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INTRODUCTION TO BACTERIOLOGY AND PATHOGENESIS

OCTOBER 12-20

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Communication is key:

Check Courses Announcements page for emails and corrections

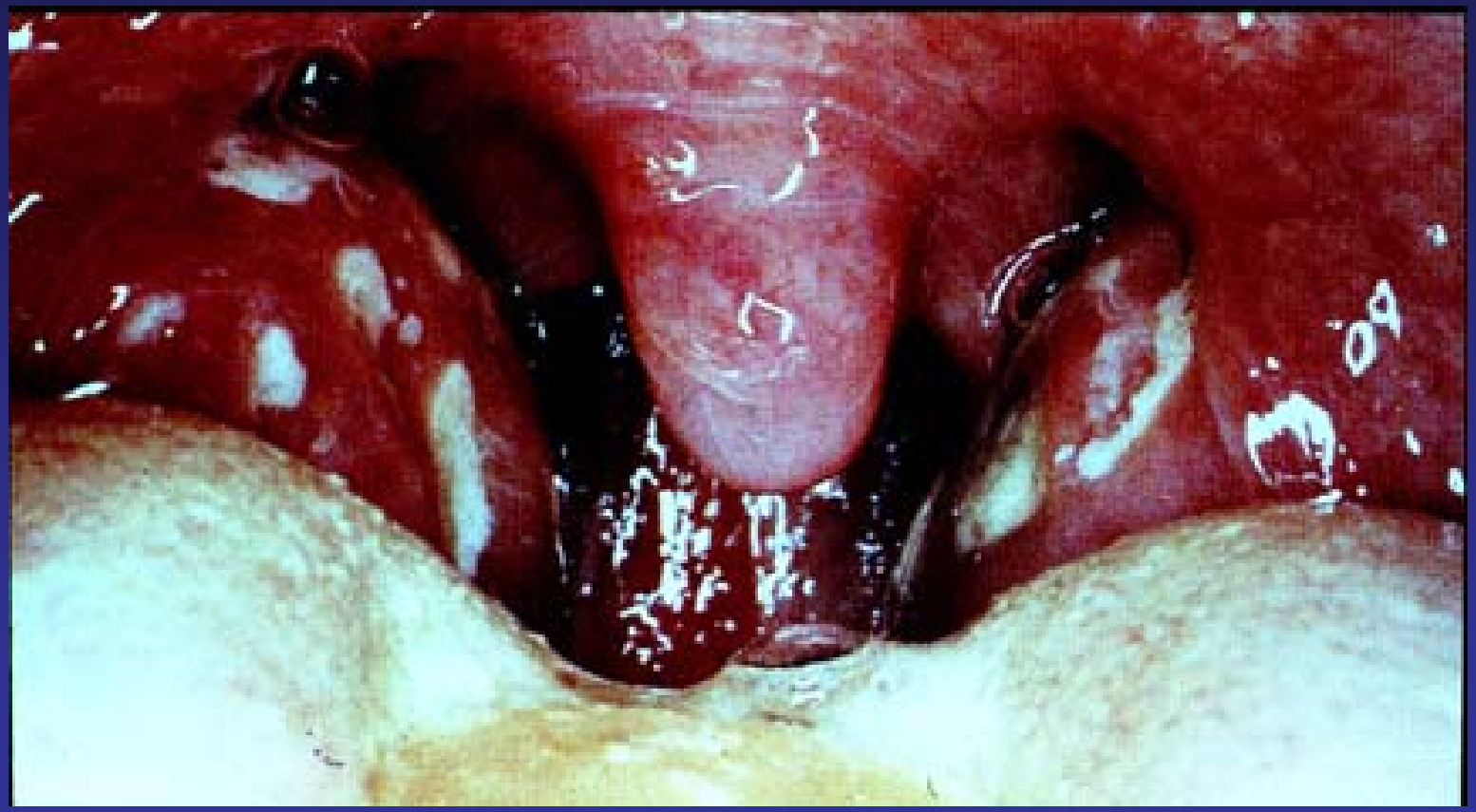
Three sections:

- Introduction to Bacteriology
- Pathogenesis of Infectious Diseases Caused by Bacteria
- Parasitology/Mycology

Note Assignments:

- Virtual Microbiology Lab
- Clinical Microbiology Conferences
- BUGS cases

A ten-year old boy experiences a sudden onset of extremely sore throat, pain on swallowing, fever of 103˚F, swollen lymph nodes in his neck, and general malaise.
If his throat looked like this ...



what is the diagnosis?

If his throat looked like this ...

what is the diagnosis?

Why is his throat so inflamed?

What is the mechanism of damage?

Are there any possible serious consequences to this disease? Why, why not?

What nonspecific defenses were available to fight this infection?

Why were they not effective?

Will specific immunity eventually clear this infection? Why, why not?

Will he be immune from the disease in the future? Why, why not?

Can this infection be treated with antibiotics? Why, why not?

What determines your choice of antibiotics to try?

Do you have to worry about the organism being resistant or becoming resistant to the antibiotics? Why or why not?

Are the boy's siblings and classmates at risk for getting this disease from him?

STAY TUNED FOR THE ANSWERS TO THESE AND MANY MORE QUESTIONS!

“The fundamental differences in the structure and physiology of bacteria as infectious agents vs. us as hosts are the bases for most of the damaging effects of infectious disease and our ability to fight infectious disease with antibiotics.”

For example, the small size and simple internal structure enable the rapid growth of bacteria to contaminate food or overcome host defenses.

BACTERIA (prokaryotes) VS. EUKARYOTES

Cell Structure:	Bacteria	Eukaryotic
Organization	Unicellular Multicellular	Unicellular/
Cell Membrane: oxidative phosphorylation, DNA replication	transport, motility,	transport
Endocytosis/Exocytosis	-	+
Intracellular Membranes: Nucleus, Golgi, Mitochondria, Endoplasmic Reticulum	-	+
Cytoskeletal:		
Microfilaments	-	+
Microtubules	-	+
Cell wall	Peptidoglycan	-
Genetics:		
Chromosomes	1 (2: Vibrios, Neisseria)	>1
Topology	Circular	Linear
Segregation	Cell membrane	Mitotic spindle
Transcription/ Translation	coupled in cytoplasm	Transcription - nucleus Translation - cytoplasm
mRNA capping, poly-A	-	+
Introns	(-)	+
Cistron structure	Polycistronic	Monocistronic
Ribosome	70S (50S + 30S)	80S (60S + 40S)
Genetic Exchange Conjugation	Transformation, Transduction,	Meiosis, Zygote fusion

Bacterial structure

- **Small** (1-8 microns)
- **Shapes** (important for identification and making diagnosis)

Others (**vibrios, filamentous, coccobacilli**)

