



TU02 – Bacteriology

Structure of the bacterial surface of Gram negative bacteria

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Team « MicrobAdapt »

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Courses organisation

Introduction

- Bacterial structures – General
- Bacterial surface : differences Gram + / Gram -

Gram negative cell wall

- Inner membrane
- Periplasm
- Outer membrane with LPS
- Cell surface appendages

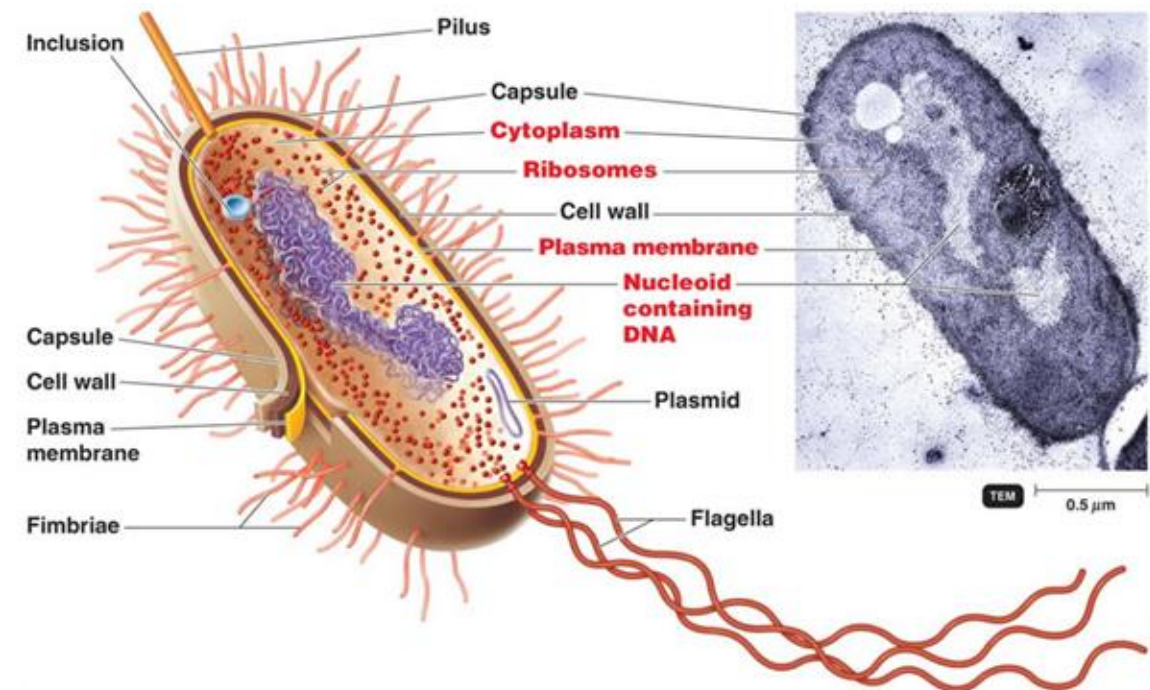
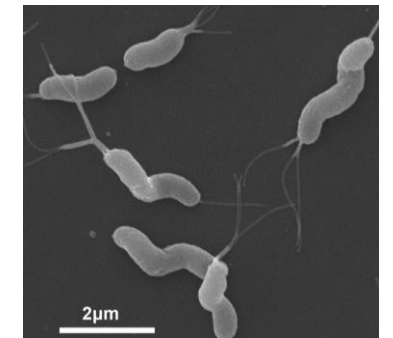
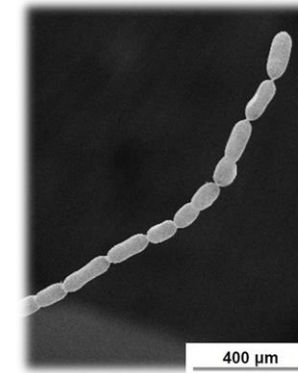
Today

Gram positive cell wall

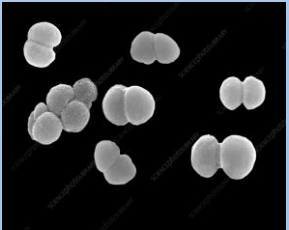
→ next course (23/01/24)

Bacterial structures

- ✓ Unicellular organisms, 1-5 μ m
- ✓ Common components with eukaryotic cells
 - Cytoplasmic membrane
 - Cytoplasm
 - DNA
 - Ribosomes
- ✓ Components specific to bacteria
 - Peptidoglycan cell wall (protection, shape)
 - Polysaccharide capsule (adhesion, resistance)
 - Fimbriae, pili, flagella



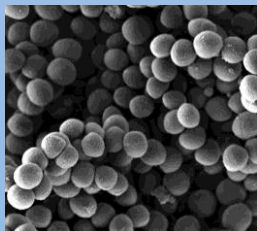
Cocci (spherical)



N. gonorrhoeae

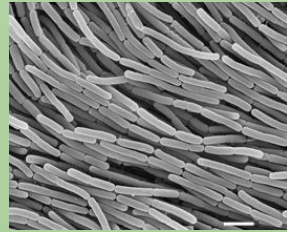


S. pyogenes



S. aureus

Bacilli (rod-shaped)



B. anthracis

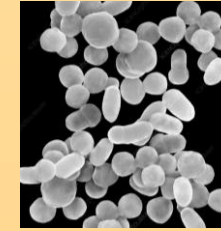


E. coli



C. perfringens

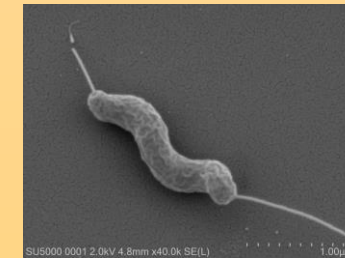
Others...



H. influenzae



V. cholerae

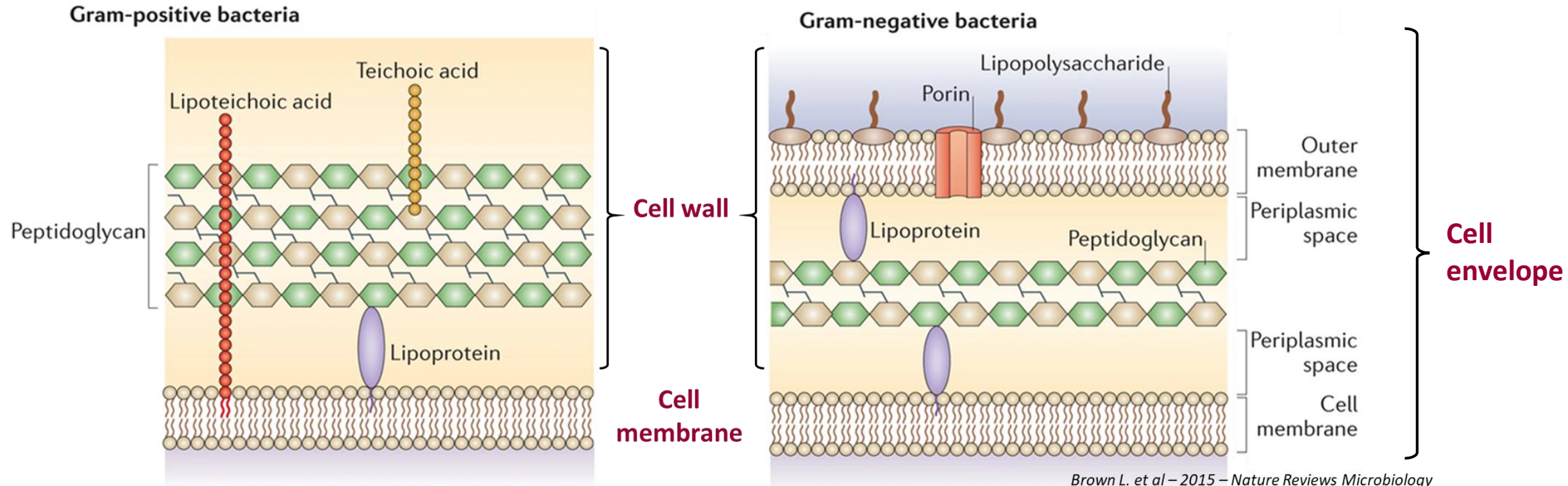


C. jejuni



T. pallidum

Bacterial surface



Cell wall

- Thick: 15-80 nm
- Several layers of PG + Teichoic acids (unique to G+ bacteria)
- Thin (10 nm) but strong /elastic
- Single layer of PG + Periplasm + Outer membrane (with LPS)

PG: disaccharides polymer / peptides monomers chains

→ **Protection** (stress, osmotic rupture, lysis), **ligands** (adherence), **receptors**

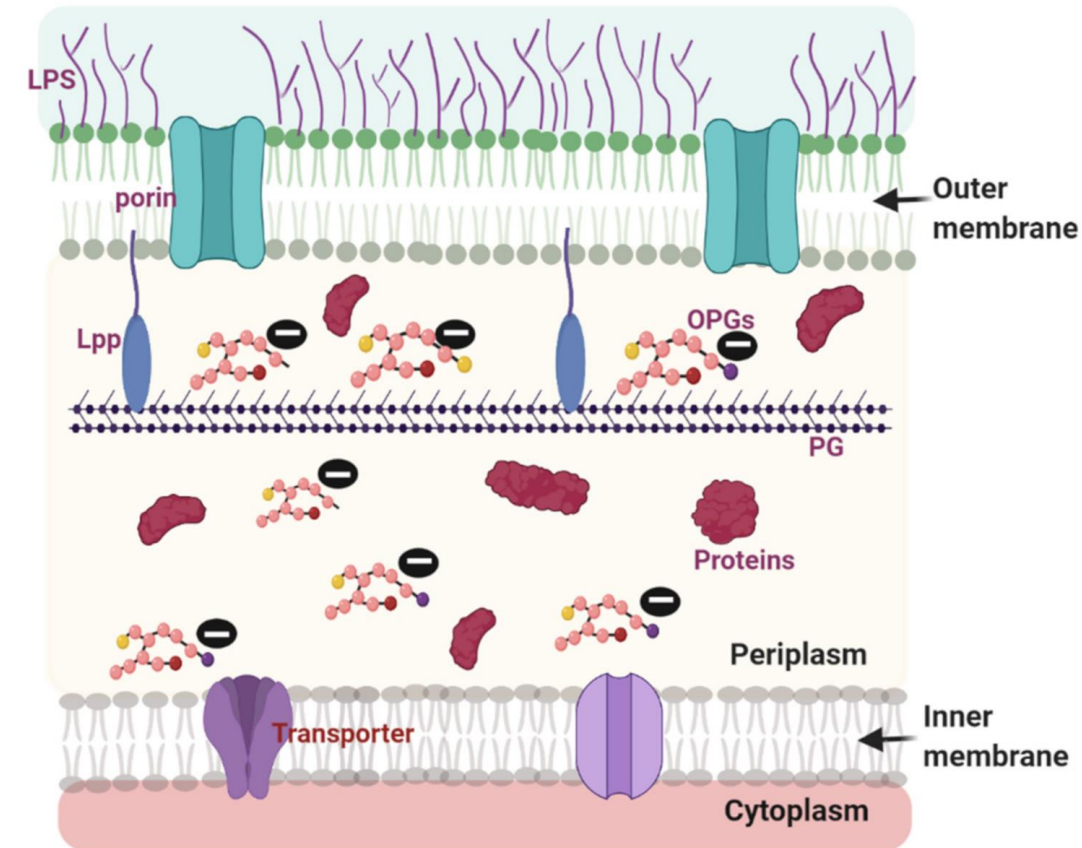
• Inner membrane

- ✓ Cytoplasmic membrane
- ✓ Symmetric phospholipid bilayer
- ✓ Selective nutrient transport, protein translocation

• Periplasm

- ✓ Contains:
 - Soluble proteins
 - Peptidoglycan mesh
 - Thin layer compared to Gram +
 - Protection against turgor pressure
 - Provides bacterial cell shape
 - Membrane-derived oligosaccharides

Duong F et al – 1997 – Cell



● Glucose	LPS- Lipopolysaccharides	⊖ Negatively charged
● Phosphoethanolamine	Lpp- Braun's lipoprotein	● Phospholipid
● Phosphoglycerol	OPGs- Osmoregulated Periplasmic glucans	● Lipid A
● Succinyl residue	PG- Peptidoglycan layer	

Alegun O et al – 2021 – Antibiotics

- **Outer membrane = Asymmetric bilayer**

- ✓ Phospholipids (inner leaflet)
- ✓ LPS (outer leaflet)

- **LPS**

- ✓ Large glycolipid
- ✓ OM major component
- ✓ Permeability barrier / « resistance »
- ✓ Composed by:

- **Lipid A**

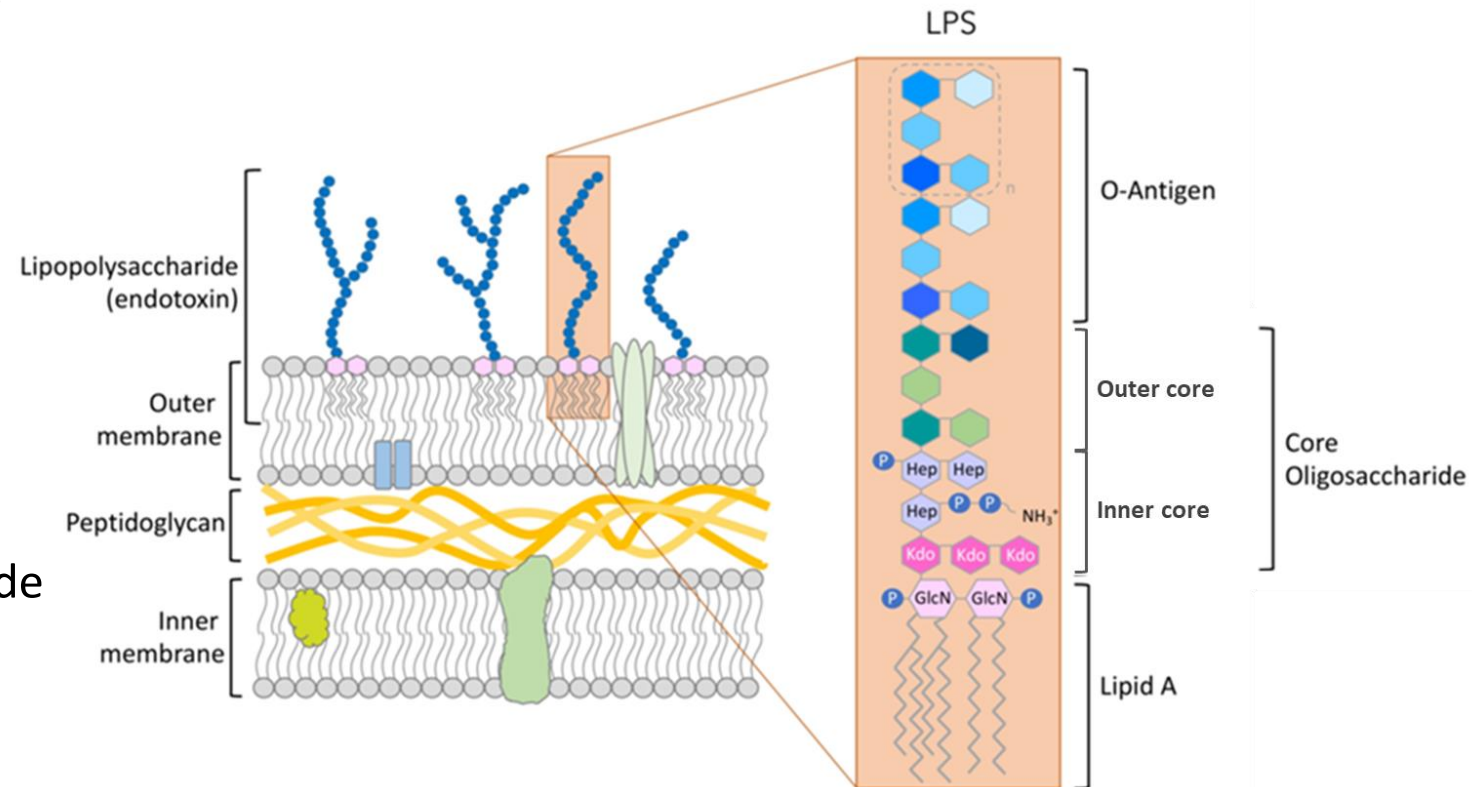
- Conserved
 - Phosphorylated glucosamine disaccharide
 - Fatty acid chains (hydrophobic anchor)

- **Core oligosaccharide**

- Inner core : unusual monosaccharides (keto-deoxyoctulosonate, heptoses)
 - Outer core : hexoses (glucose, galactose) +/- phosphates, phosphoethanolamine, ...

