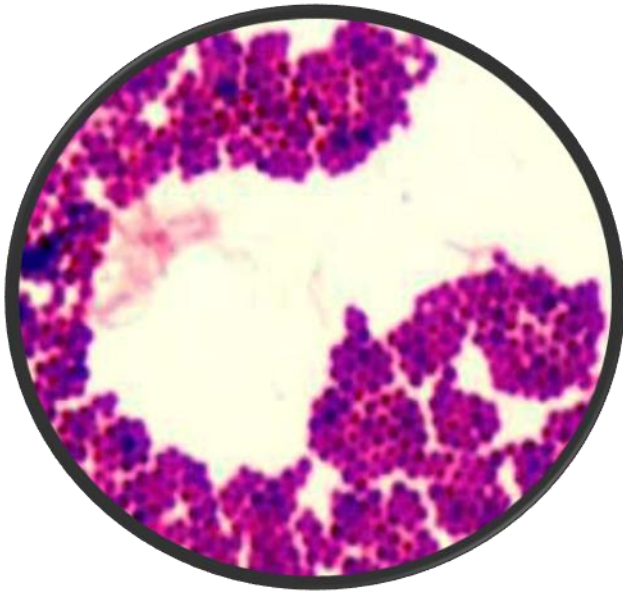


Gram Stain

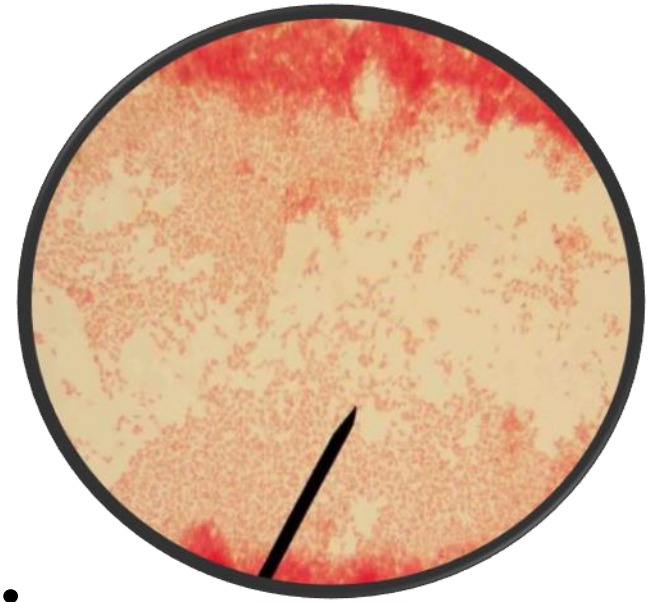
- Distinguishes between two large groups of microorganisms:
 - purple staining, Gram-positive bacteria
 - pink staining, Gram-negative bacteria
- Let's see how Gram Staining reveals the difference between Gram+ and Gram- cell wall structure...

GRAM STAINING PROCEDURE
Crystal violet (1 min) > *rinse*
Iodine (1 min) > *rinse*
Acetone Alcohol (10-15 sec) > *rinse*
Safrinin (1 min) > *rinse & blot dry*