Envelope structure is unique to prokaryotes

Cell wall

- rigid structure surrounding the cell membrane

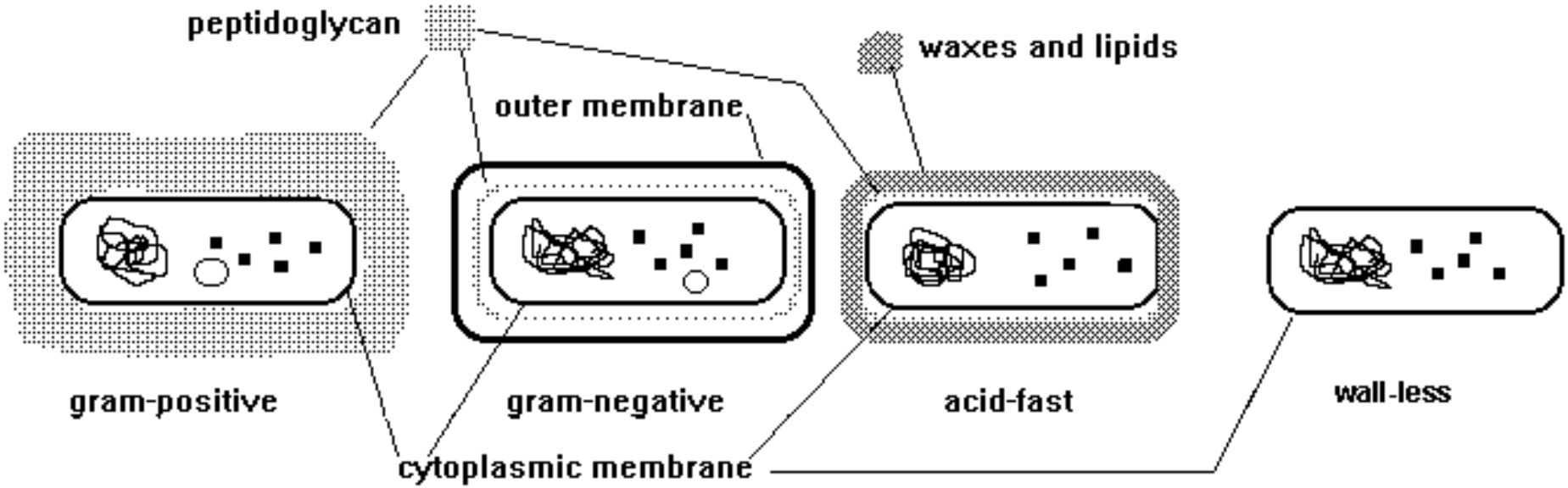
Functions:

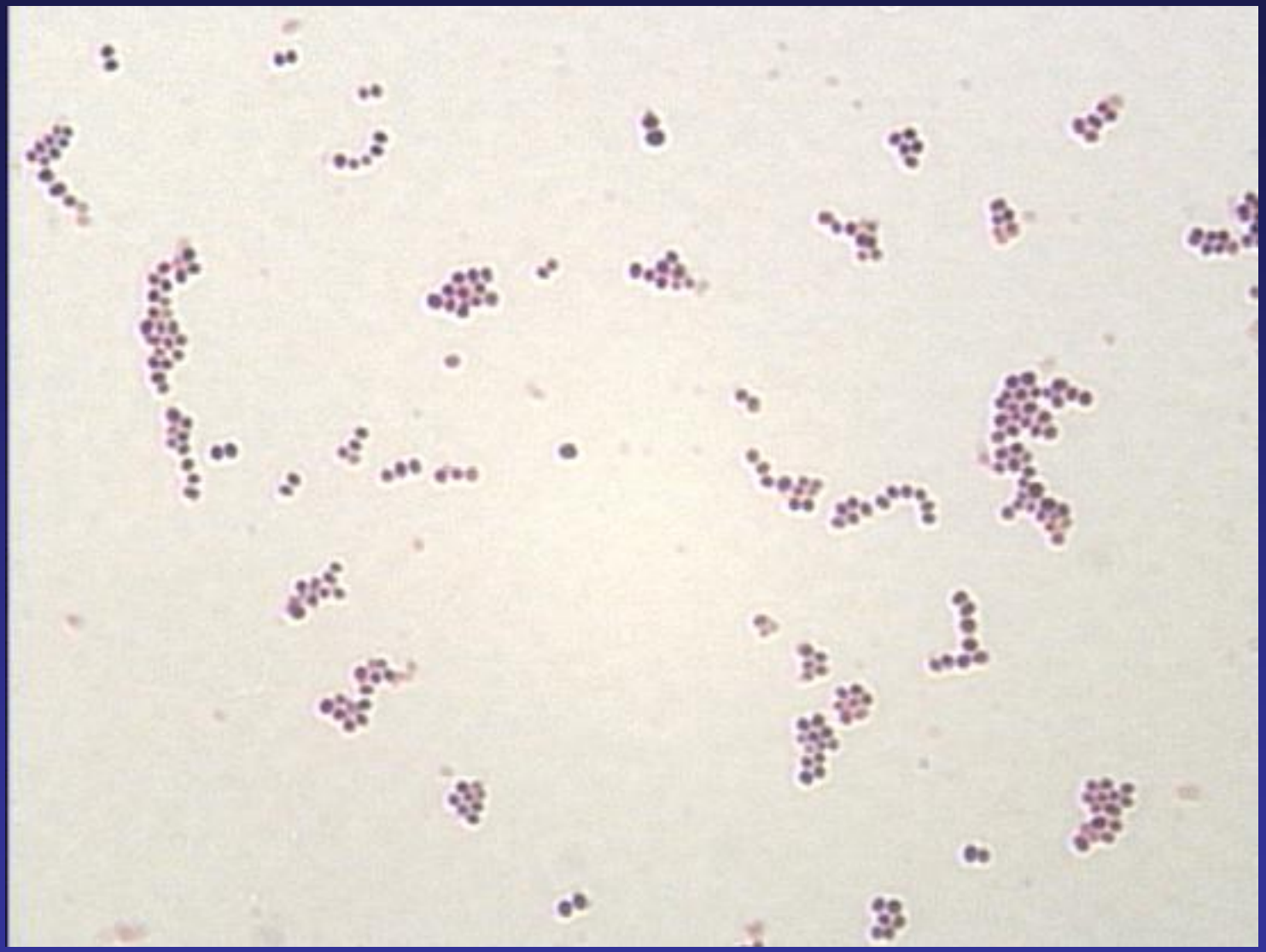
- prevent osmotic lysis
- protect cell from external stresses (host)
- contributes to virulence
- target for antimicrobials

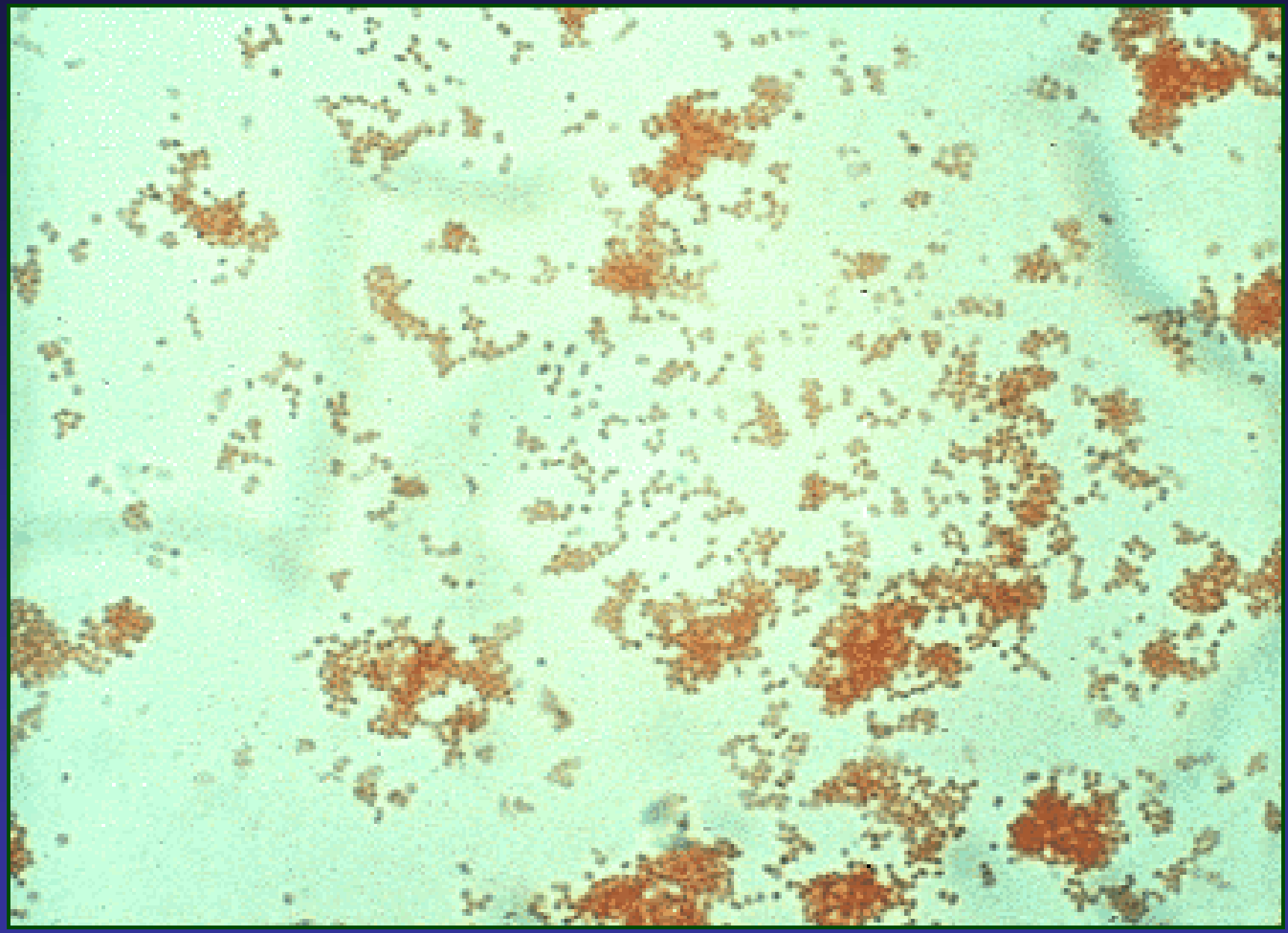
Gram stain
gram-positive (blue)
gram-negative (pink)