- **O-antigen**

- Repeating units of 1-6 sugar residues



Hsu Y.M. – 2020 – AB Biosciences

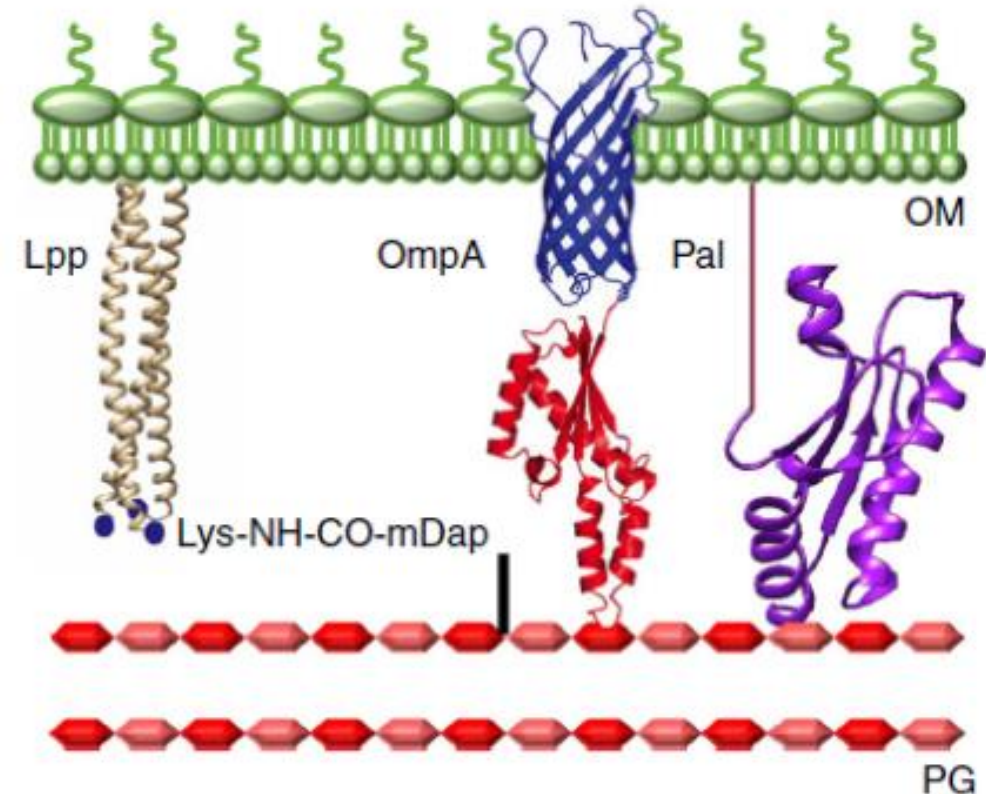
- **Attached to PG via different proteins**

- ✓ Lpp (BLP) → **covalent** attachment

- Braun lipoprotein
 - Covalently linked to PG (via C-ter)
 - Homotrimer anchored in the OM (N-ter)
 - Control of the periplasmic space's width
 - Regulation of mechanical properties of cell envelope
 - In *E. coli*, some *Enterobacteriaceae*, *Vibrio* and *Pseudomonas*

- ✓ Other proteins → **non-covalent** bond

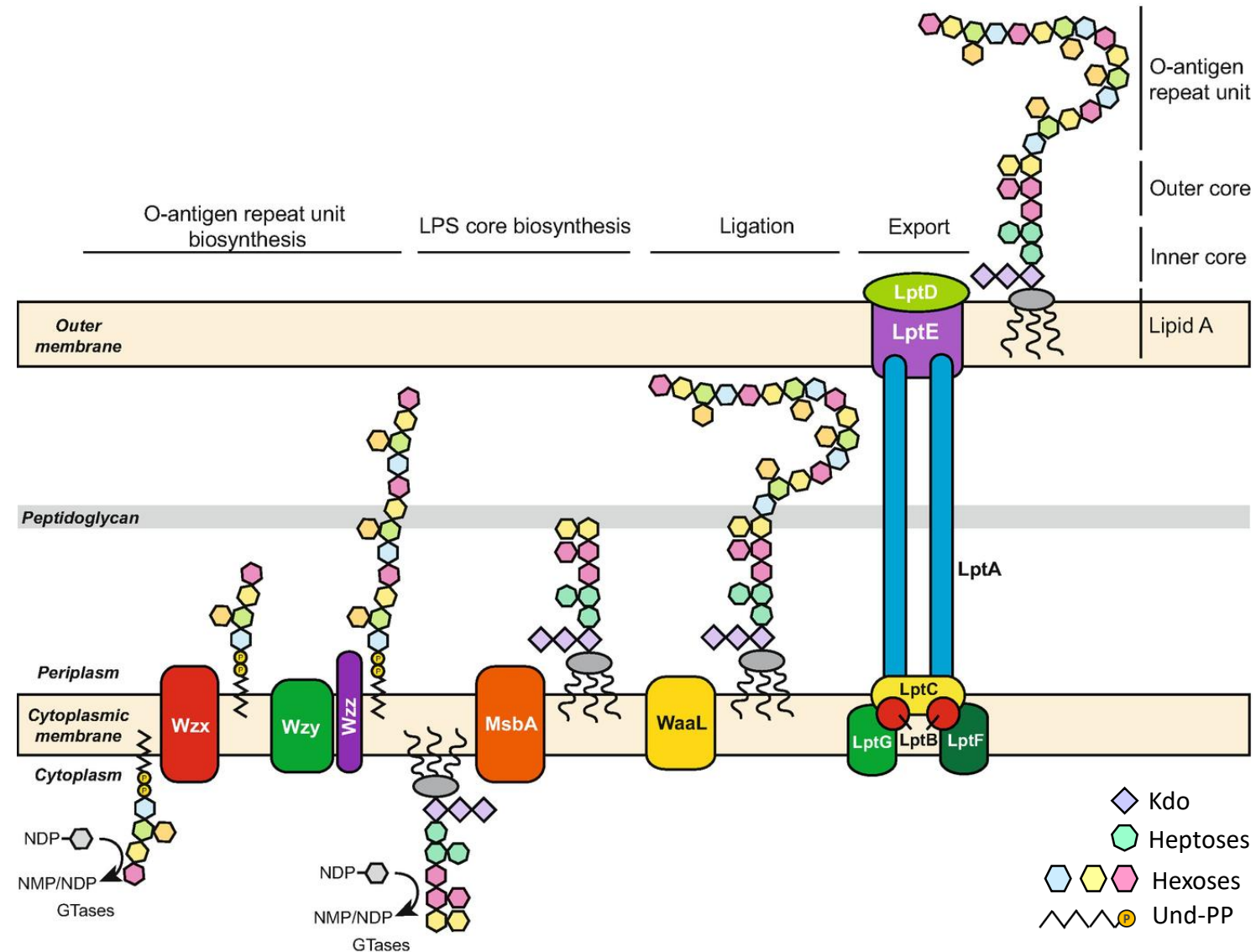
- Pal : OM lipoprotein
 - OmpA : OM protein



Mathelié-Guinlet M. et al – 2020 – Nature Comm

LPS biosynthesis

- **3 cellular compartments (C, IM, P)**
- **2 independent pathways / assembly line**
- **LPS core** (Lipid A / inner, outer core sugars)
 - ✓ Cytoplasm
 - ✓ Lipid A + [IC = Kdo + heptoses] + [OC = hexoses]
 - ✓ Transport across IM: MsbA (ABC transporter)
- **O-antigen**
 - ✓ Cytoplasm: Und-PP + sugar-P
 - ✓ Transport across IM: Wzx (flippase)
 - ✓ IM: extension (3 different pathways)
- **Ligation**
 - ✓ IM (periplasmic side)
 - ✓ LPS core + O-antigen: WaaL (ligase)
- **Transport to cell surface: Lpt complex**



Yates L.E. et al – 2018 – In: . Advances in Biochemical Engineering/Biotechnology

Lipid A / endotoxin

- *V. cholerae* heat-killed lysates: toxic shock (guinea pigs)
⇒ « **endotoxin** »
- Isolation of a « **pyrotoxin** » from many Gram - bacteria (rabbits)
- **Endotoxin**
 - ✓ Pyrogenic properties
 - ✓ Altered host immunity (leukocytosis / leukopenia)
- **LPS**
 - ✓ Lipid A = « active component »
 - ✓ Heat-stable (250°C – 30 min), released after lysis
 - ✓ Powerful immunostimulator: innate immunity activation