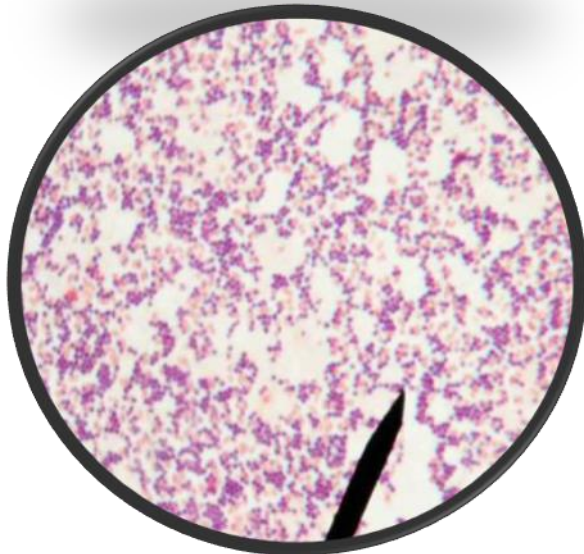


Staphylococcus epidermidis

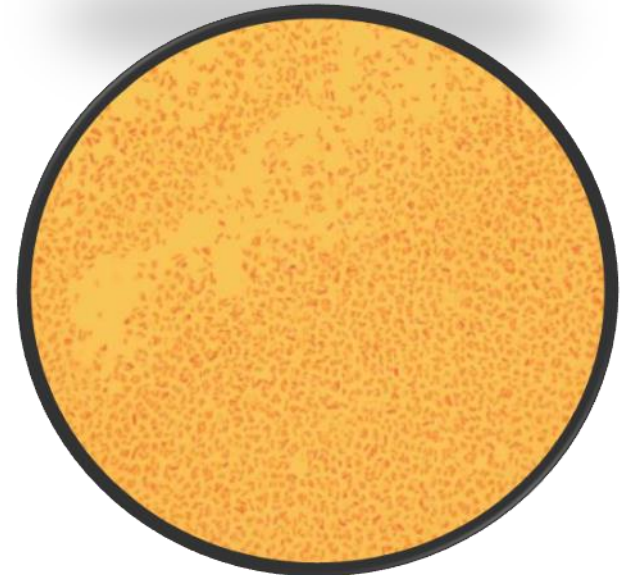


Escherichia coli

Gram Stain Examples

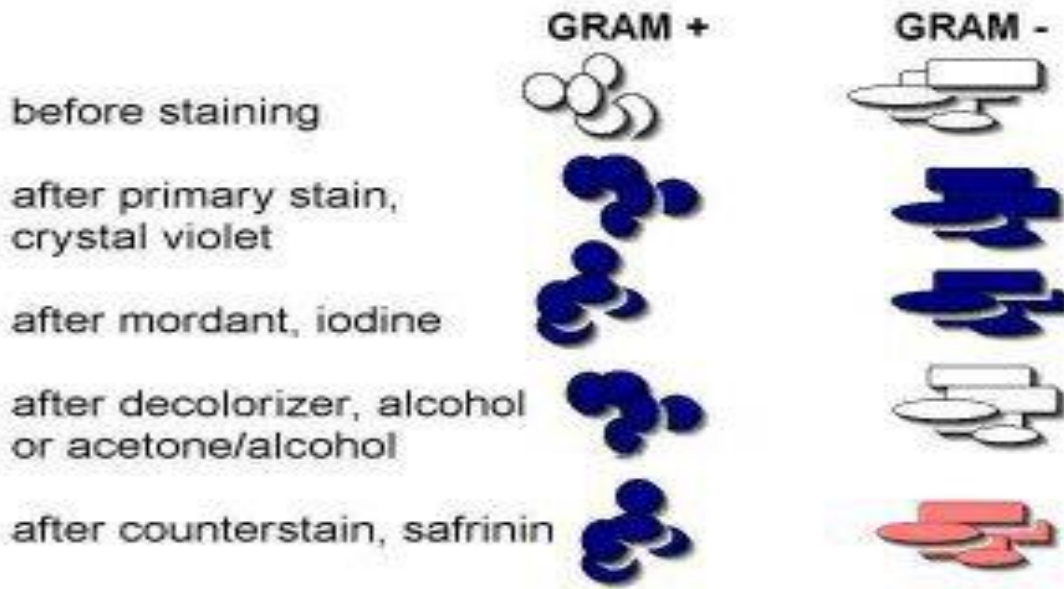


Mixed Sample of *S. epidermidis* & *E. coli*



REVIEW!