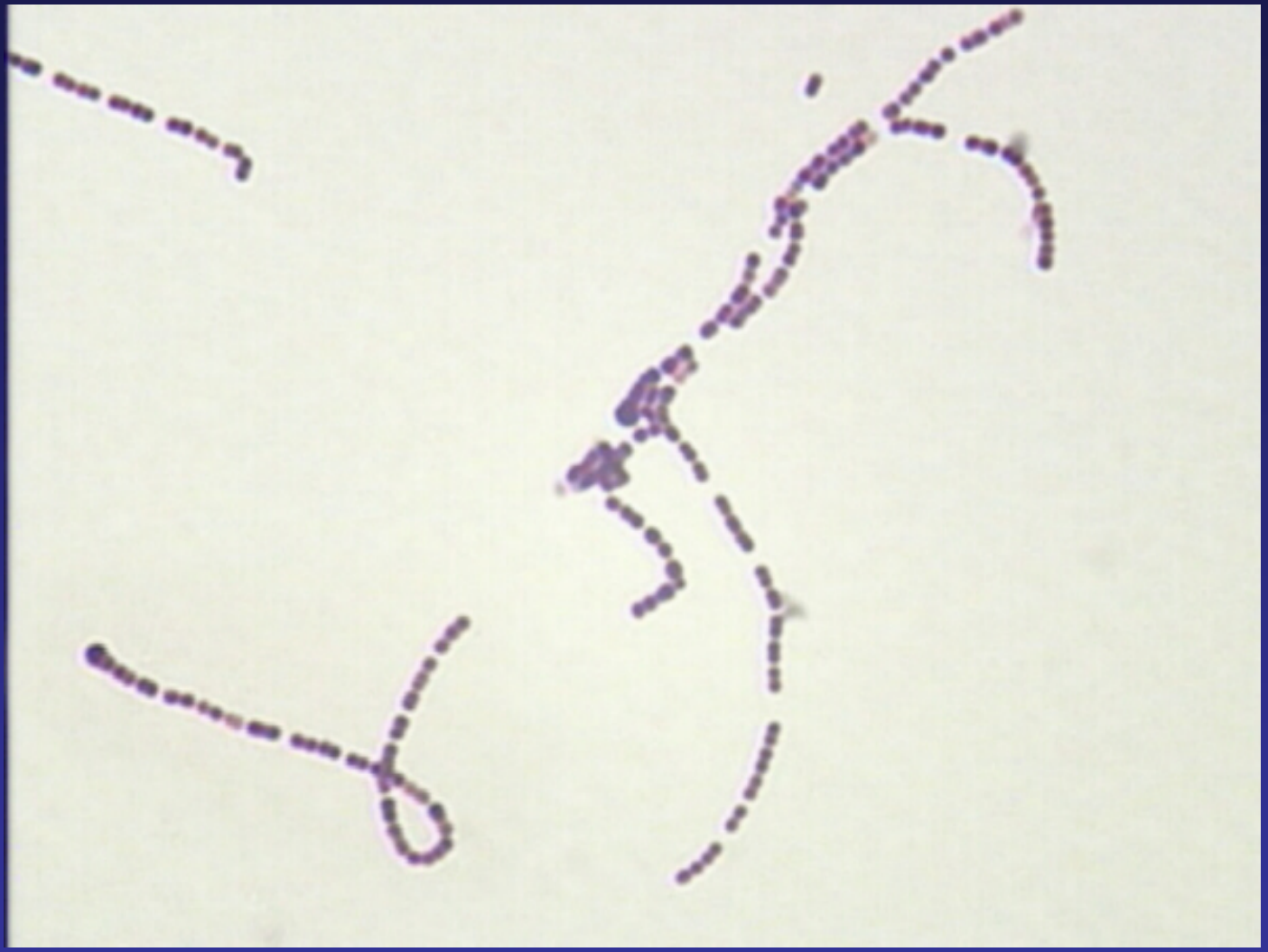
Acid fast stain
acid fast (red on blue)

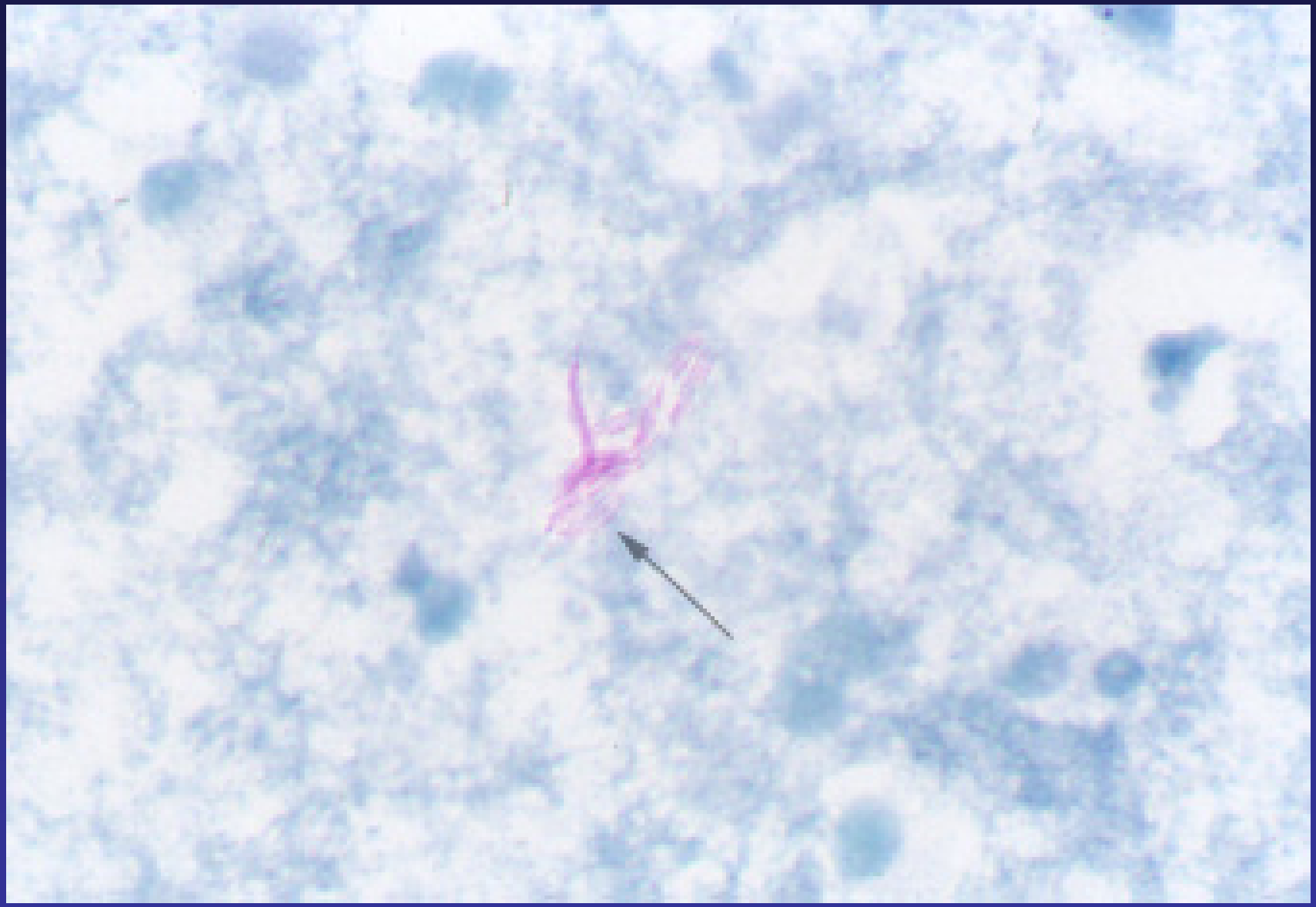
Wall-less (can't stain - need special stain)