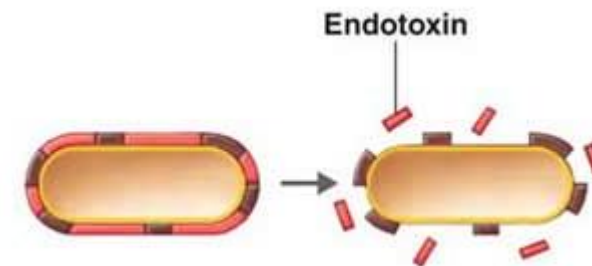
Richard Pfeiffer
(1858–1945) - Germany



Eugenio Centanni
(1863–1942) - Italy

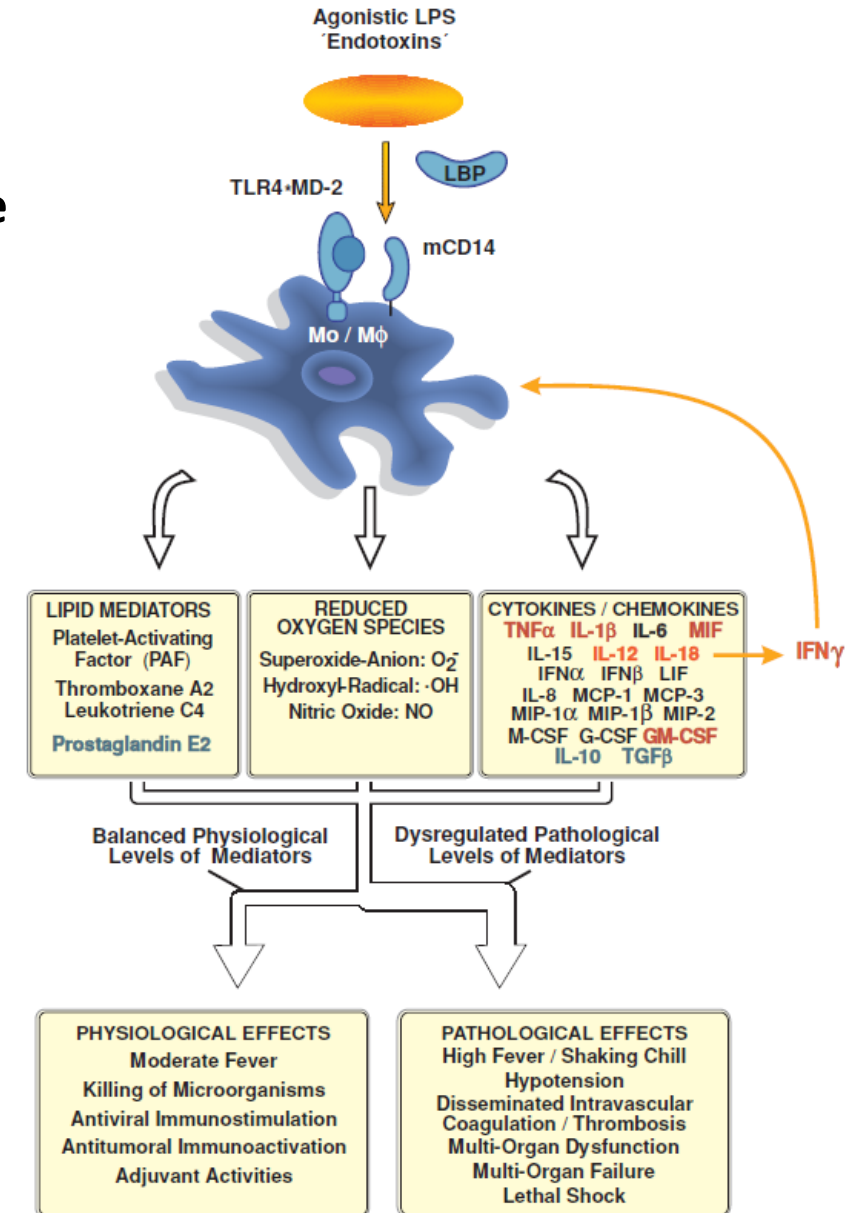


Hans Buchner
(1850–1902) - Germany



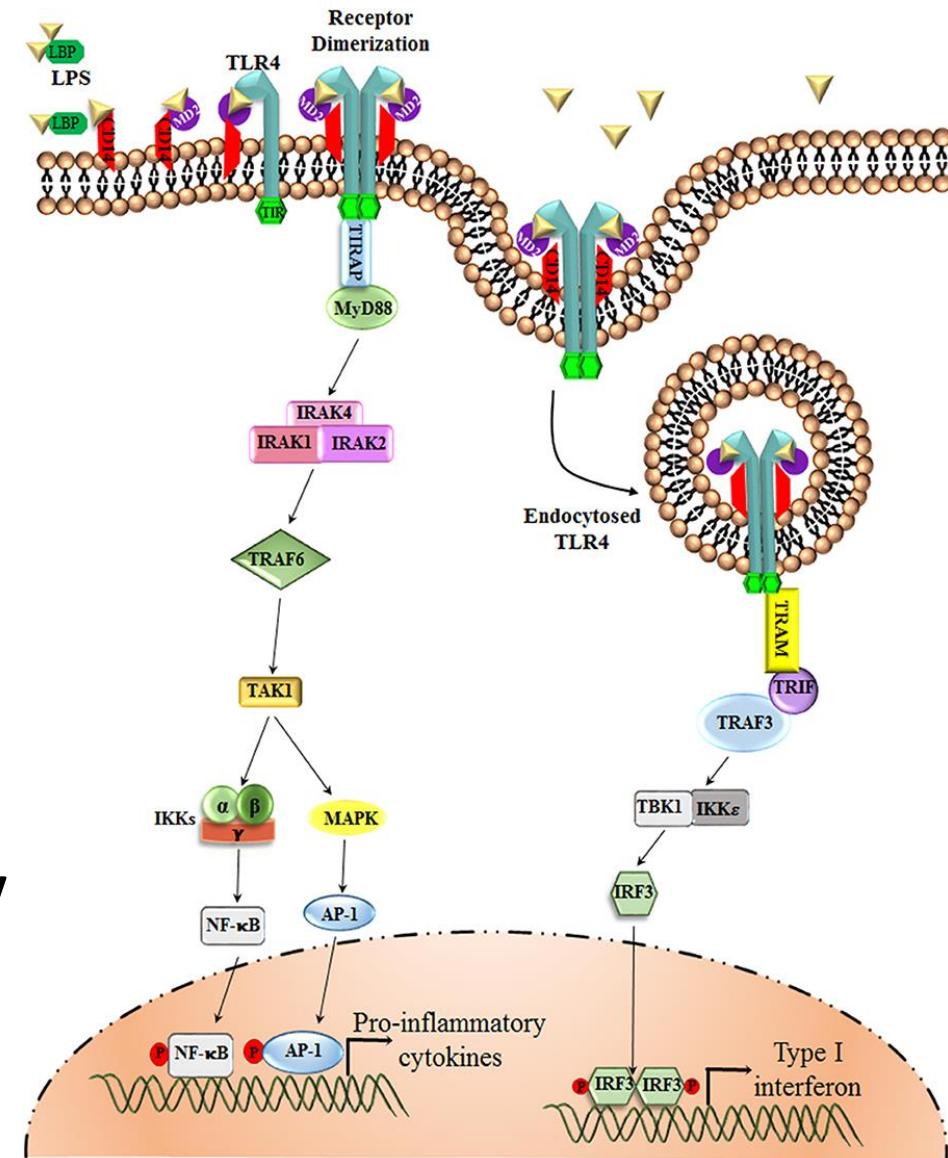
Lipid A / endotoxin

- **Lipid A = PAMP (pathogen associated molecular pattern) ⇒ innate immunity activation**
 - ✓ Lipid A / **LBP** (LPS binding protein)
 - ✓ **CD14**
 - ✓ **TLR4 signaling** ⇒ endogenous mediators release
- « Physiological effects »: antimicrobial defense
- « Pathological effects »
 - ✓ High fever
 - ✓ Sepsis, toxic shock



TLR4 signaling by lipid A / endotoxin

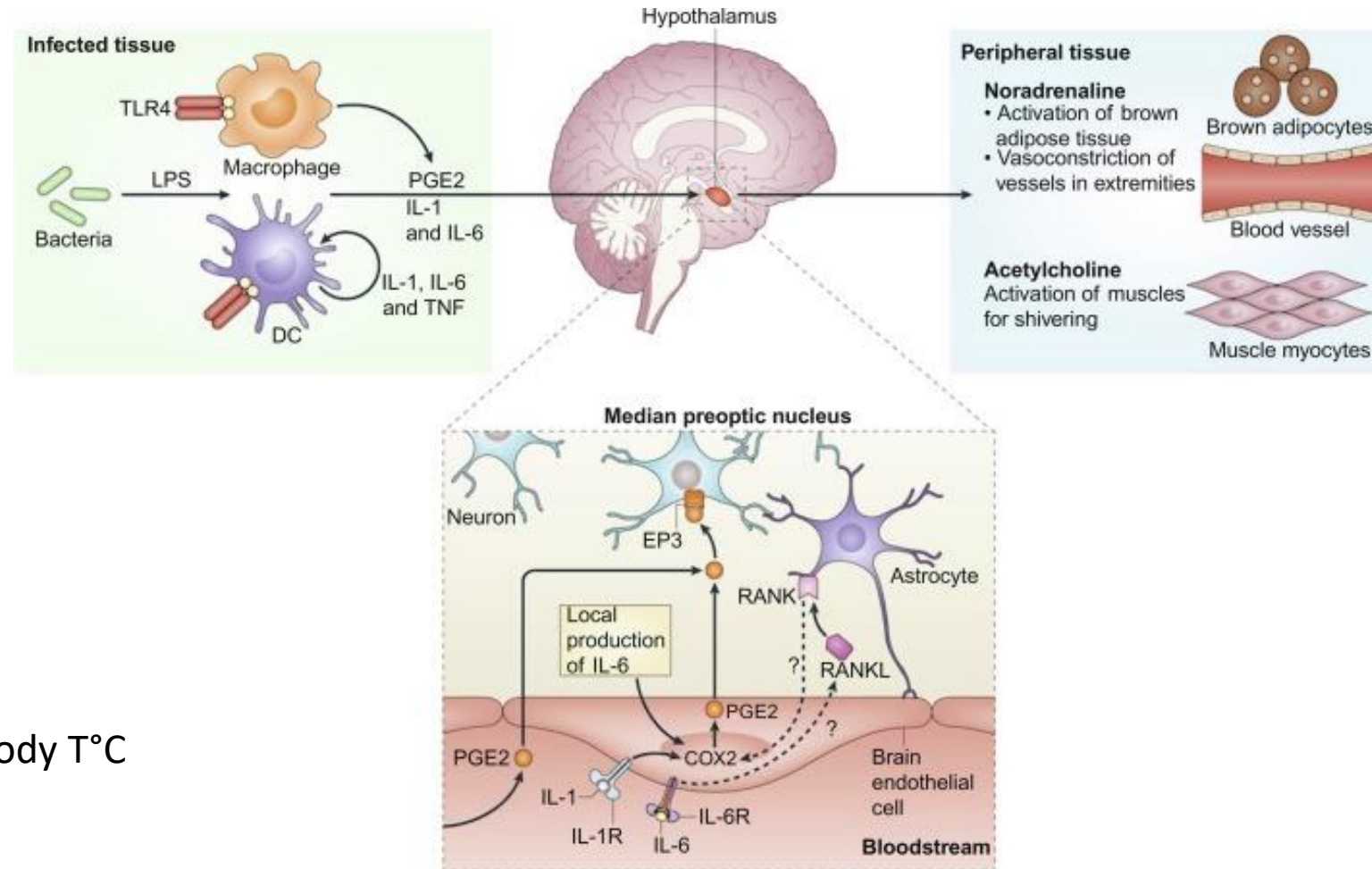
- **LBP** : binds LPS and transfers to CD14+/MD-2
- **CD14 +/- MD2**: accessory proteins
- **TLR4**: recognition, dimerization and endocytosis
- **TLR4 signaling**
 - ✓ **MyD88-dependent pathway**
 - **IRAK** and **TRAF6** activation
 - Transcription factor (**NF-κB**, **AP-1**) phosphorylation and translocation
 - **Cytokines** transcription
 - ✓ **MyD88-independent pathway / TRIF-dependent pathway**
 - **TRAF3** activation
 - Transcription factor (**IRF-3**) phosphorylation and translocation
 - **Type I interferons** (IFN α , IFN β) transcription



Firmal P. et al – 2020 – Front Immunol

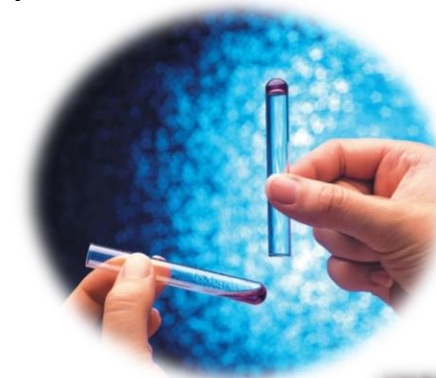
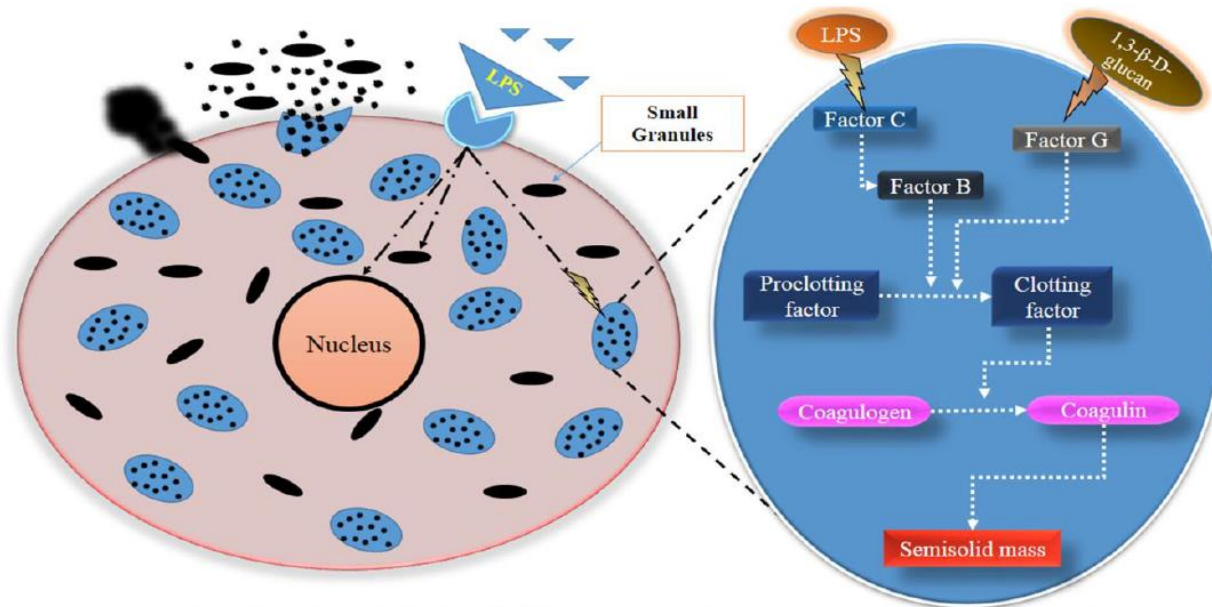
TLR4 signaling by lipid A / endotoxin

- **Activation of immune cells**
 - **PGE2 and pyrogenic cytokines (IL-1, IL-6, TNF α) release \rightarrow fever**
 - **IL-6 (hypothalamus)**
 - COX-2 synthesis
 - **PGE2 production \rightarrow fever**
 - **EP3 receptor (PGE2 receptor)**
 - Sympathetic nervous system
- \Rightarrow **thermogenesis** } \nearrow body T $^{\circ}$ C
- **Nad:** adipose tissue, vasoconstriction
 - **ACH:** shivering



TLR4 signaling by lipid A / endotoxin

- Pyrogenic activity \Rightarrow **highly toxic**
- **Control of parenteral pharmaceutical drug products**
 - *Limulus* amoebocyte lysate (LAL) assay (USA, 1977)



- Recombinant Factor C assay ?