Check out this short video on [Gram Staining](#)



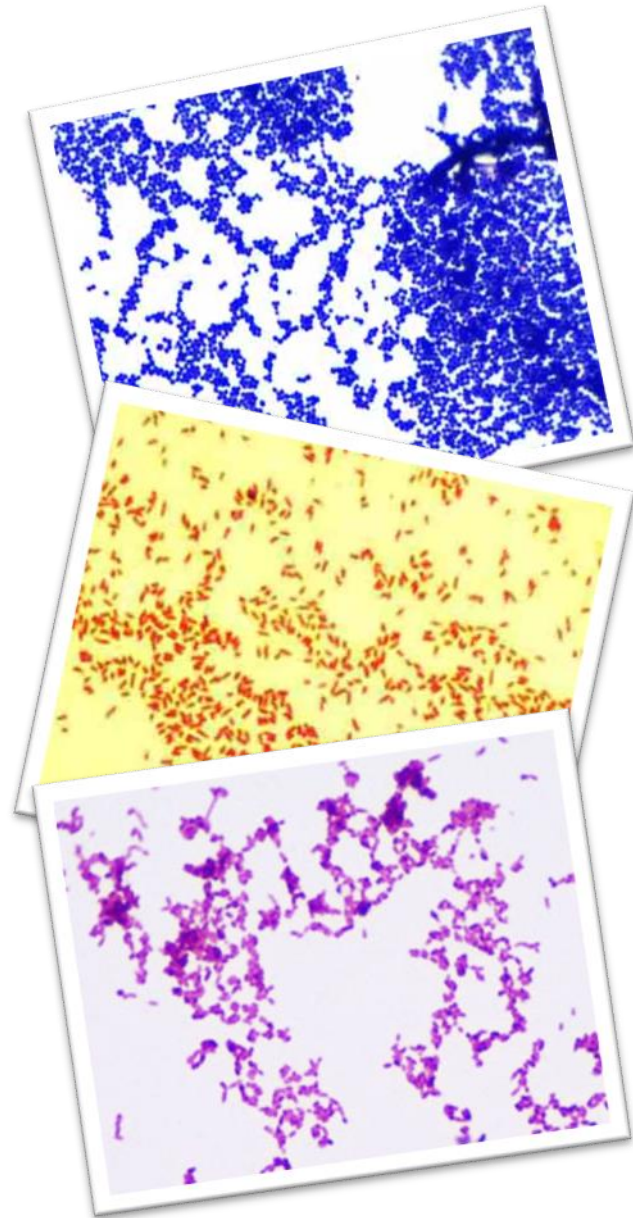
Q: How does the Gram stain reveal the difference between Gram+ and Gram- cell wall structure?