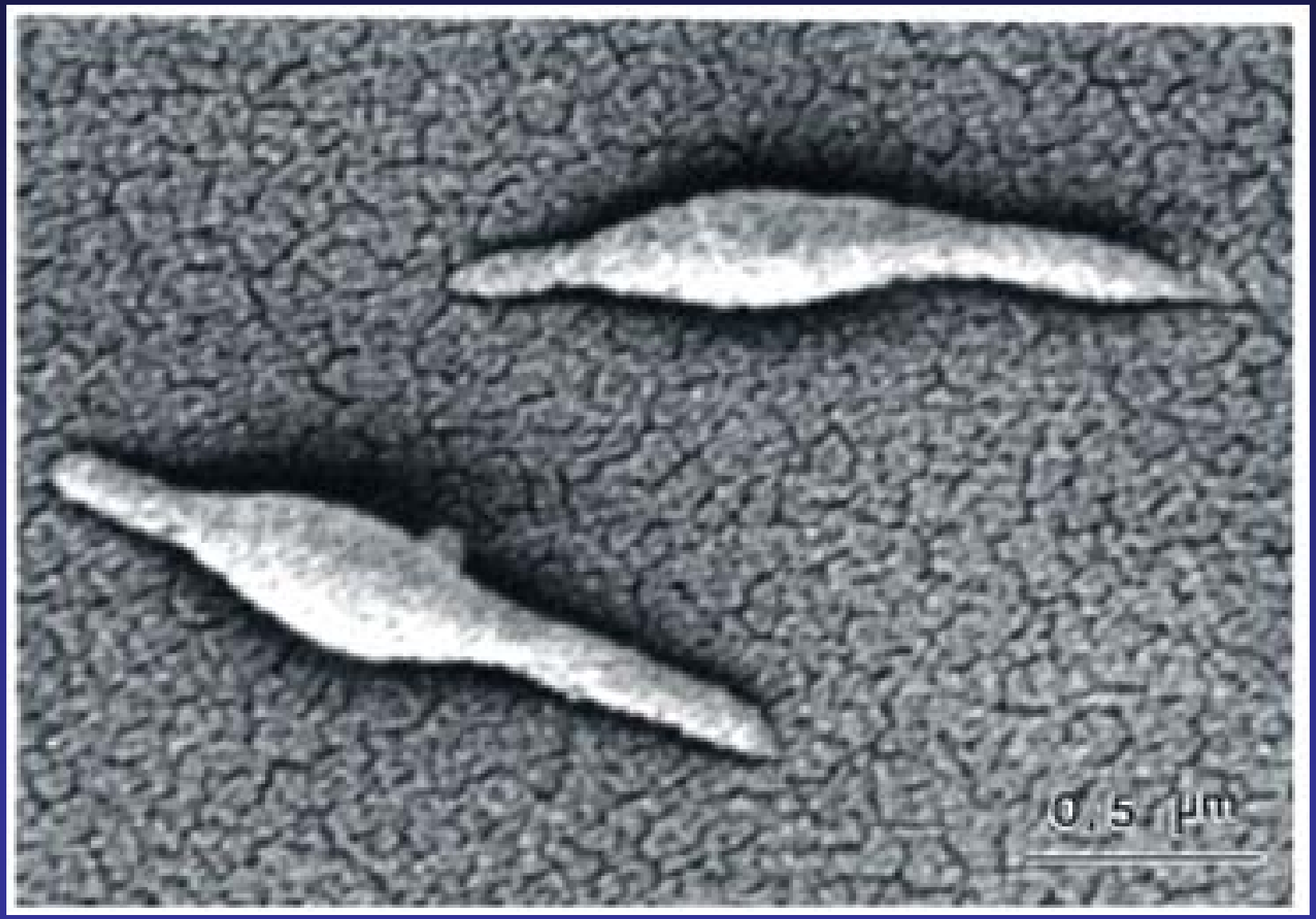






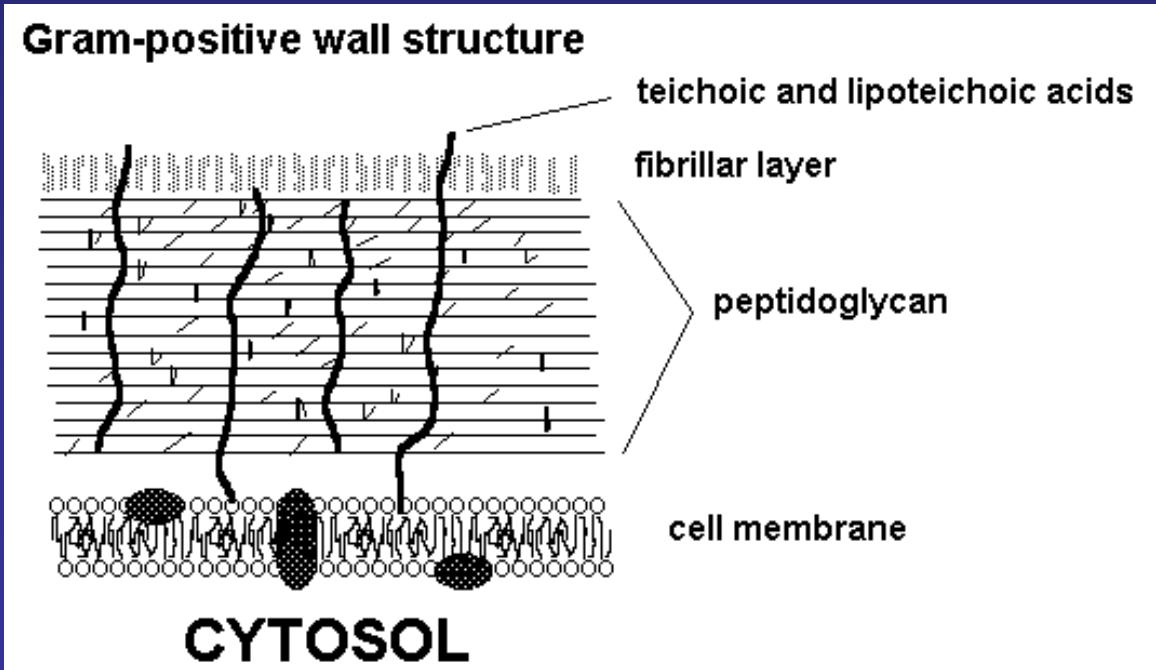





0.5 µm

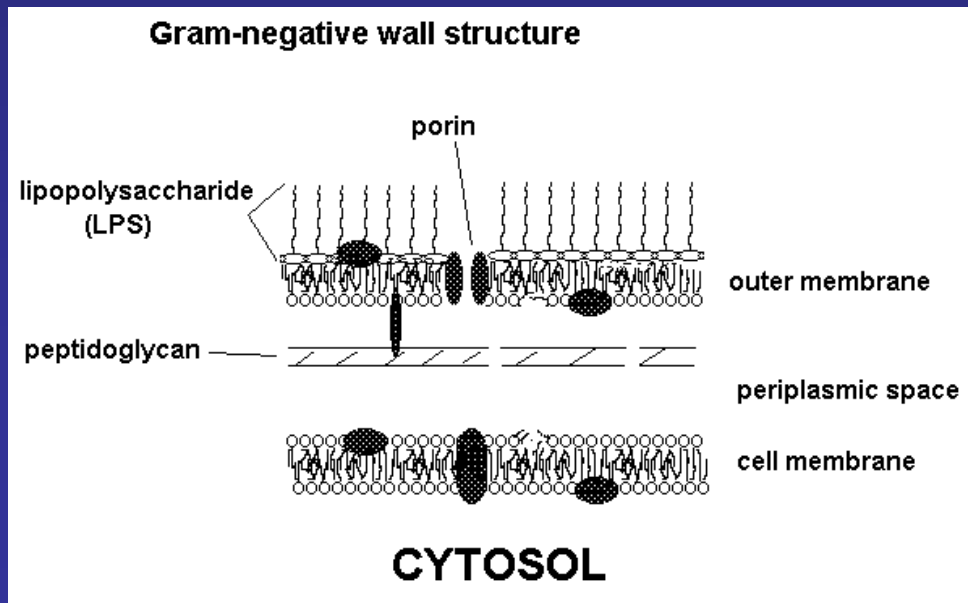
Gram-positive structure

- **thick peptidoglycan** cell wall (40+ layers of chain link fence)
- resist lysis by complement, but still can be opsonized
- **teichoic acids and lipoteichoic acids** (polymer of ribitol or glycerol – phosphates), antigenic classification
- other proteins and carbohydrates (e.g., M protein fibrillar layer and Group A carbohydrate capsule of *Streptococcus pyogenes* contribute to virulence).



Gram-negative structure

- the **outer membrane** - a second lipid bilayer
- **periplasmic space** between inner (cytoplasmic) and outer membrane
- **single layer of peptidoglycan** in periplasmic space
- special outer membrane proteins (**porins**) enable diffusion across outer membrane
- outer surface of the outer membrane contains unique lipid component - **lipopolysaccharide (LPS)**, which is extremely important in pathogenesis



Acid fast structure - (Mycobacteria)

- most similar to gram-positive bacteria
- cell wall composed of **fatty acids** and **waxes** which contribute to virulence
- hydrophobic components difficult to stain, but once stained, retain stain (resistant to acid decolorization)
- **mycolic acid, Wax D, cord factor, arabinogalactans, and sulfolipids** (mycobacterial virulence factors)