O-antigen

- **Highly variable**

- ✓ Inter and intra-species variation

- ✓ Polysaccharide chain

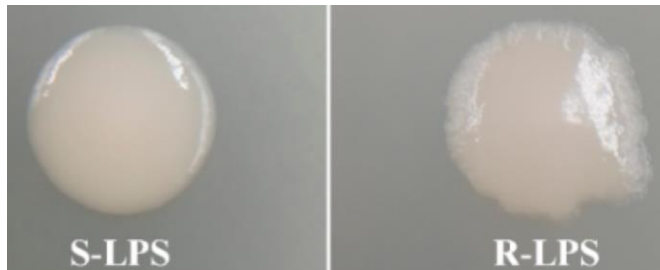
- **Length:** up to 40 repeating units

- **Nature:** > 20 different sugars

- **Order:** arrangement

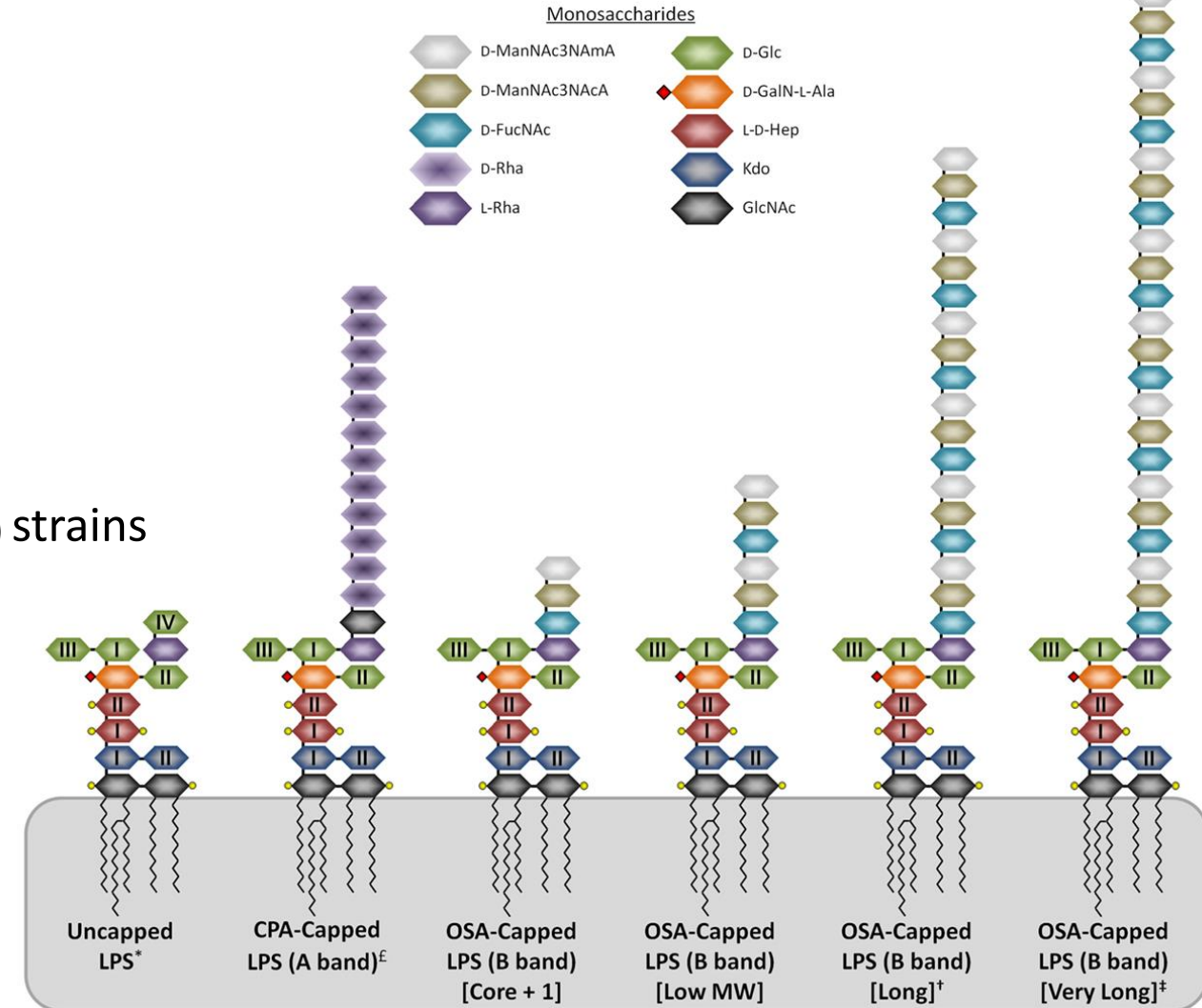
- **Union:** linkages

- ✓ **Smooth** (LPS with O-antigen) / **Rough** (LPS without O-antigen) strains



Meng J. et al – 2020 – Molecules

DIVERSITY OF SURFACE LPS GLYCOFORMS IN A SINGLE CELL *Pseudomonas aeruginosa*

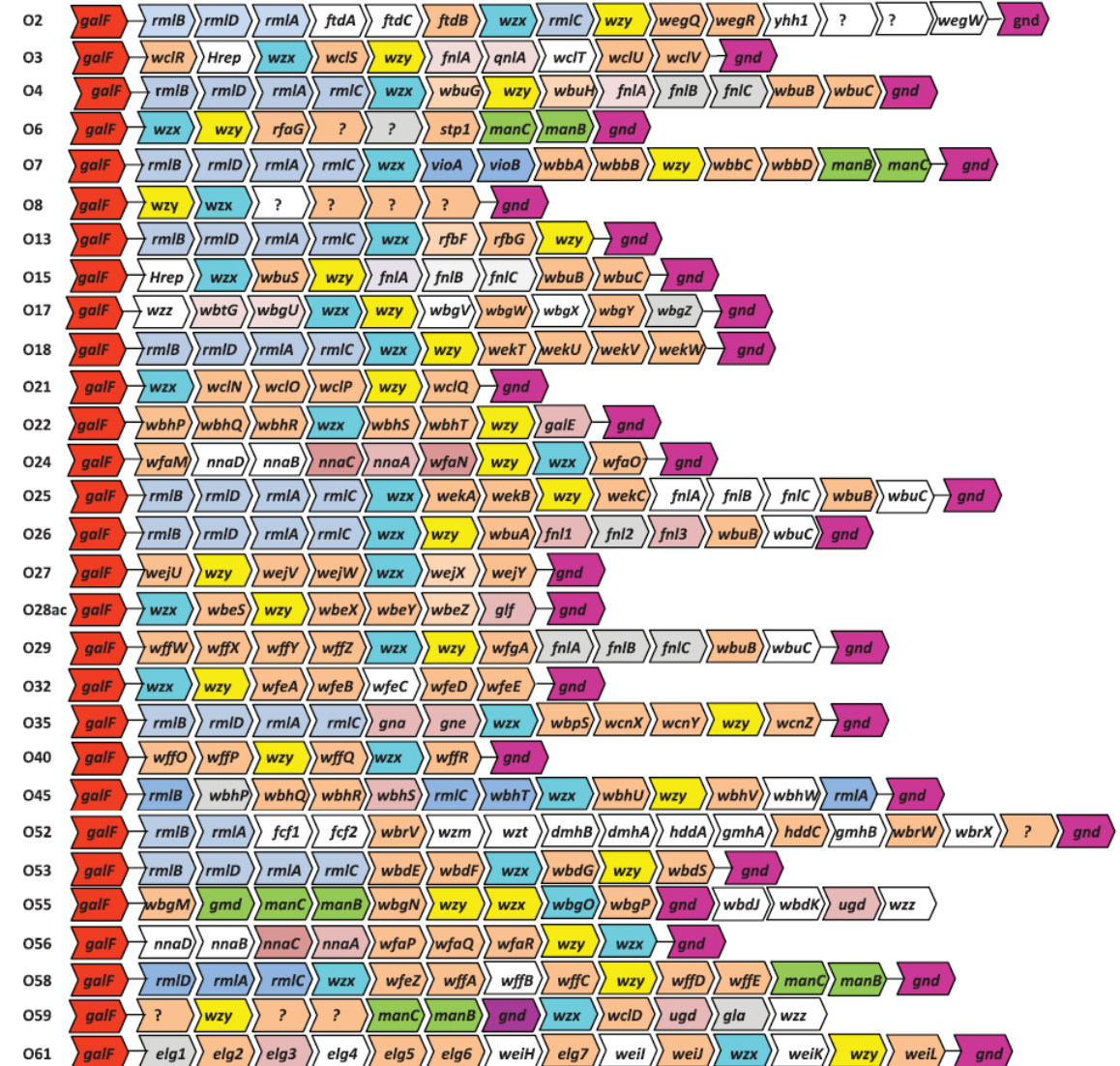


Lam J.S. et al – 2011 – Front Microbiol

O-antigen

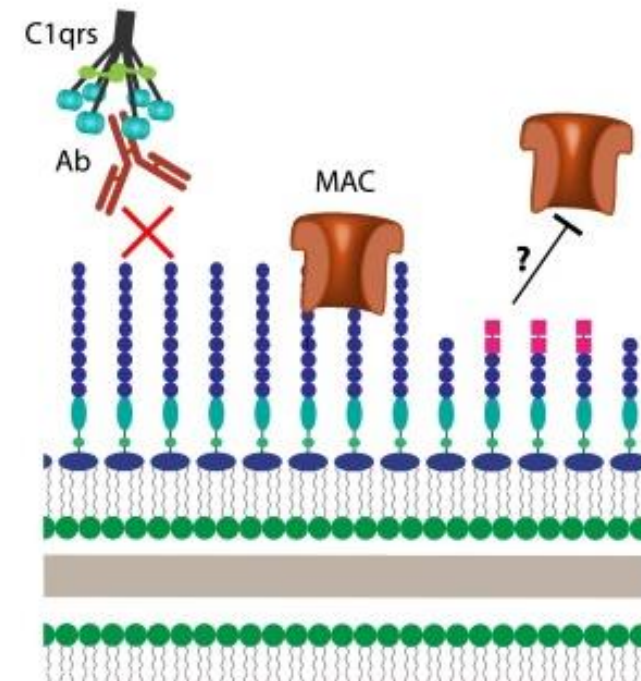
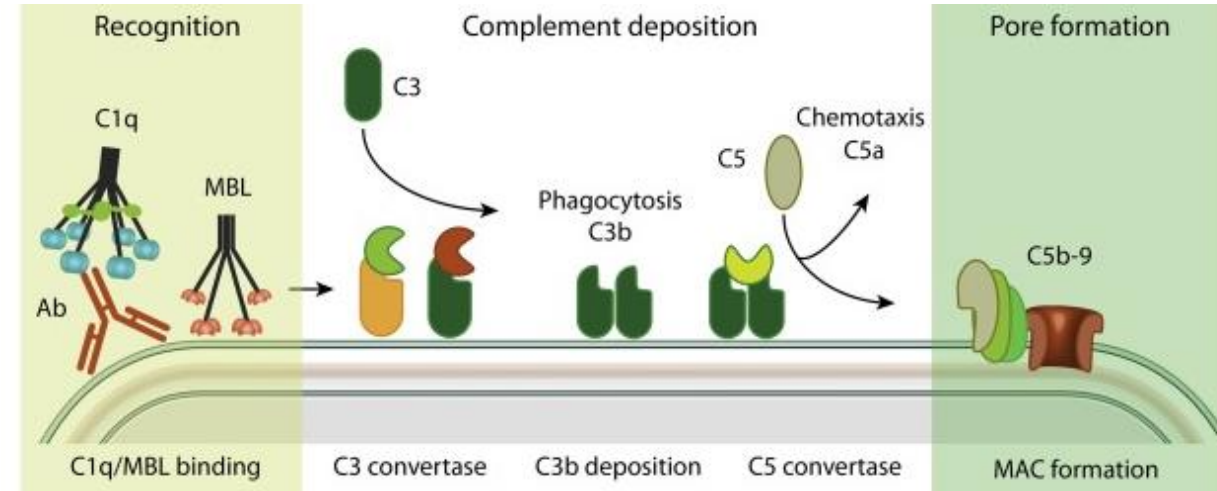
- Genetic variation
- O-antigen gene cluster
 - ✓ Chromosome
 - ✓ 3 main gene classes
 - Nucleotide sugar synthesis genes
 - Sugar transferase genes
 - O-unit processing genes

High variability in *E. coli* strains



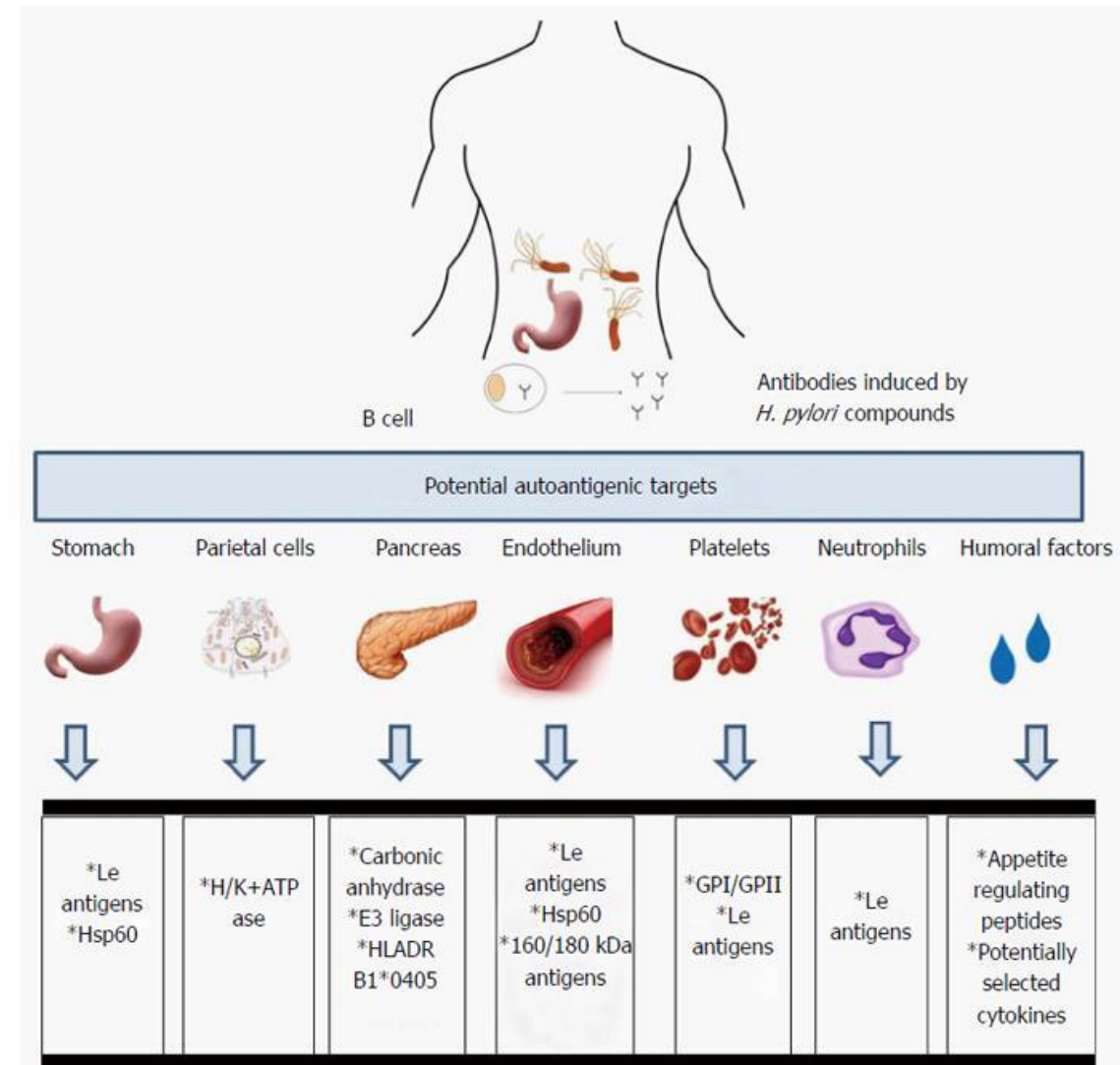
O-antigen

- **Immunodominant part of LPS (non-toxic)**
 - ✓ Immune response
 - ✓ Host-bacteria interaction influence
- **Complement system (plasma proteins)**
 - ✓ **Recognition / Labeling / Killing**
 - ✓ Classical complement pathway (3 different pathways)
 - C1q : Ag/Ab complexes recognition
 - C3 protein cleavage (C3 convertase)
 - C3b deposition (bacterial surface)
 - C3b recognition ⇒ **phagocytosis**
 - **MAC** (membrane attack complex) **formation** ⇒ **pore** (outer membrane G- bacteria)
- **Resistance (*K. pneumoniae*)**
 - ✓ O-antigen chain elongation → C3b /MAC deposition away from surface
 - ✓ Epitopes blocking recognition by C1q