Bacterial Cell Wall

Function: Shape and protection

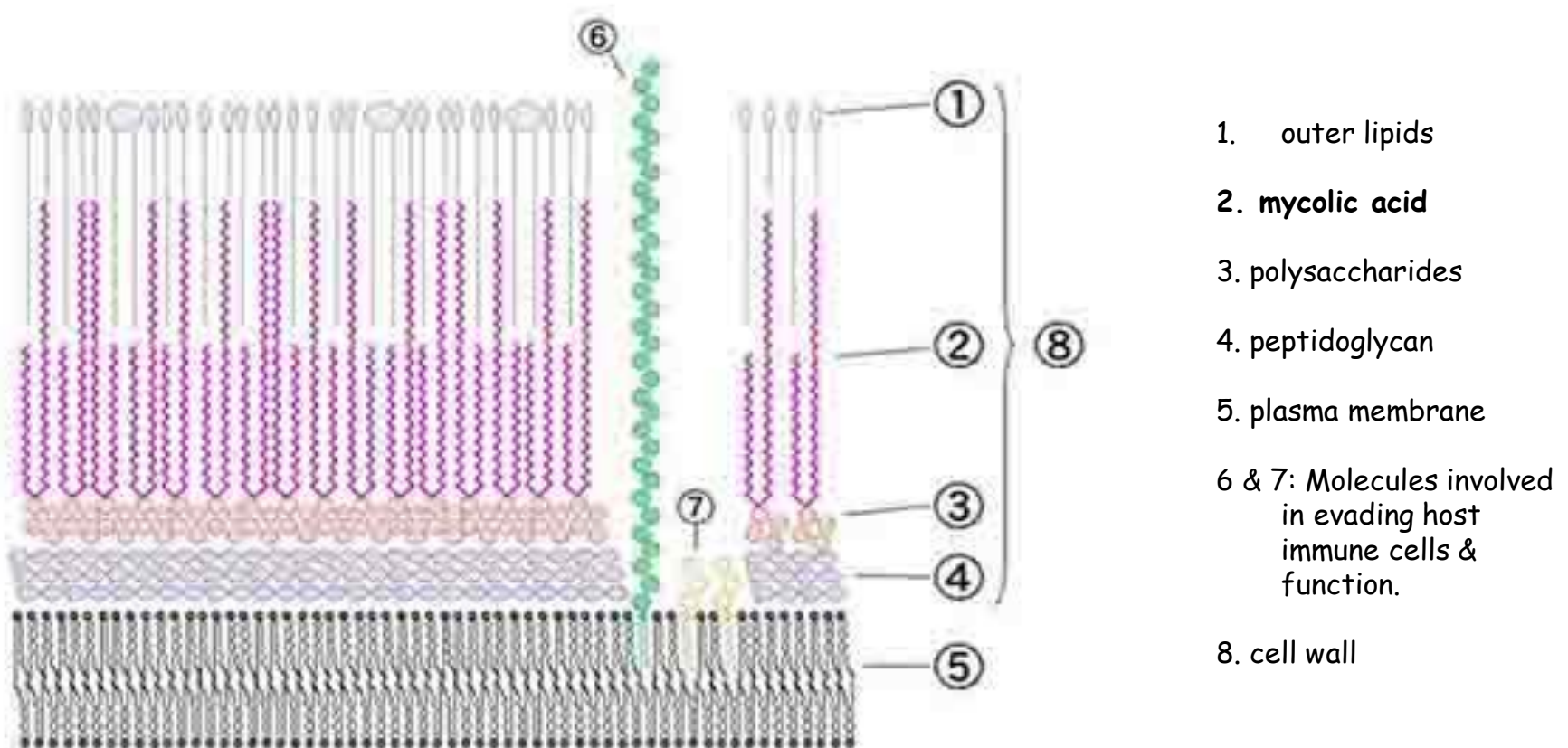
Structure: Distinguishes groups of bacteria

- Cells that Gram stain
 - Gram positive and Gram negative
- **Cells that resist Gram stain**
 - Genus *Mycobacterium* and *Nocardia*
 - Stained using Acid-fast staining techniques
- Cells that lack cell walls
 - Will retain counterstain (second color applied during differential staining).



Images: Gram positive bacteria , Gram-negative bacteria & Acid fast bacteria, all under oil immersion @1000XTM, T. Port

Mycobacterial Cell Wall

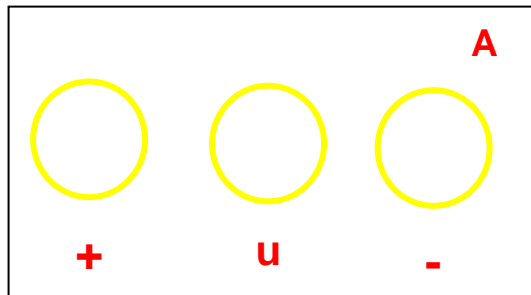


Because of waxy cell wall, these bacteria are "tough"; able to survive exposure to acids, alkalis, detergents, oxidative bursts, lysis by immune system, and many antibiotics.

Acid-fast Stain

- For staining cells resistant to Gram staining:
 - purple staining, Nonacid-fast cells (NAF)
 - bright pink staining, Acid-fast cells (AF)