Wall-less structure - (Mycoplasma)

- No cell wall (no peptidoglycan)
- No outer membrane
- Incorporation of cholesterol from host
- Very labile
- No definite shape
- Small genome
- Simplest free-living organisms (artificial life?)

Peptidoglycan (murein layer)

- **unique to prokaryotes**
- **antimicrobials:**
  - β-lactams: penicillins and cephalosporins, vancomycin, bacitracin

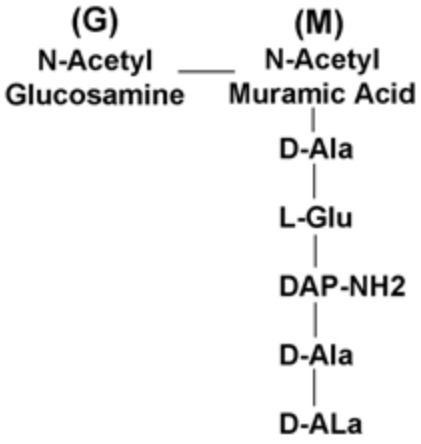
- enzyme **lysozyme** hydrolyses backbone

- **composition - murein backbone with unusual peptide chain**
  - N-acetyl glucosamine - N-acetyl muramic acid
  - pentapeptide with L and D amino acids

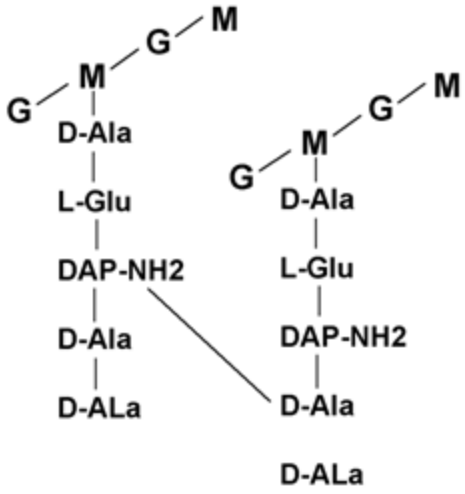
Peptidoglycan Structure

Gram Negative

Building Block

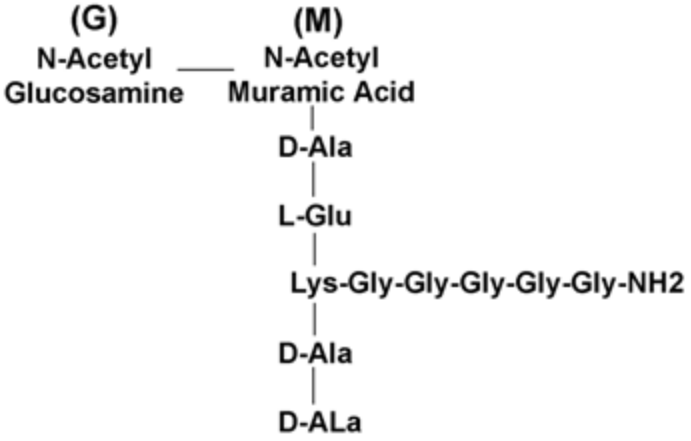


Crosslinking (Transpeptidation)



Gram Positive

Building Block



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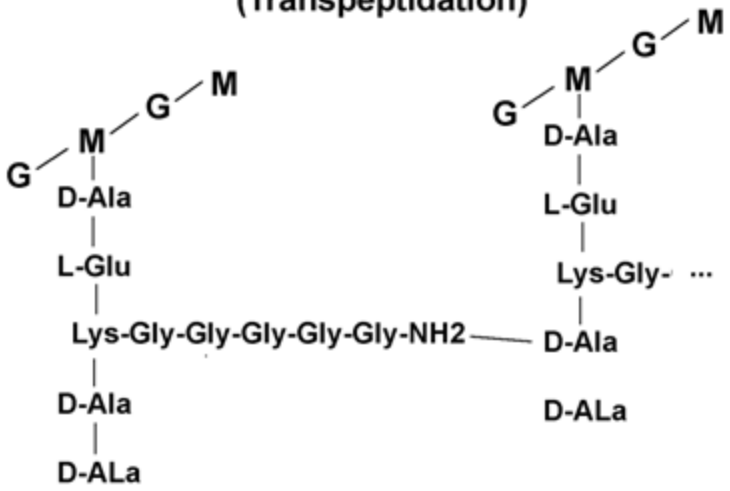


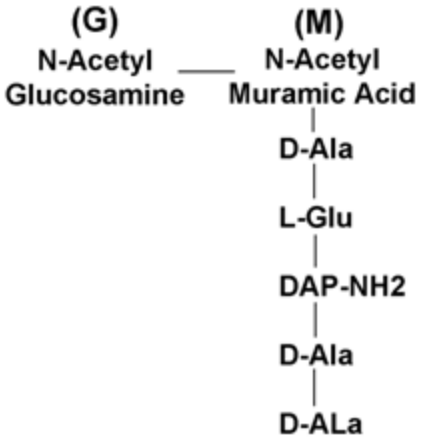
synthesis

- build blocks in cytoplasm
- **transport through cytoplasmic membrane**
  - **(bacitracin-sensitive)**
- polymerize backbone
- **cross-link peptides**
- **third amino acid** - NH2 side chain (**lysine** [gram-positives] or **diaminopimelic acid** [gram-negatives]) peptide bond displaces terminal amino acid (D-alanine) of adjacent peptide chain, crosslinking chains and conferring rigidity