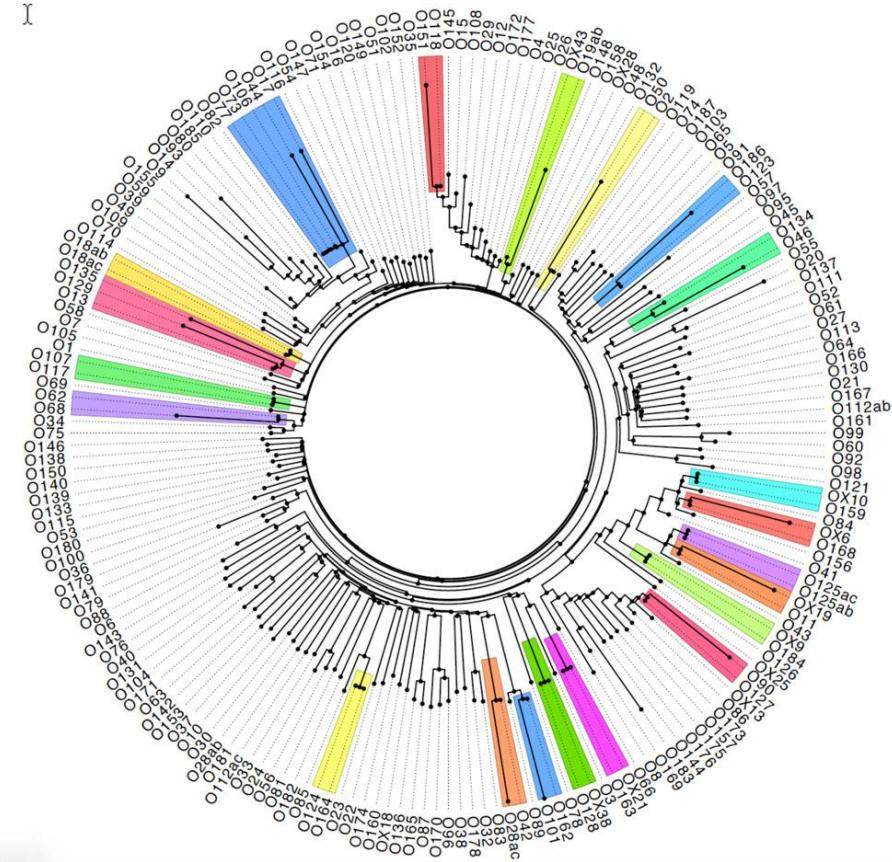
O-antigen

- **Molecular mimicry**
= bacterial / host Ag resemblance
- O-antigen of *H. pylori* similar to Lewis blood groups X (Lex) Ag (expressed in human gastric epithelium)
- **Diverging pathogenic roles**
 - ✓ Bacteria protection ⇒ **infection persistence**
 - ✓ **Auto-immune inflammation ?**
eg: HLA-B27 ⇒ reactive arthritis

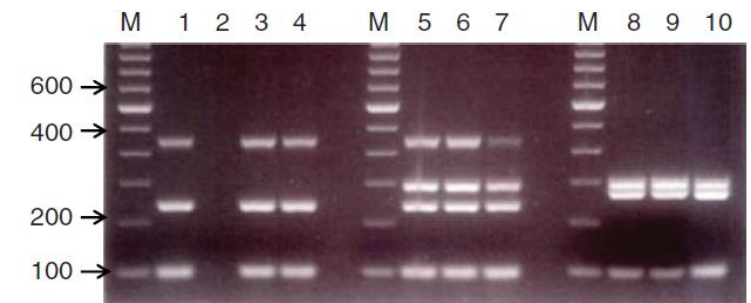
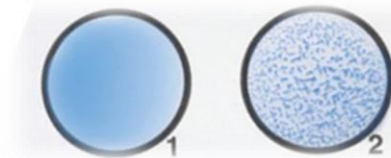


O-antigen: serogroups

- O-antigen: basis for **serological classification** (G- bacteria)
 - ✓ Immunogenic
 - ✓ Highly variable
- **Serotyping**
 - ✓ Classification / Epidemiology / Tracing
 - ✓ **O, H** (flagellar) \pm **K** (capsule) **antigens**
 - Eg: *E. coli* (183), *V. cholerae* (>200), *Shigella* (34)
- **Serotyping techniques**
 - ✓ **Agglutination / clumping** (rabbit antiserum)
 - ✓ Molecular approaches (RFLP, PCR, microarrays)



DebRoy C - 2016 - PLOS One



O-antigen: virulence

O157:H7

STEC: bloody diarrhea, HUS
1993 (USA), 1996 (Japan),
2005 (France)



O145 2010 (USA)



O26

STEC: HUS
2019 (France)



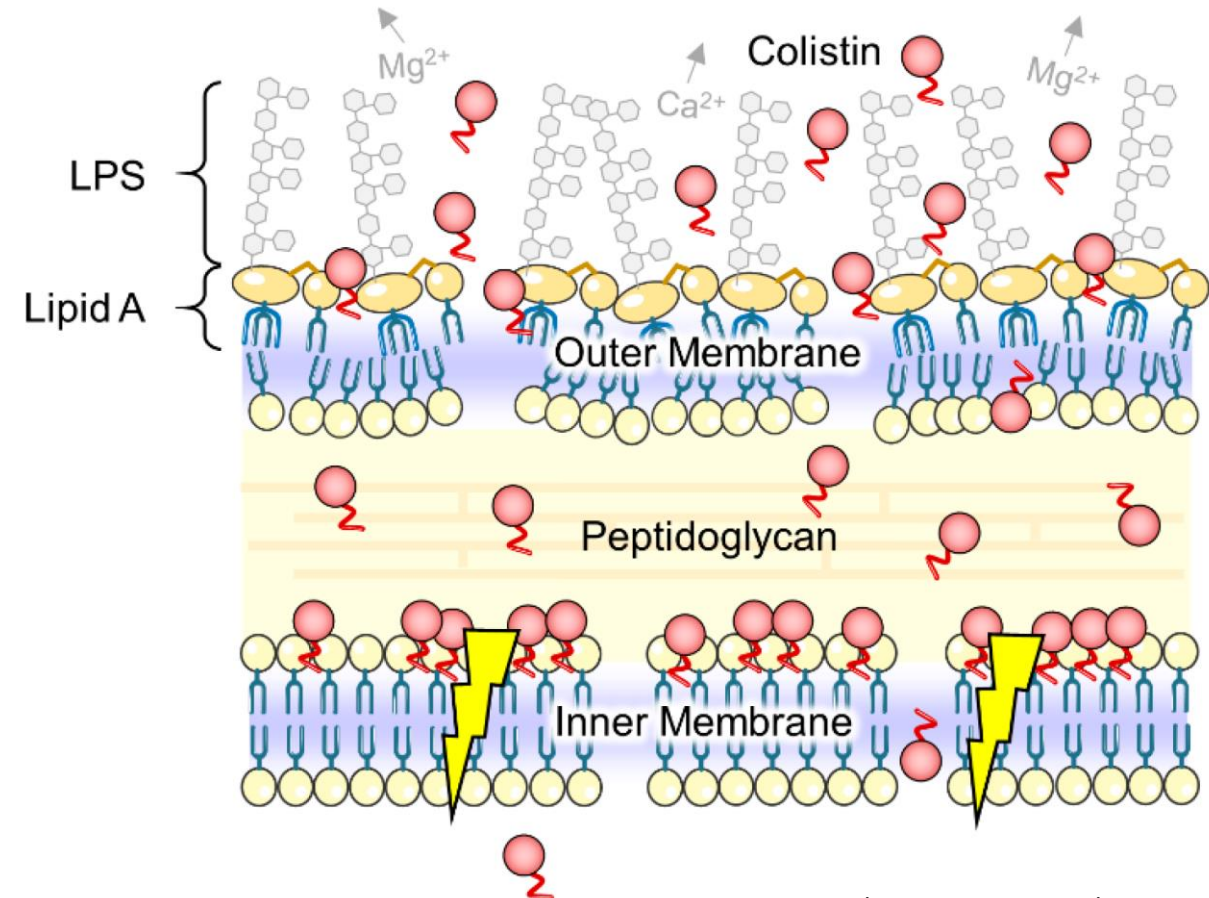
O104:H4

EHEC
2011 (Germany/Europe)



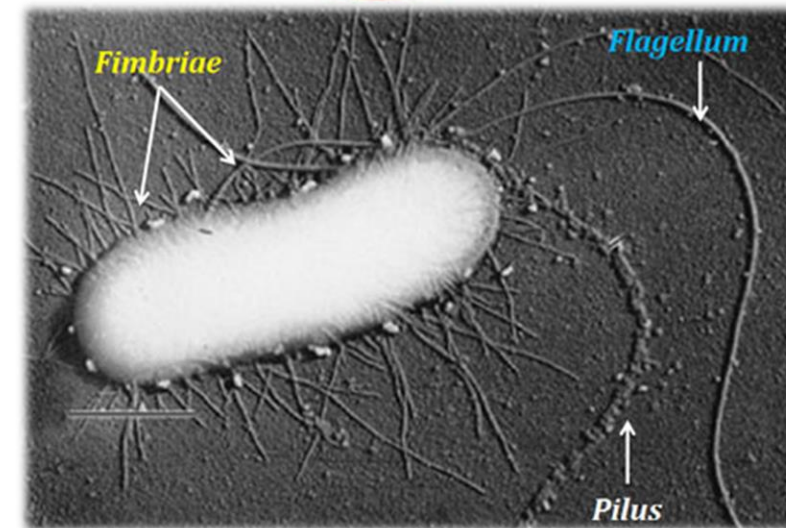
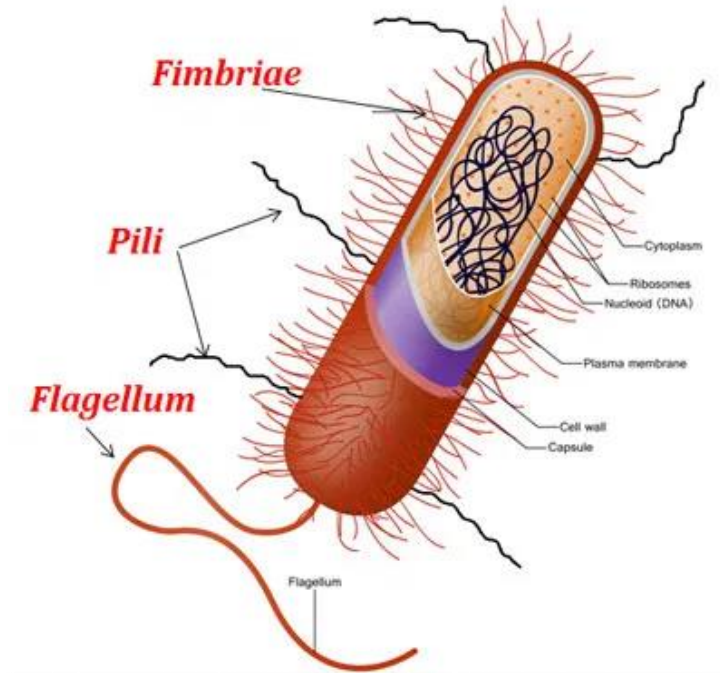
LPS: a therapeutic target ?

- **Polymyxins:** cationic antimicrobial peptides
 - ✓ Polymyxin B, colistin
 - ✓ Active against gram - bacteria (*P. aeruginosa*, *Klebsiella*, *E. coli*, ...)
 - ✓ **Inner membrane disruption**
 - ✓ **Outer membrane disorganization**
 - LPS (-): stability
 - Colistin (+): Mg^{2+} and Ca^{2+} displacement, LPS bonding
- ⇒ **Increased OM permeability**
- **Inhibitors of LPS biosynthesis ?**



Richter P. - 2019 - Antibiotics

- Extracellular structures \Rightarrow growth and survival
- **Cell surface appendages**
 - ✓ Projecting filamentous structured
 - ✓ Attached to cell envelopes
 - ✓ Link to external environment
 - ✓ Diverse cellular functions: motility, attachment, cell-to-cell interaction, secretion ...
 - ✓ 2 morphology categories
 - **Pili / Fimbriae**: thinner, hair-like structures \Rightarrow adhesion, gene transfer
 - **Flagella**: long, whip-like structures \Rightarrow motility



Pili / fimbriae

• Pili / Fimbriae

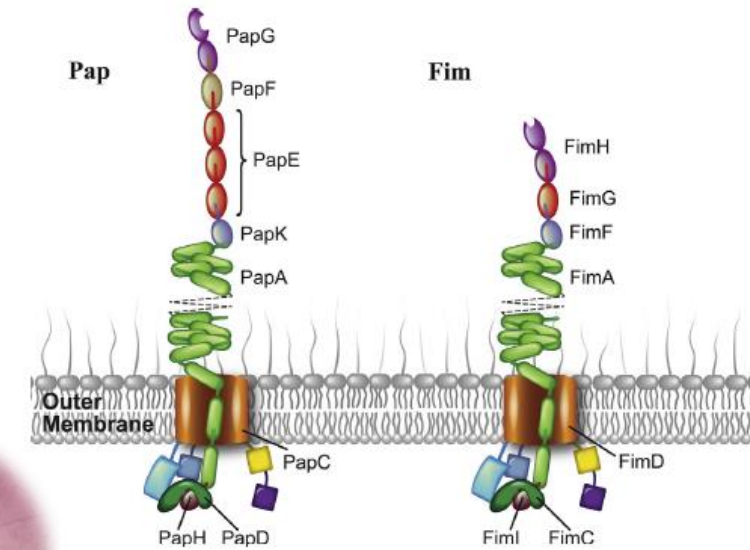
- ✓ Thin, protein tubes (cytoplasmic membrane)
- ✓ Hundred to thousands units of pilin
- ✓ Different classes (pilin sequence, structural subunits, genes, assembly mechanism, function)

• Chaperon-usher pili

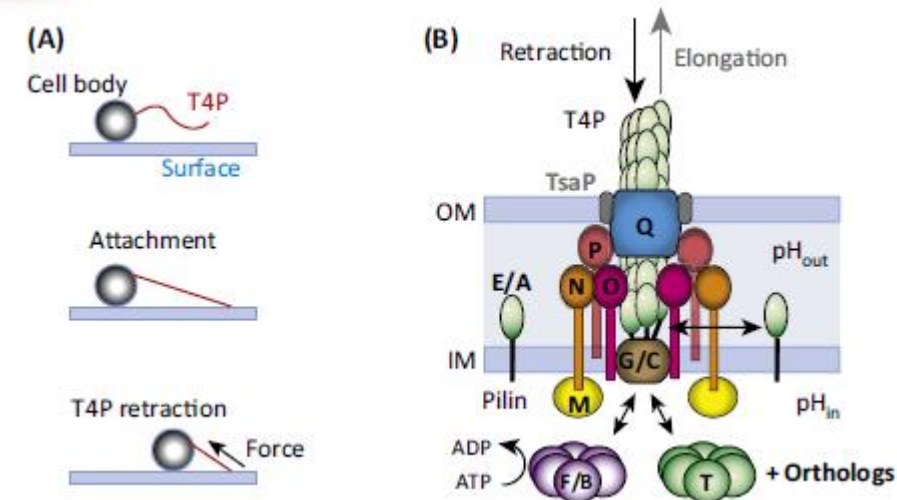
- ✓ G- bacteria: *Escherichia*, *Shigella*, *Salmonella*, ...
- ✓ Adherence, cell invasion, biofilm formation (« virulence factor »)
- ✓ 1-2 μm long – 2-4 nm width
- ✓ UPEC: type I pili (FimH adhesin / bladder), P pili (PapG adhesin / kidney)