Q: *Specifically what does this stain reveal about a bacterium's cell wall?*



ACID-FAST STAINING PROCEDURE

Blotting paper

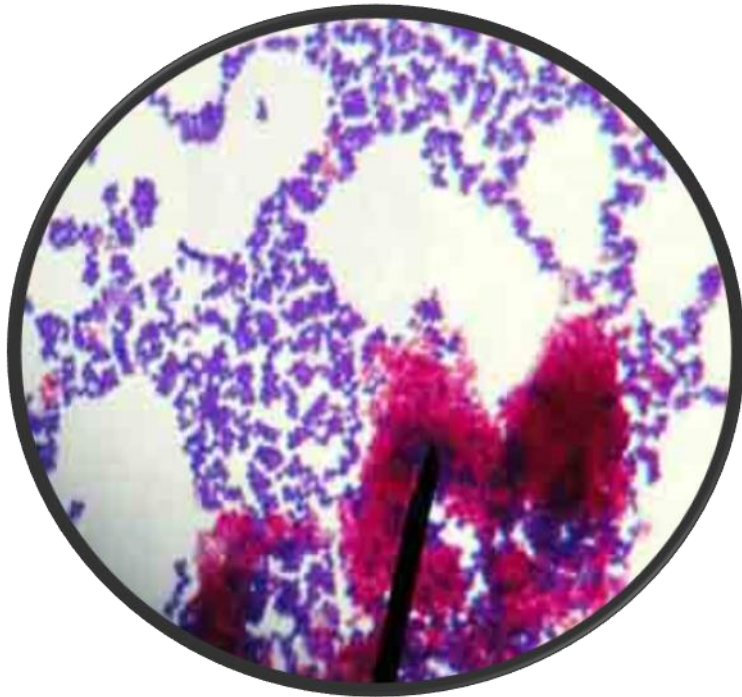
Ziehl's carbol fuchsin (3 – 5 min heat) > *rinse*

Acid Alcohol (10 – 15 sec) > *rinse*

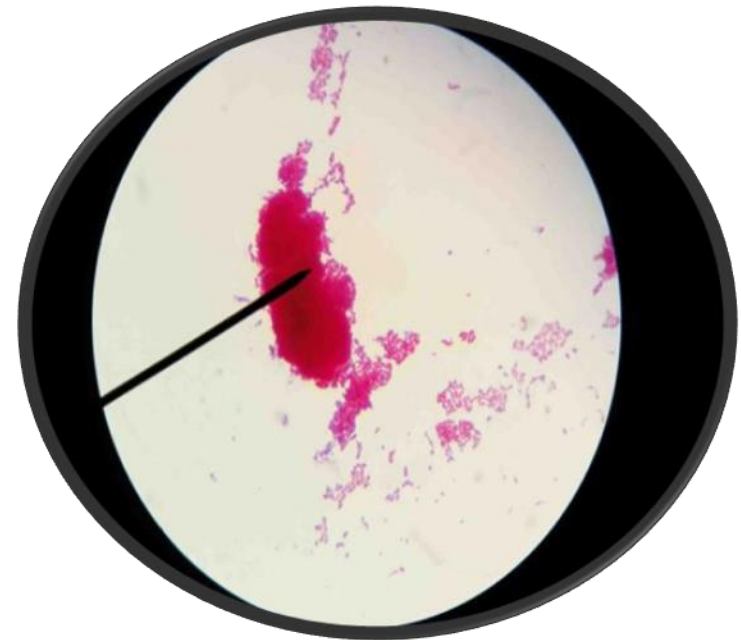
crystal violet (1 min) > *rinse & blot dry*

	Acid Fast Organisms	Not Acid Fast Organisms
Create a smear of organism you are testing. Cover smear with a blotting paper.		
Saturate paper with Ziehl's carbol fuchsin (say <i>fyook-sin</i>). Heat 3 – 5 minutes. Remove blotting paper.		
Rinse slide with tap water, then decolorize the smear for 10 - 15 seconds with acid alcohol. Rinse.		
Apply crystal violet for 1 minute, wash, blot dry.		