Peptidoglycan Structure

Gram Negative

Building Block



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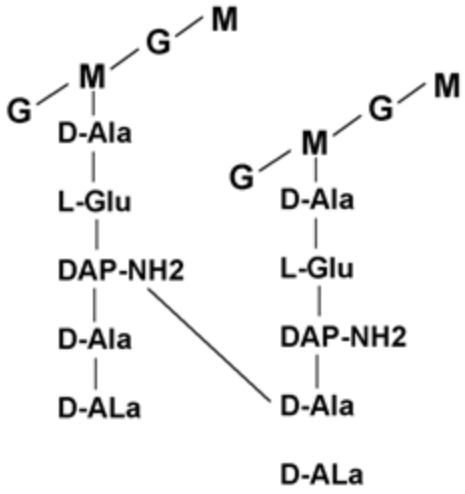
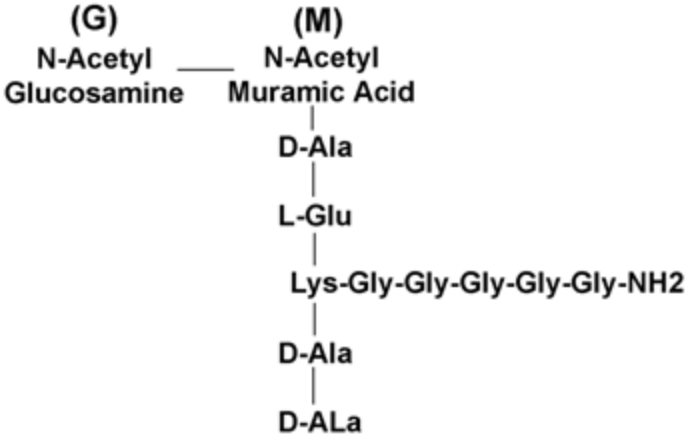


Gram Positive

Building Block



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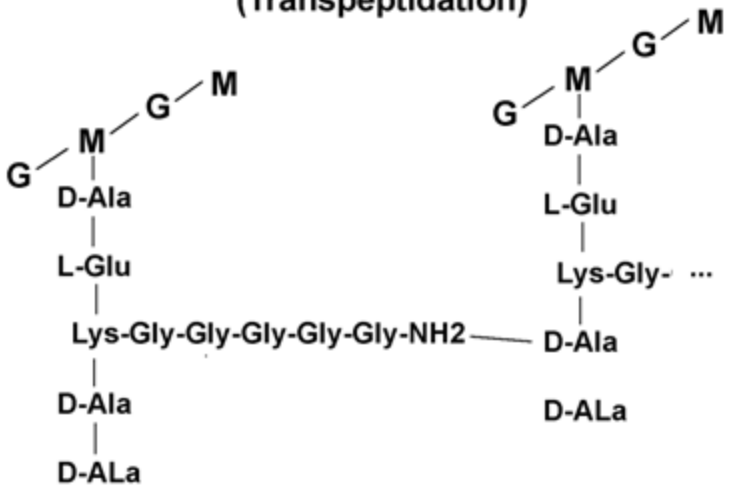


- **Penicillin-binding proteins (PBPs)** - perform crosslinking, etc.
- some gram-positive cells - **pentaglycine bridge** to form cross-links
- **muramyl dipeptide** - highly **inflammatory** and chemotactic
- **recognized by TLR-2**

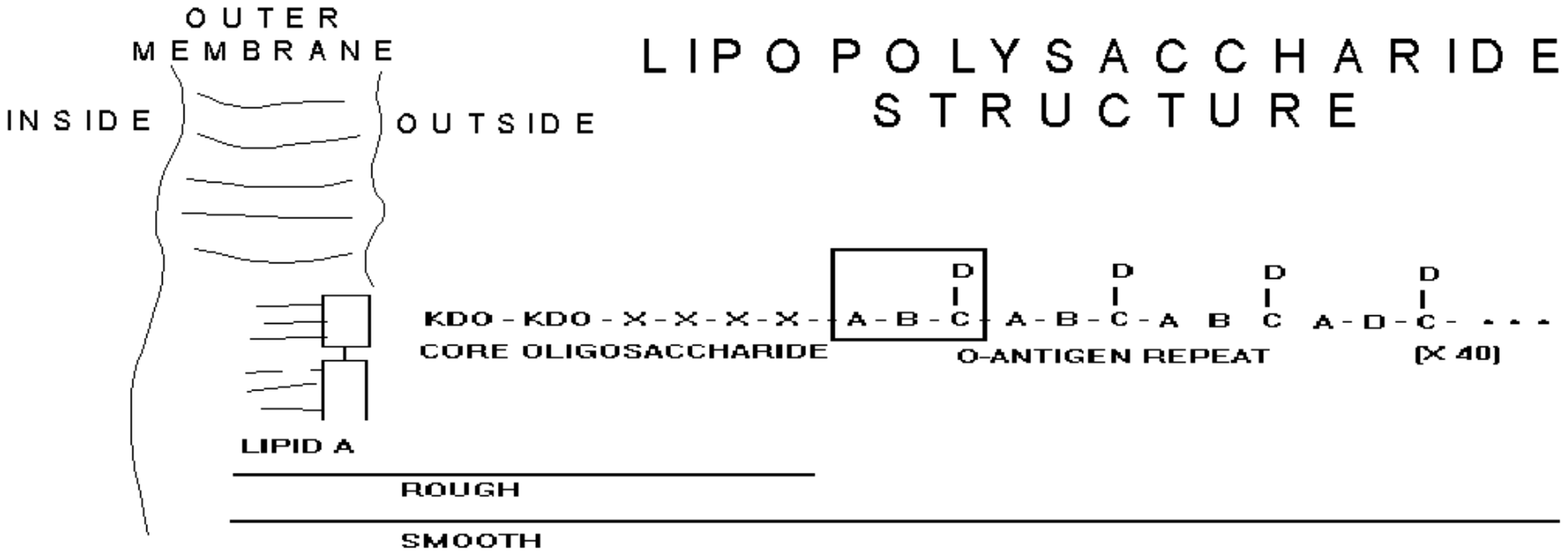
Lipopolysaccharide (LPS) – Endotoxin

The most important part of gram-negative bacteria

- **lipid A**
  - embedded in membrane = **endotoxin activity**
  - unique C14 fatty acid - **β-hydroxy myristic acid**, phosphates, glucosamine
  - **recognized by TLR4**

- **core oligosaccharide**
  - highly conserved among different bacteria
  - unique components - **KDO and heptose**

LIPOPOLYSACCHARIDE STRUCTURE



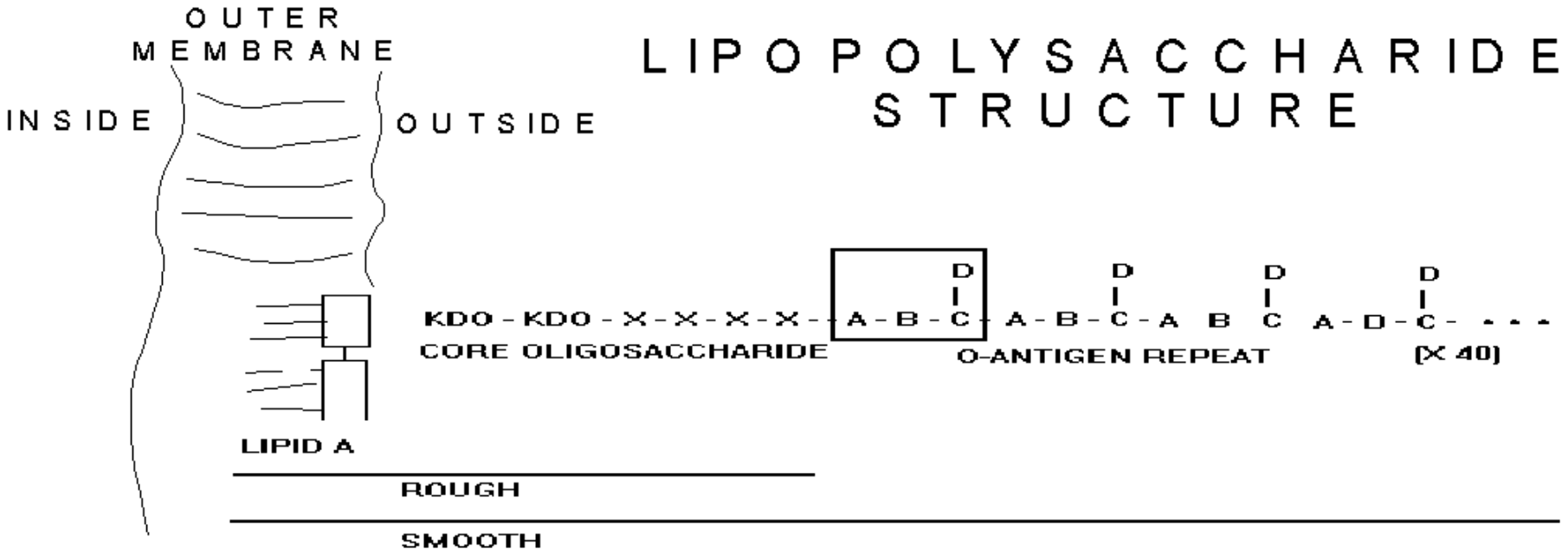
- **O antigen**
  - may be present or not, depending on species