• Type IV pili

- ✓ G- bacteria: *Pseudomonas*, *Neisseria*, *Vibrio*, ...
- ✓ Adherence, biofilm formation, DNA uptake, microcolony formation
- ✓ 1-4 μm long – 5-8 nm width
- ✓ Dynamic: polymerization/depolymerization \Rightarrow force / movement



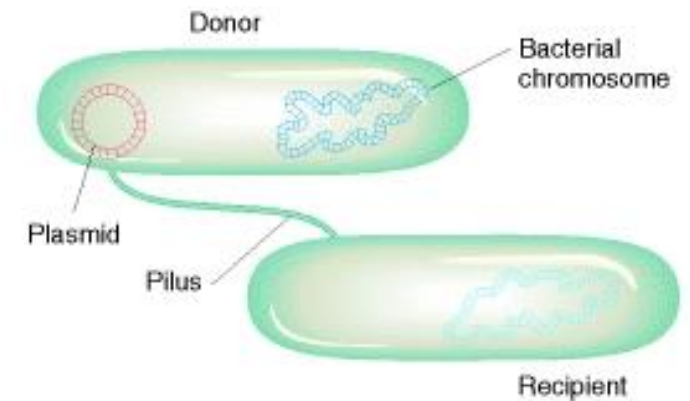
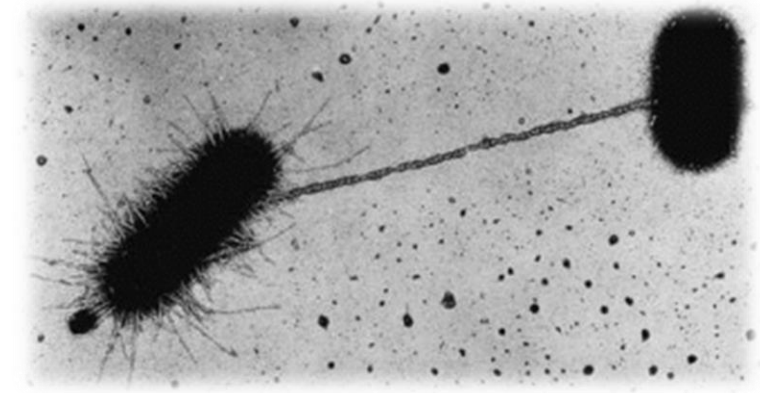
Kalas V – 2013 - Elsevier



Maier B - 2015 - Trends in Microbiol

Type IV secretion pili

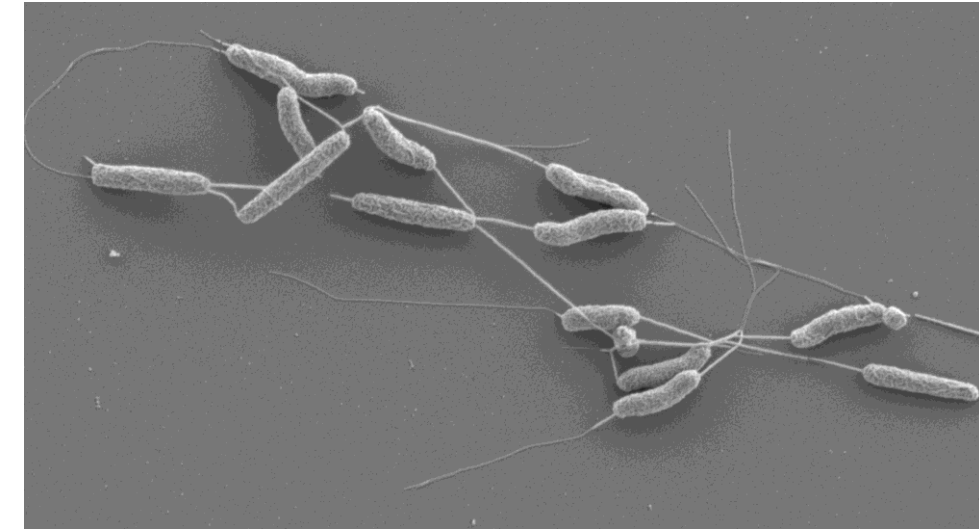
- **T4SS:** secretion machineries
- **In and out transport:** DNA, proteins, nucleoproteins complex
 - ✓ Conjugation (« conjugative pili »): donor / recipient cell
 - ✓ DNA uptake and release
 - ✓ Delivery of effectors proteins
- **F-pilus (*E. coli*)**
 - ✓ Plasmid F
 - ✓ Dynamic (extension/retraction)
 - ✓ Horizontal gene transfer
 - ✓ Antibiotic resistance gene propagation



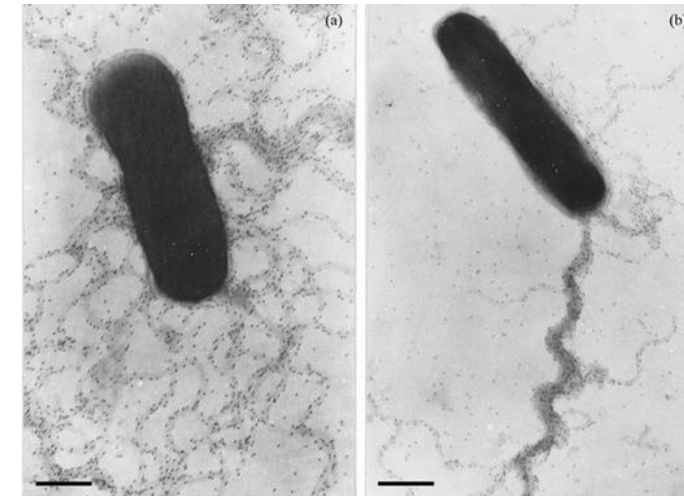
Griffiths AJF -2000

Flagella

- Main organelle for **motility**
- G- and G+ **bacilli**
- **Complex filamentous structures**
 - ✓ Attached to cell envelope
 - ✓ 5 – 15 μm long
 - ✓ 12 – 30 nm width
- **Observation**
 - ✓ Transmission electron microscopy
 - ✓ Special flagella stains (ex: phosphotungstic acid)

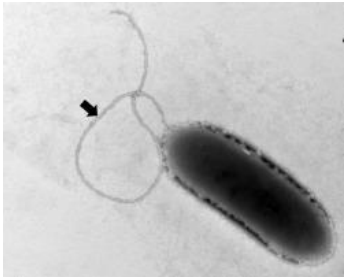


Campylobacter jejuni (Gram-)



Clostridioides difficile (Gram+)

Flagella: location



P. aeruginosa



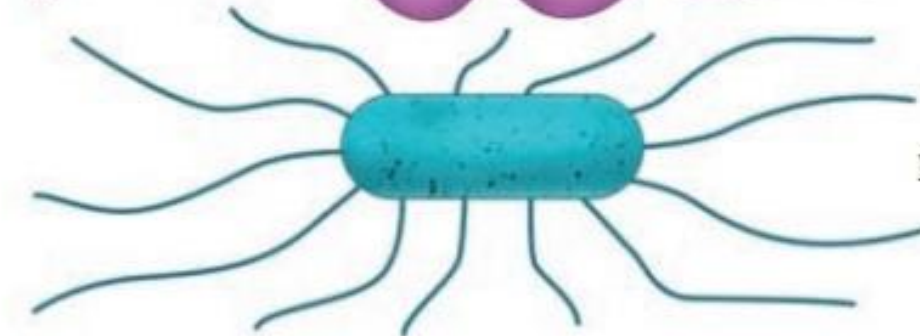
Monotrichous



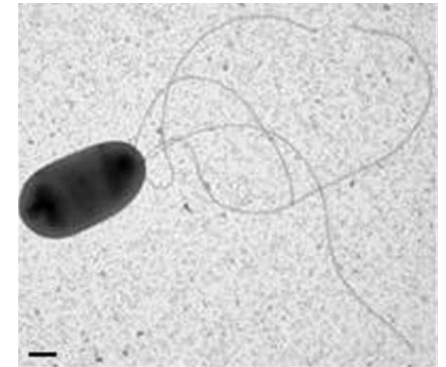
Lophotrichous



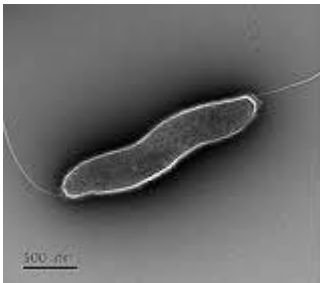
Amphitrichous



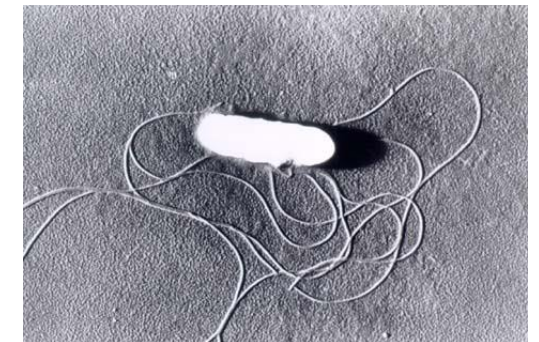
Peritrichous



P. fluorescens



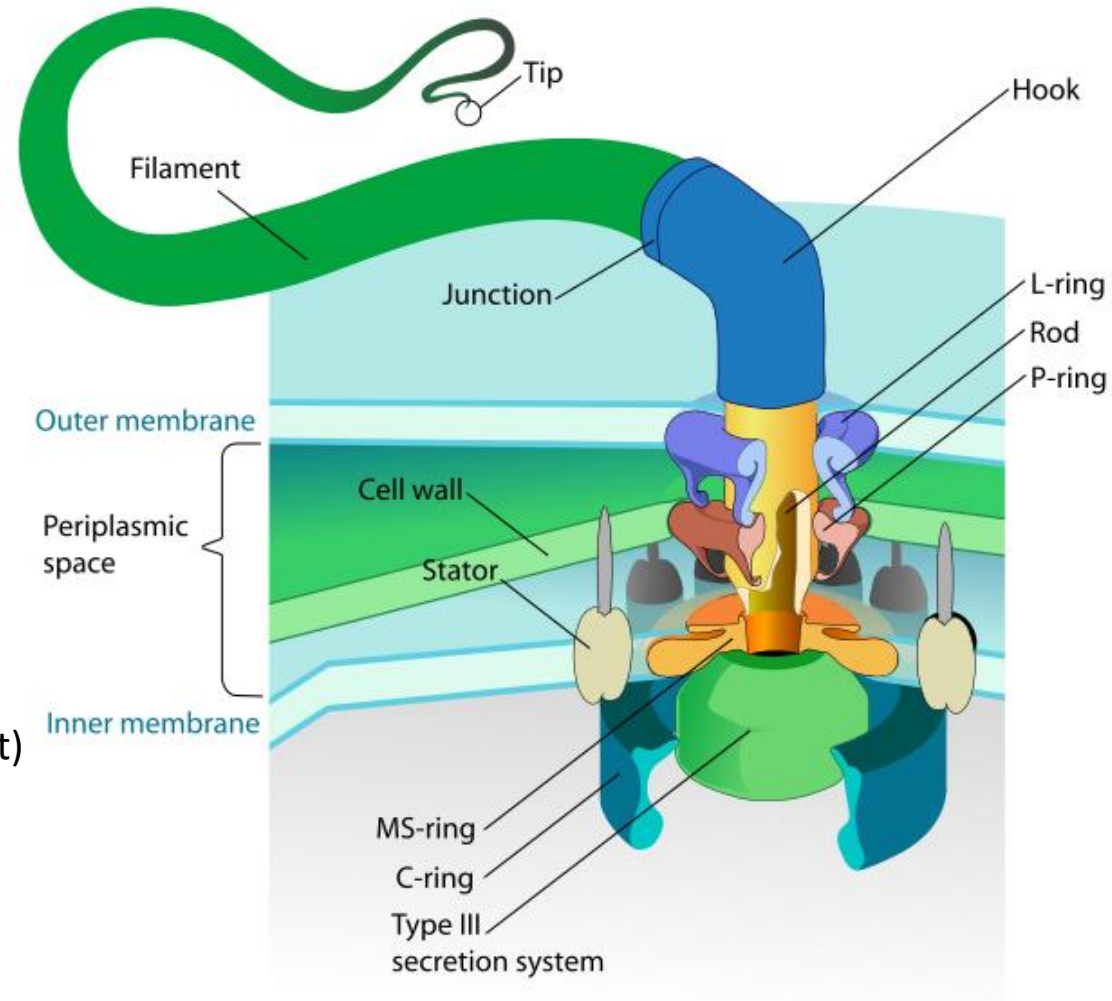
A. serpens



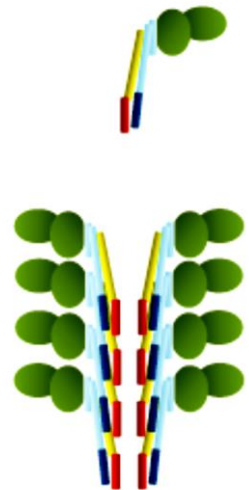
S. typhi

Flagella: structure

- **Large macromolecular complex** (30 different proteins)
- **Filament**
 - 20 nm width, up to 15 μm
 - Up to 30,000 flagellin subunits
 - « Cap »: FliD pentamer
- **Hook** : energy transmitter
- **Basal body / motor** (T3SS)
 - Ring complexes (M, C, P, L)
 - Rod
 - Stator (MotA, MotB)
 - Export apparatus
- **Motility**
 - Stator: torque/twist generation (H^+ or Na^+ gradient)
 - C-ring rotation \Rightarrow direction change
 - MS-ring > rod > hook > filament rotation
 - Filament: torque \Rightarrow thrust \Rightarrow cell motility