Acid Fast Stain Examples



Mixed sample of *Mycobacterium smegmatis* & *Micrococcus luteus*



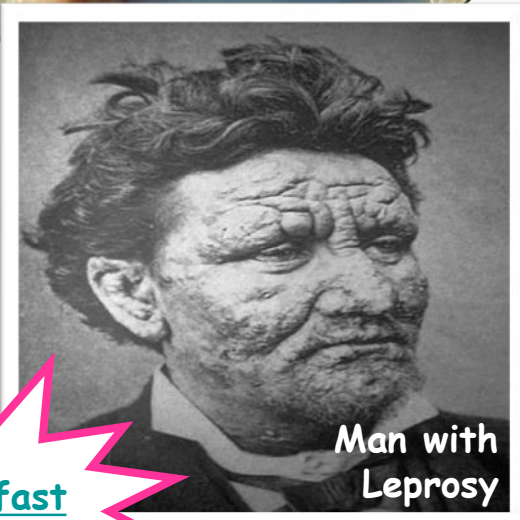
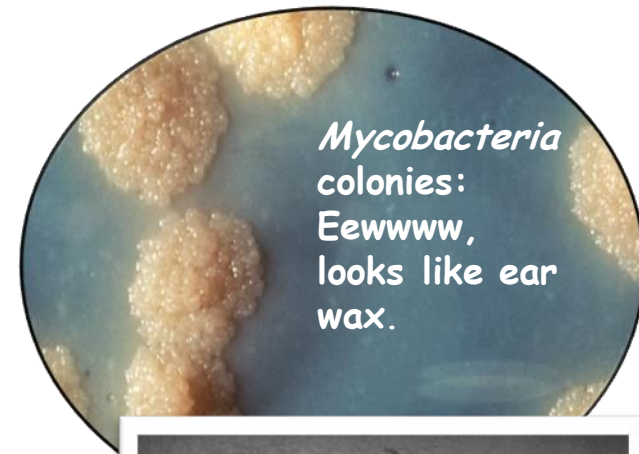
Mycobacterium smegmatis

Bacterial Genus: *Mycobacterium*

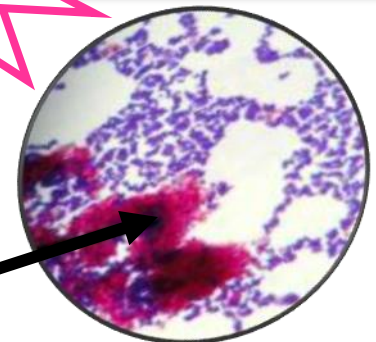
GRAM-variable, obligate aerobe, bacillus-shaped

Q: Why Gram variable?

- Both **leprosy** and **tuberculosis** caused by *M. leprae* and *M. tuberculosis* respectively, have plagued mankind for centuries.
- Thought that *M. tuberculosis* and *M. leprae* evolved from a soil bacterium that infected cows, then made jump to humans about the time of animal domestication, 10,000 years ago.
- *M. tuberculosis* doubles population every 18-24 hours,
- *M. leprae* doubles population about every 14 days.
- **Q:** What might be the impact of generation time on the course of the infectious diseases these microbes cause?



The pink is our lab friend *Mycobacterium smegmatis*



REVIEW!

Check out this short video on [Acid Fast Staining](#)

Ziehl-Neelson Stain Kiriyoun Modification

Acid Fast
Organisms

Not Acid Fast
Organisms

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Saturate paper with Ziehl's carbol fuchsin (say *fyook-sin*). Heat 3 - 5 minutes. Remove blotting paper.



Rinse slide with tap water, then decolorize the smear for 10 - 15 seconds with acid alcohol. Rinse.



Apply crystal violet for 1 minute, wash, blot dry.



Q: *How does acid fast staining reveal the difference between AF and NAF cell wall structure?*