  - **repeating units** of 3 to 5 sugars

  - **smooth** with O antigen
  - **rough** without (ending at core)
  - LPS of bacteria without O antigen
  - sometimes called lipooligosaccharide (LOS)

  - **antigenic** and highly **variable** among species and strains

LIPOPOLYSACCHARIDE STRUCTURE



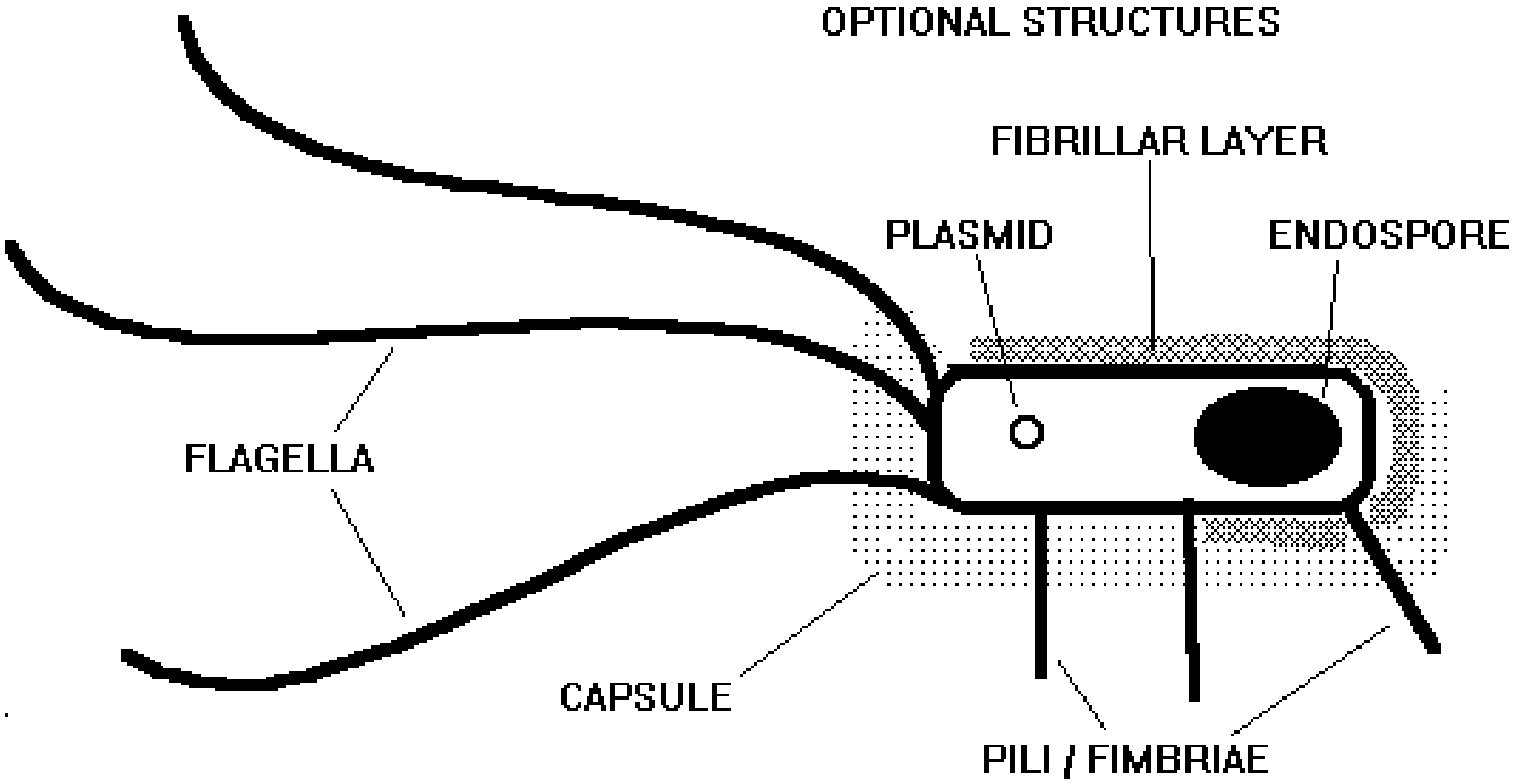
Other optional gross structural components

- **Capsule** (slime layer), K antigen
  - not impermeable
  - **Both** gram-positive and gram-negative bacteria can make capsules

  - **polysaccharide**
  - (exception: *Bacillus anthracis* (anthrax) poly-glutamate)

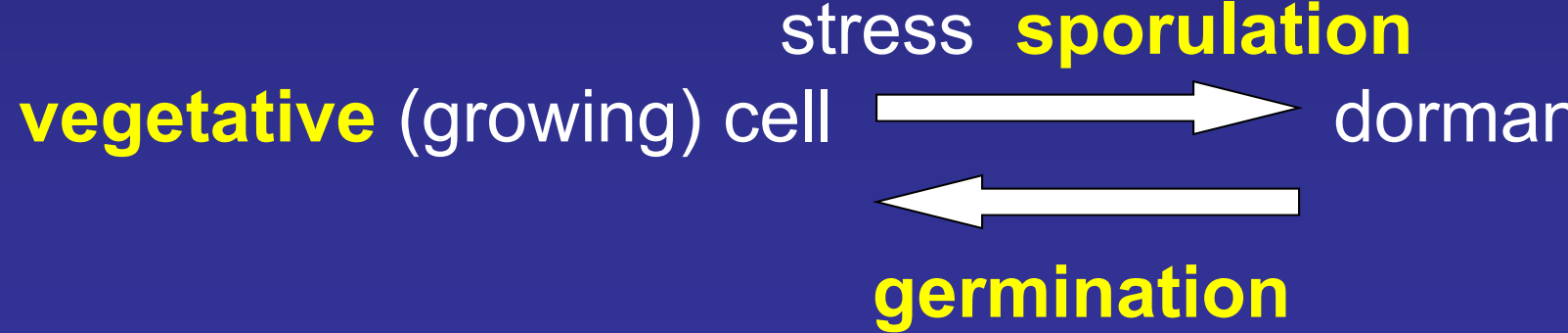
  - **virulence - inhibit complement - phagocytosis**

- **glycocalyx** - extracellular polysaccharide; **biofilms;** technically not a capsule


OPTIONAL STRUCTURES
FIBRILLAR LAYER
PLASMID
ENDOSPORE
FLAGELLA
CAPSULE
PILI / FIMBRIAE

- **Flagella** - H antigen
  - propeller
  - motility and chemotaxis
  - recognized by TLR5
- **Pili/fimbriae**
  - hair-like; protein; 2 unrelated functions:
  - **adherence**
  - **genetic exchange** (not related to adherence fimbriae)
- **Fibrillar layer**
  - protein coat on surface
  - virulence (e.g., M protein of *Streptococcus pyogenes* is anti-phagocytic, others involved in adherence to host cells)