Flagellin
monomer

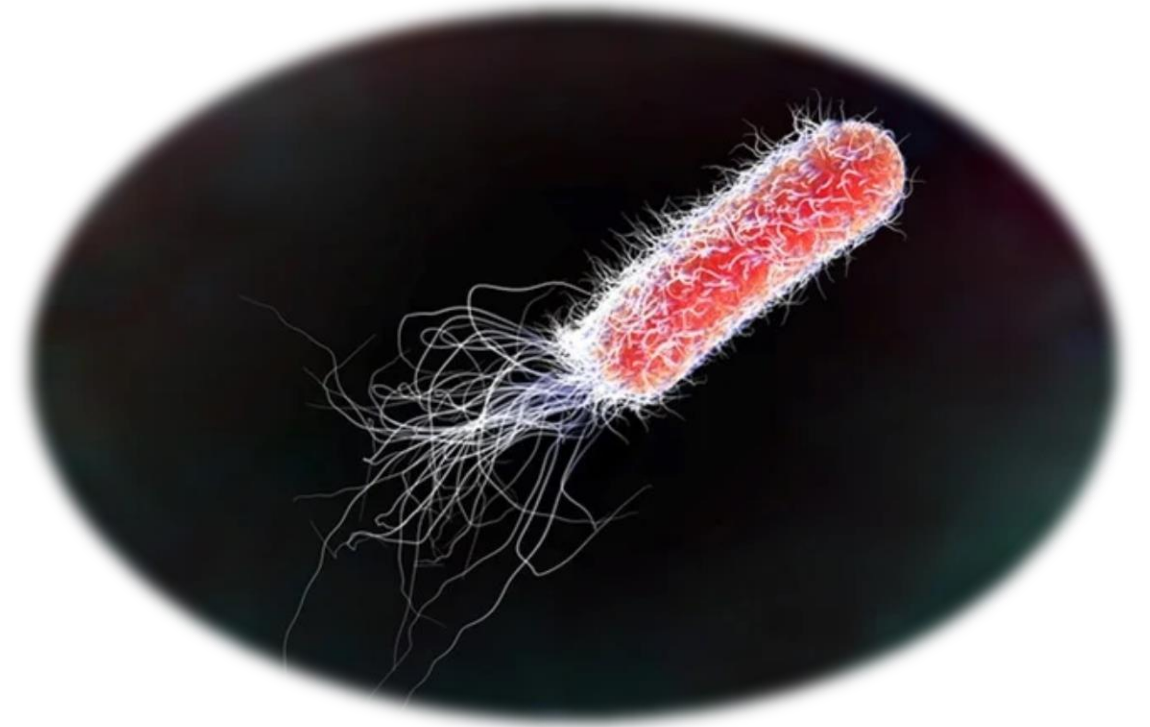


Flagellin
polymerization

Flagella: virulence factor

Flagella are involved in bacterial pathogenicity by

- **motility** (movement, chemotaxis)...
- **...but non only**
 - ✓ Adhesion (bacterial adhesins)
 - ✓ Biofilm
 - ✓ Protein export (T3SS)
 - ✓ Innate immune response



Flagella: motility and virulence

INFECTION AND IMMUNITY, Apr. 2002, p. 1984–1990
0019-9567/02/\$04.00+0 DOI: 10.1128/IAI.70.4.1984-1990.2002
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Vol. 70, No. 4

Helicobacter pylori Uses Motility for Initial Colonization and To Attain Robust Infection

Karen M. Ottemann* and Andrew C. Lowenthal

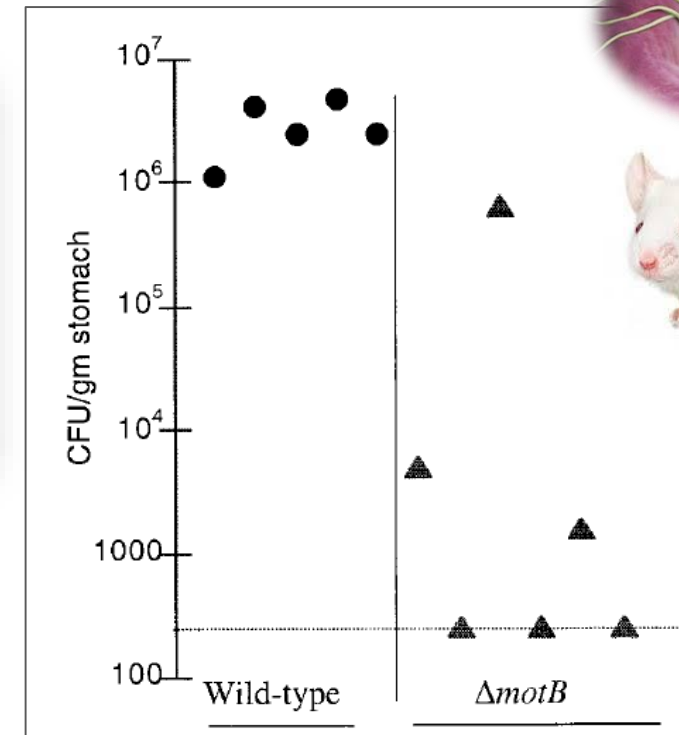
Departments of Environmental Toxicology and Molecular, Cell and Developmental Biology,
University of California at Santa Cruz, Santa Cruz, California 95064

- *Helicobacter pylori* Δ *motB* mutants

- ✓ Flagellated but non motile

- ✓ Reduced colonization

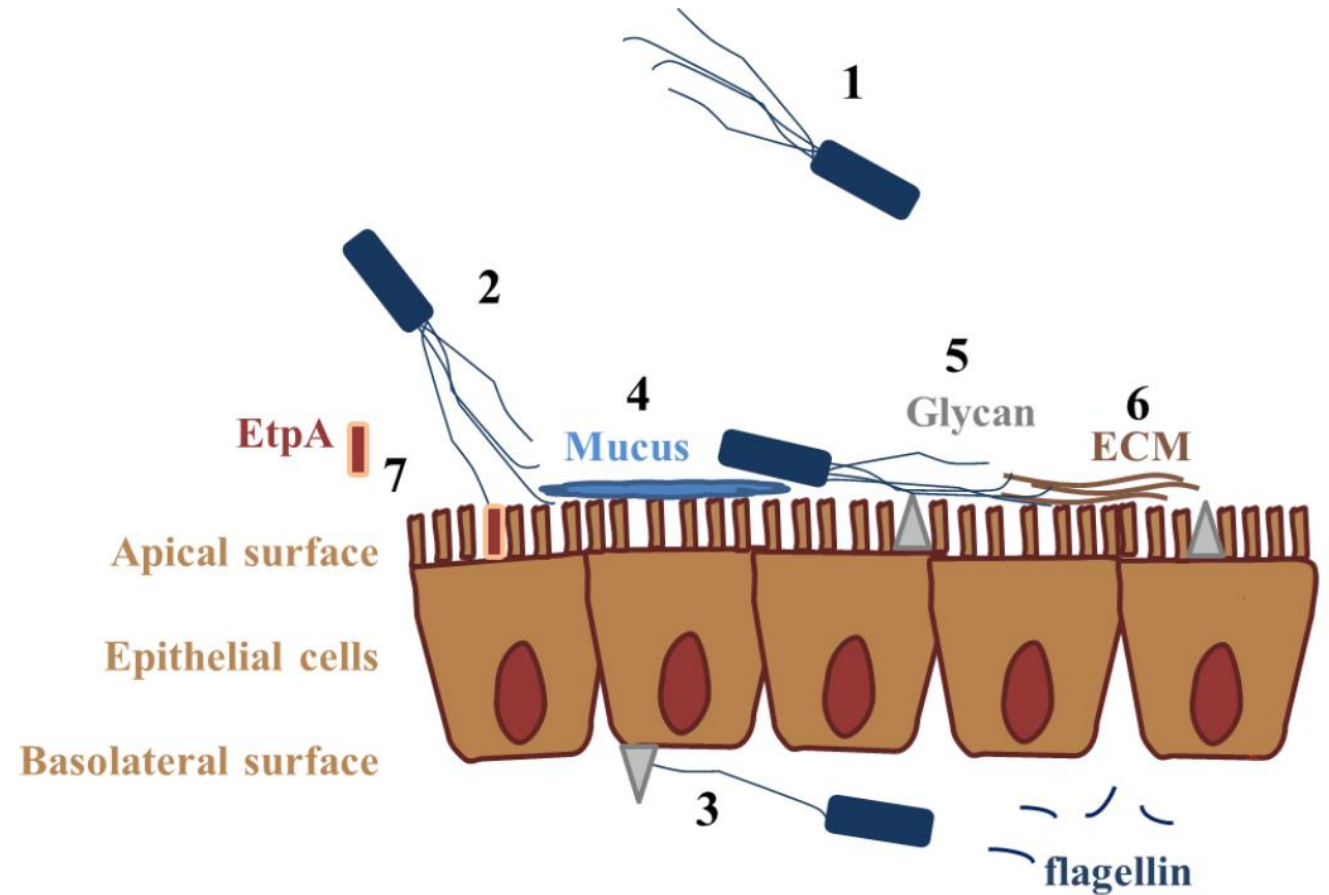
- ✓ Increased ID₅₀



Strain	Infecting dose	No. of mice infected/ no. inoculated	CFU (10 ⁶)
SS1	1.3×10^6	4/4	2.7 ± 1.0
	1.3×10^5	4/4	1.9 ± 0.28
	1.3×10^4	2/2	3.1 ± 1.5
	1.3×10^3	4/4	1.6 ± 0.67
	1.3×10^2	4/4	0.58 ± 0.15
SS1 Δ <i>motB</i>	5×10^7	2/5	0.14 ± 0.07
	5×10^6	3/5	0.036 ± 0.018
	5×10^5	0/4	0
	5×10^4	0/4	0

Flagella: adhesion

- **Indirect mechanism:** motility
- **Direct mechanisms**
 - ✓ Epithelial cell lines binding
 - ✓ Mucus / mucins
 - ✓ Glycans (mucus or cells)
 - ✓ Extracellular matrix proteins
 - ✓ Secreted 2-partner adhesin (EtpA)