Bacterial Cell Wall

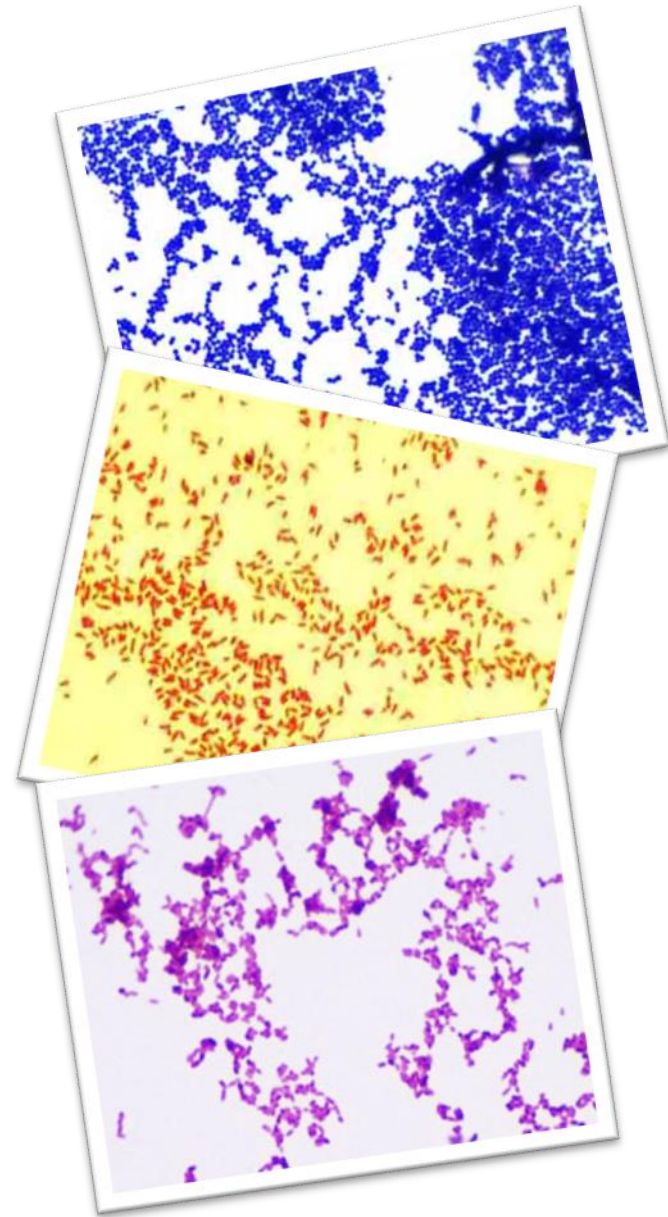
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➤ Cells that lack cell walls

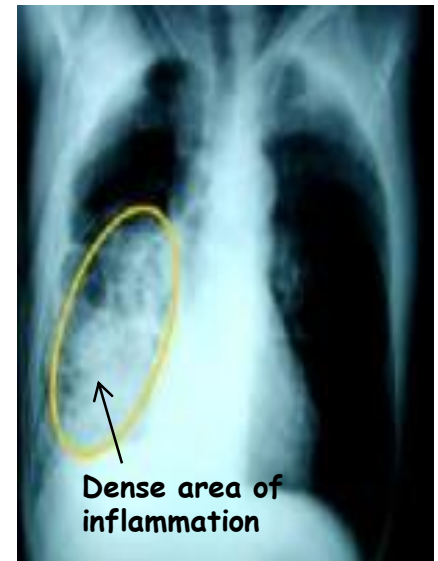
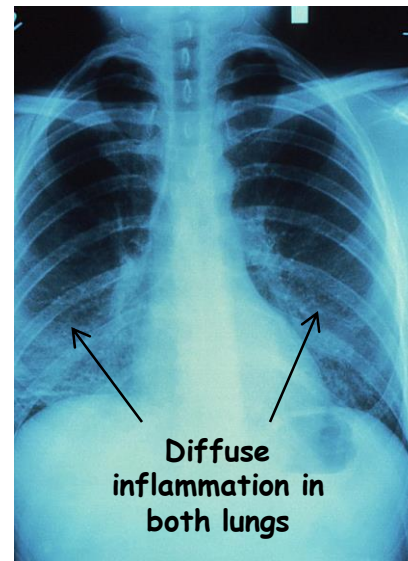
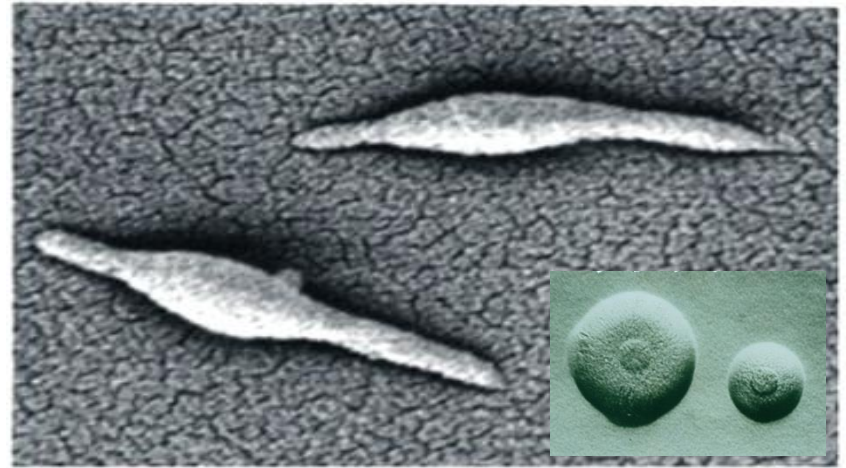
- Will retain counterstain (second color applied during differential staining).
- Called **L-form** or **cell wall-deficient (CWD)** bacteria. Sometimes difficult to detect and grow in standard lab conditions.