- **Spores**
  - certain **gram-positives only** - both aerobic and anaerobic
  - metabolically **inactive**
  - **resistant to heat (boiling), desiccation**
  - need **autoclave (121˚C, 15 min)**
  - Contain **dipicolinic acid**
  - developmental stage in response to stress:



- **Plasmids - Non-chromosomal DNA**
  - usually circular
  - can be transmissible between cells by **genetic exchange (conjugation)**
  - some encode **virulence properties, antibiotic resistance**

Cytoplasmic/Inner Membrane

- **similar** to eukaryotic **plasma membrane** and **mitochondrial membrane**

- **little usefulness as target for antibiotics**

- carries out **many functions**
  - **transport:** facilitated diffusion, active transport, group translocation (phosphotransferase – carbos)
  - **electron transport and oxidative phosphorylation**
  - **energy production**
  - **motility**
  - **replication**

Nucleoid - Chromosome – DNA

- **Single, circular structure (haploid genome)**
  - **Vibrios have 2 different chromosomes**
- < eukaryotic chromosomes, ~ 3,500 genes
- **Not in nucleus - no nuclear membrane.**
  Transcription in cytoplasm with translation
- **Supercoiling - DNA gyrase - DNA replication**
  - **Nalidixic acid** and other **quinolones** inhibit gyrase and DNA replication
  - **Metronidazole** - binds to DNA after metabolism by anaerobes, inhibiting DNA replication

Ribosomes

- **similar but different from ours**
- **70S** ribosomes composed of **50S** and **30S** subunits
- **co-transcription-translation**
- **target of many useful antimicrobials:**
  - **aminoglycosides**
  - **tetracyclines**
  - **chloramphenicol**
  - **macrolides - erythromycin**

Metabolism

- **(review intermediary metabolism for other exams)**
- **The "meaning of life" for bacteria is growth = replication** - they don't just sit around
- Colony Forming Unit (CFU)
- Replication = synthesizing a bacterial cell
- Most **metabolic pathways** are similar if not identical to ours, therefore not targeted by antibiotics
- See "Breathing Problem" BUGS case

- **Oxygen and bacterial physiology and growth**

- **aerobes** - grow well in the presence of oxygen; they tolerate oxygen and oxidative products of metabolism

  - **strict or obligate aerobes** require oxygen
  - **facultative anaerobes** - grow well in presence or absence of oxygen (aerobes)

- **anaerobes** - grow best in the absence of oxygen
  - **microaerophilic** or **aerotolerant** - tolerate ↓oxygen
  - **obligate anaerobes** - cannot tolerate oxygen or oxidative products of metabolism

- **processing samples and ordering culture** tests

Unique functions

- acquisition of **iron** by **siderophores** - important for **virulence,** (no antibiotics yet)
- **folic acid metabolism** (1 carbon donor: DNA synthesis, etc.)
- **humans** get folic acid as a **nutrient**
- **bacteria** must **synthesize**
- **sulfanilamide** is a **PABA** analog that inhibits **dihydropteroate synthetase**
- **trimethoprim** inhibits **dihydrofolate reductase**

Transcription

- **RNA polymerase - ααββ'δ**
  - ααββ' – core
  - δ – binds to promoters
- inhibited by **rifampin**

- **regulation** of protein synthesis is primarily at level of **initiation of transcription** involving regulatory DNA binding proteins to turn on/off genes in response to environmental conditions (remember the Lac operon)

- **polycistronic operons**, several genes transcribed from same promoter and regulated by the same conditions

- some genes regulated in response to **stress** - **heat shock proteins** involved in survival (also involved in **autoimmune reactions**)

- **Quorum sensing** - regulation in bacterial communities such as biofilms
  - small **inducer molecules** are secreted
  - when concentration in environment reaches threshold (quorum has been attained), **gene expression** changes

Note: Review lactose operon and Lambda phage regulation for "other" exams.

Translation

- co-transcription/translation in cytoplasm
- ribosomes - smaller / antibiotics

Growth

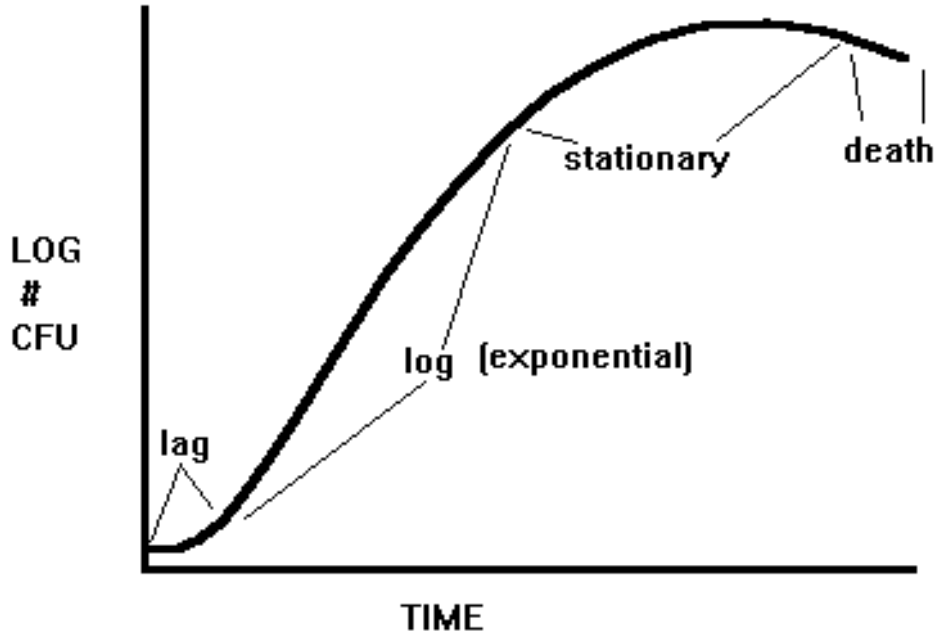
- **Fast** - as little as 10 min. generation time (*Vibrio vulnificus*) as long as 24 hr. (*Mycobacterium tuberculosis*)

- **Phases: lag, log (exponential), stationary, death**

Calculating yield: $\mathbf{N_t = N_0 \times 2^g}$
g = number of generations

Simple rule of thumb:
3 gen. = 10X increase

Note: You might be asked to perform some simple growth calculations on exams.



Biofilms - communities on solid/liquid environments

- change metabolism
  - **glycocalyx** holds the cells together
  - **slow** metabolism and growth.
  - resistant to antibiotics and host defenses
  - **planktonic** bacteria are free, individual – NOT in biofilm.
  - **quorum sensing**

- Contaminated devices (catheters, artificial valves, etc.)

- Body - tooth plaque, heart valves

Temperature:

- **Mesophiles** - grow best at our **body temperature - 37˚C**
- **Special growth temperatures:**
  - *Campylobacter* - 42˚C
  - *Listeria* and *Yersinia enterocolitica* - 4˚C

Bacterial culture:

- **provide all necessary things for growth**

- **Fastidious** organisms require many nutrients
- **Simple** requirements can make everything from scratch

- **Some bacteria cannot be cultured in vitro**
  - ***Chlamydia* and *Rickettsia*** - tissue culture like viruses
  - ***Treponema pallidum, Mycobacterium leprae*** not at all, require animal infection

- Cannot predict virulence by growth (some slow or non-culturable bugs can still kill you!).

Sterilization and disinfection

- **Sterilization - no viable organisms at all**
- **Disinfection - pathogens reduced < infectious levels**
- **Methods**
  - sterilization – autoclave (≥121°C, 15 lb/sq in, 15 min), UV irradiation, gamma irradiation, filtration, phenolics
  - disinfection - antiseptics - detergents, ethanol, halogens (Cl), peroxide, betadyne
  - which method on what (e.g. a wound, a scalpel, i.v. fluid, bacterial waste) - need for sterility vs. ability to sterilize
  - spores are very resistant
  - Purell and other hand sanitizers - ethanol in gel
- **WASH YOUR HANDS ! WASH YOUR HANDS !**