Images: Gram positive bacteria , Gram-negative bacteria & Acid fast bacteria, all under oil immersion @1000XTM, T. Port

Meet the Microbe: *Mycoplasma pneumoniae*

- **Pleiomorphic** shaped bacteria with no cell wall.
- Cause of primary atypical pneumonia (walking pneumonia).
- **Transmission:** Airborne droplets
- **Pathogenesis:** Bacterial cells attack and destroy ciliated epithelial cells of respiratory track.
- **Treatment:**
 - Often clears with no intervention.
 - With no cell wall, these organisms are resistant to the effects of beta-lactam antibiotics.
 - If antibiotic needed, treatment of choice is Erythromycin or Tetracycline (both protein synthesis inhibitors).



Images: [Mycoplasma pneumoniae](#) cells, Microbe Wiki; [Mycoplasma pneumoniae colonies](#); X-ray showing atypical pneumonia, PHIL 14372; [Typical pneumonia](#)

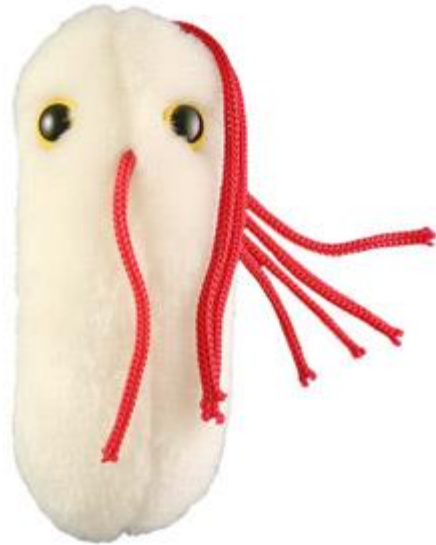
Confused?

Here are links to resources that further explain bacterial cell wall & differential staining:

- [Differential Stain Laboratory](#) Main Page on the Virtual Microbiology Classroom of [Science Prof Online](#).
- [Gram Stain](#) Interactive Tutorial. This is an extremely useful tutorial that shows, step-by-step, what happens in Gram-positive and Gram-negative cells during Gram staining.
- [Acid-fast Stain](#) Animated Tutorial. The staining procedure depicted in this tutorial differs a bit from how we do it in lab, but this tutorial is still very useful. Shows the steps of the staining procedure and the resulting color of Acid-fast and Nonacid-fast cells.
- Videos of differential staining procedures: [Gram](#), [Acid-fast](#), [Endospore](#)
- [Drug Resistant TB: Past, Present & Future](#), Chang et al (2010) [Official Journal of the Asian Pacific Society of Respirology](#), DOI: 10.1111/j.1440-1843.2010.01738.x



Smart Links

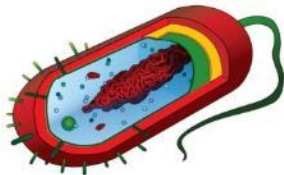


Are microbes intimidating you?

Do yourself a favor. Use the...

Virtual Microbiology Classroom (VMC) !

The VMC is full of resources to help you succeed, including:



- practice test questions
- review questions
- study guides and learning objectives

You can access the VMC by going to the Science Prof Online website

www.ScienceProfOnline.com