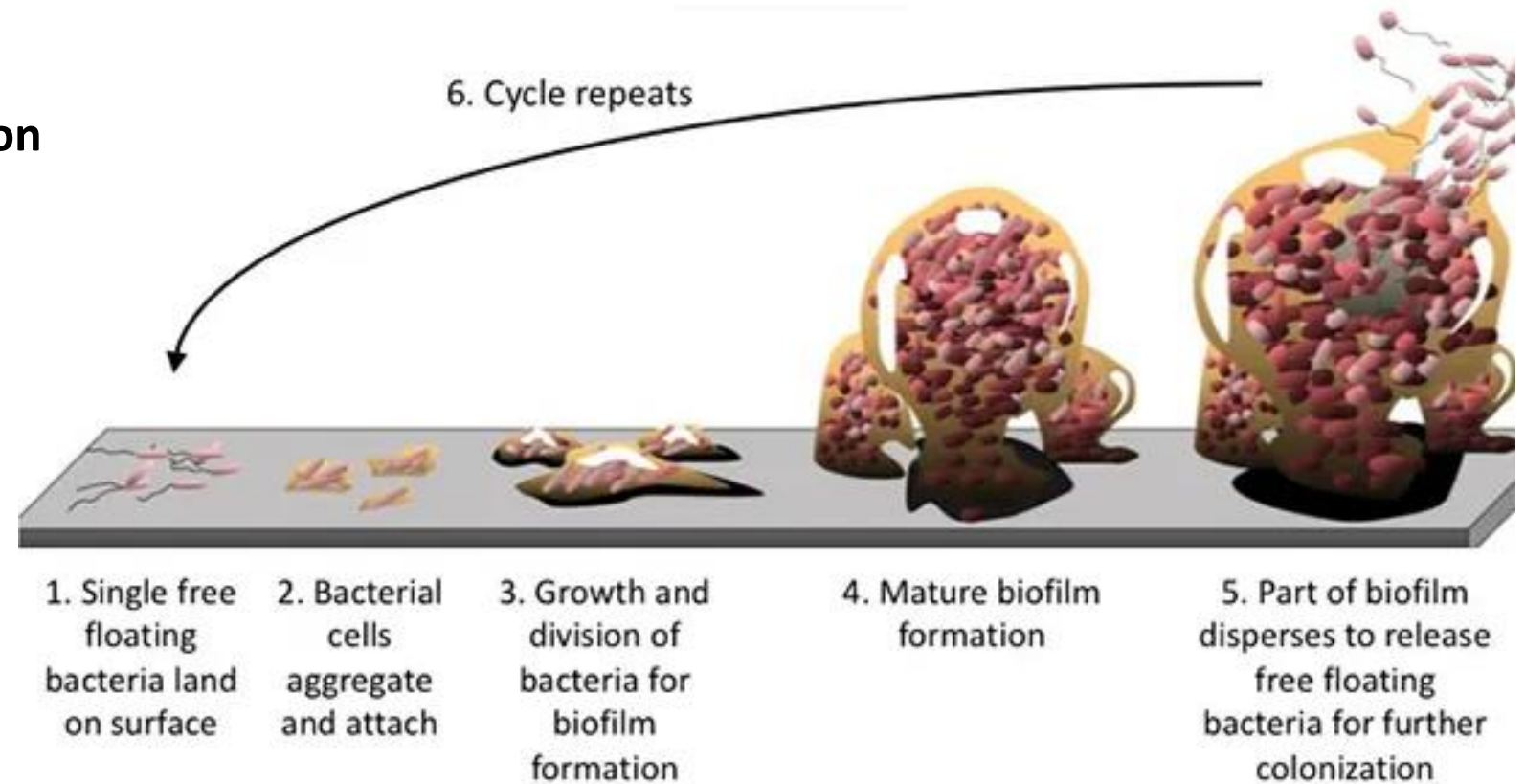


Haiko J - 2013 - Biology

Flagella: biofilm formation

- Microorganisms community attached to solid surface

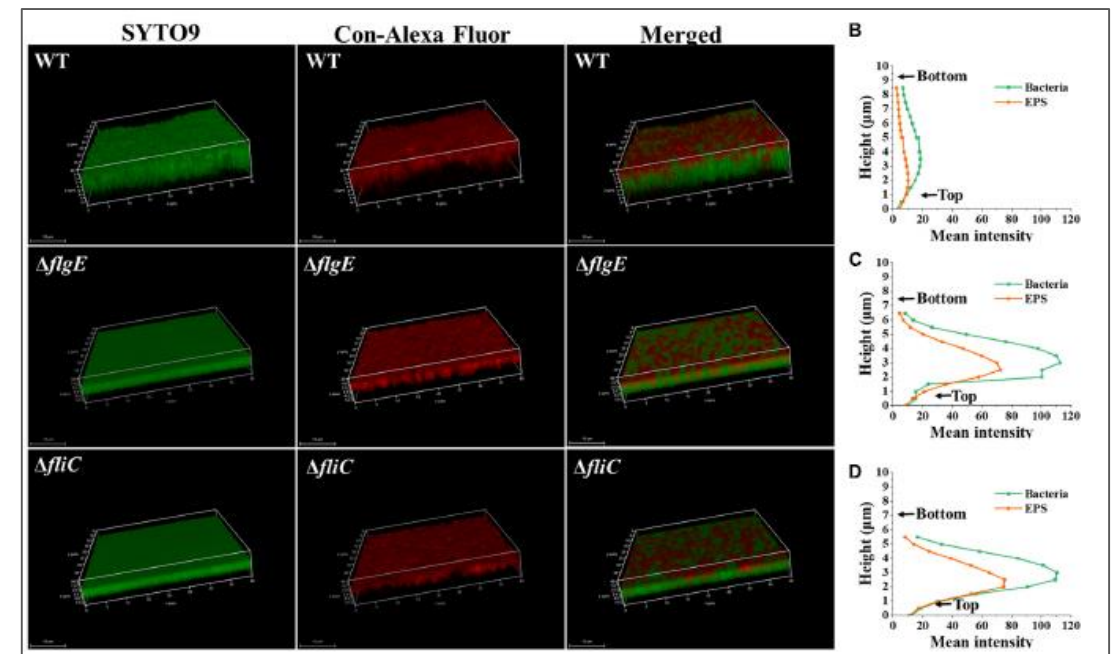
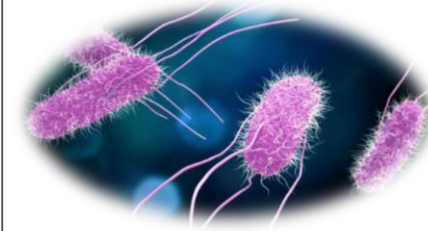
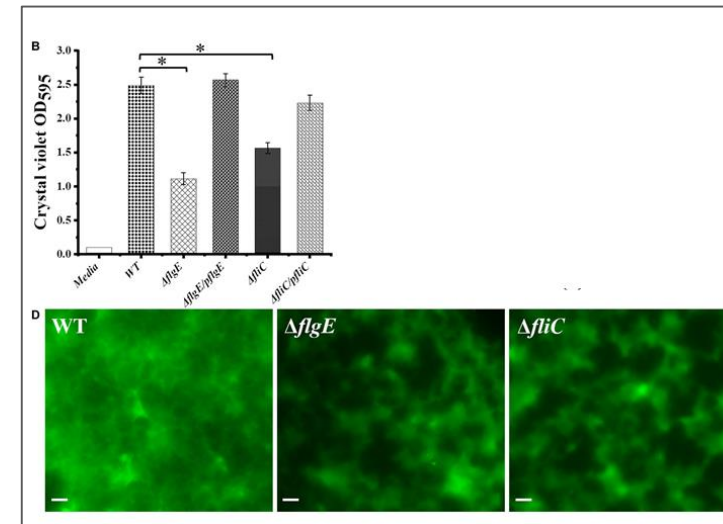
- **Bacteria transport**
- **Initial reversible attachment/adhesion**
- **Extracellular matrix production**
 - ⇒ monolayer / irreversibly attached
- Multilayers = **microcolony**
- **Mature biofilm**
 - « mushroom » structures
 - Polysaccharides
- **Dispersion**
- ...



Flagella: biofilm formation



- *Salmonella enterica* mutants ($\Delta fliC$, $\Delta flgE$)
- Reduced biofilm formation (early stages: 6h)
- Denser mature biofilm (48h)
 - ✓ More bacteria
 - ✓ More extracellular polymeric substrates (EPS)

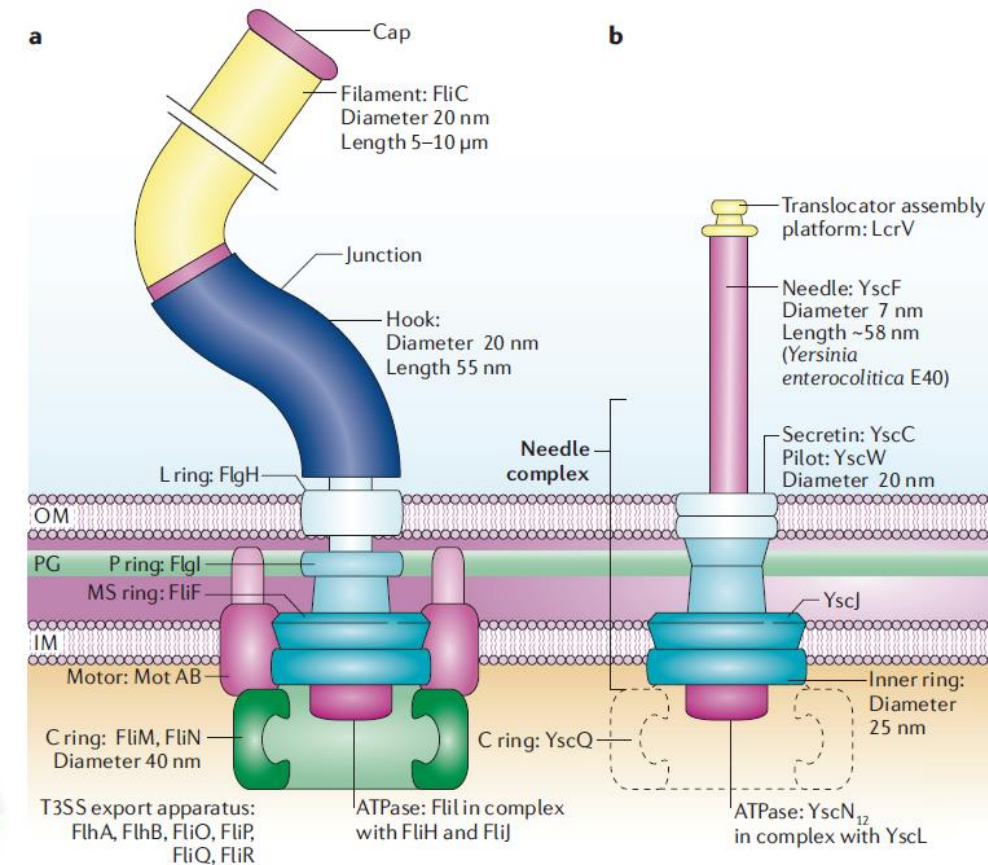
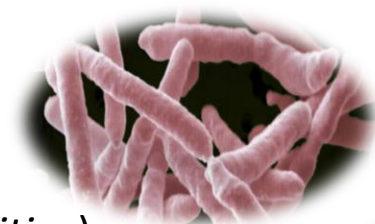


Flagella: protein export

- **Flagella export apparatus = special T3SS**
- Similarity with virulence-related T3SS « **injectisome** »
 - ✓ G- bacteria
 - ✓ Intracellular bacterial proteins delivery

- **Flagellar protein export**
 - ✓ **Filament biogenesis**
 - ✓ **Virulence factor export**

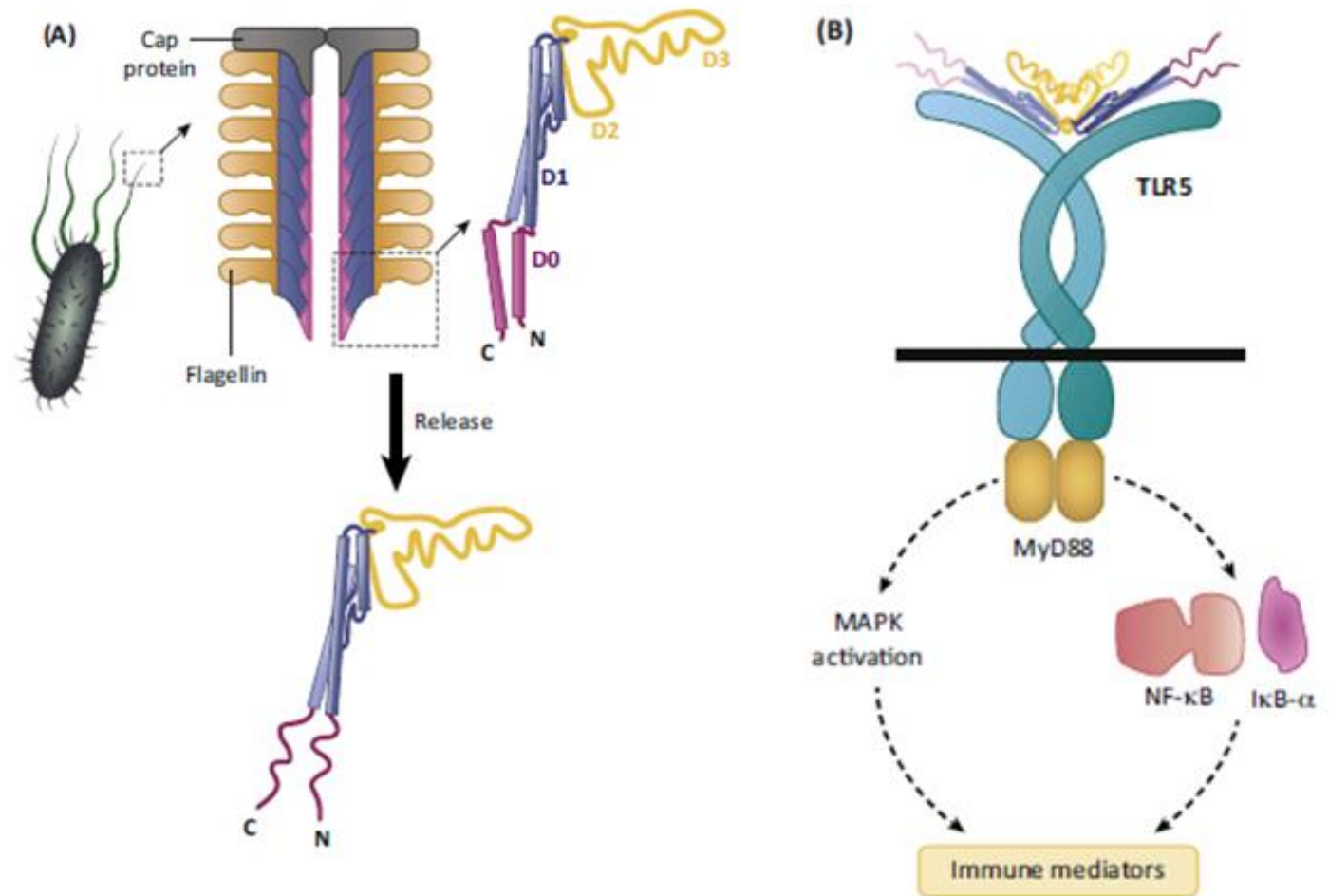
- Phospholipase YplA (*Y. enterocolitica*)
- Hemolysin HBL and PC-PLC (*B. thuringiensis*)
- Campylobacter invasion antigen Cia and FspA proteins (*C. jejuni*)



Cornelis GR - 2006 - Nat Rev Microbiol

Flagella: TLR5-induced immunity

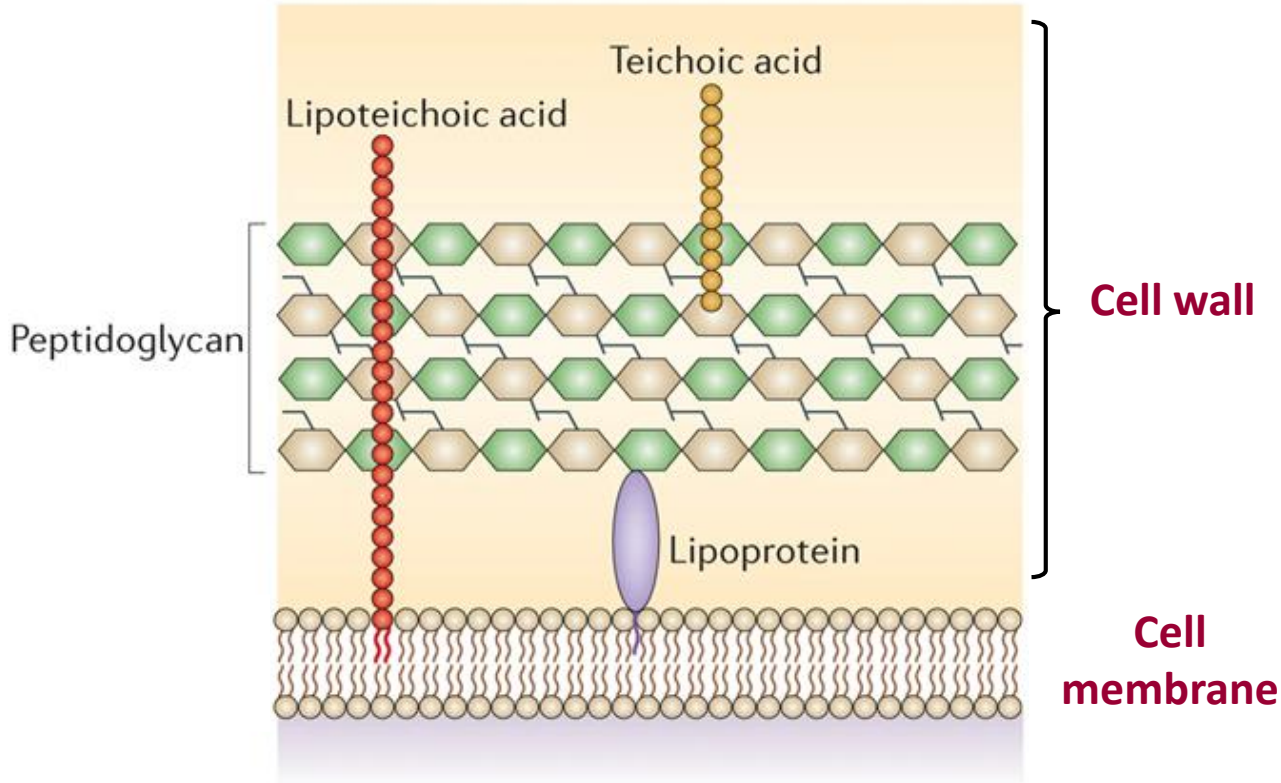
- **PAMP / TLR5 specific ligand**
- **2-4 domains**
 - ✓ Central region
 - Hypervariable
 - Exposed
 - ✓ N-ter, C-ter domains
 - Highly conserved
 - D0 and D1 domains: polymerization
 - **Conserved 89-96 motif: TLR5 binding**
 - Monomer
 - Alternative motif \Rightarrow no TLR5 activation
- **TLR5 signaling pathway**
 - ✓ MAPK / NF κ B
 - ✓ Pro-inflammatory cytokines



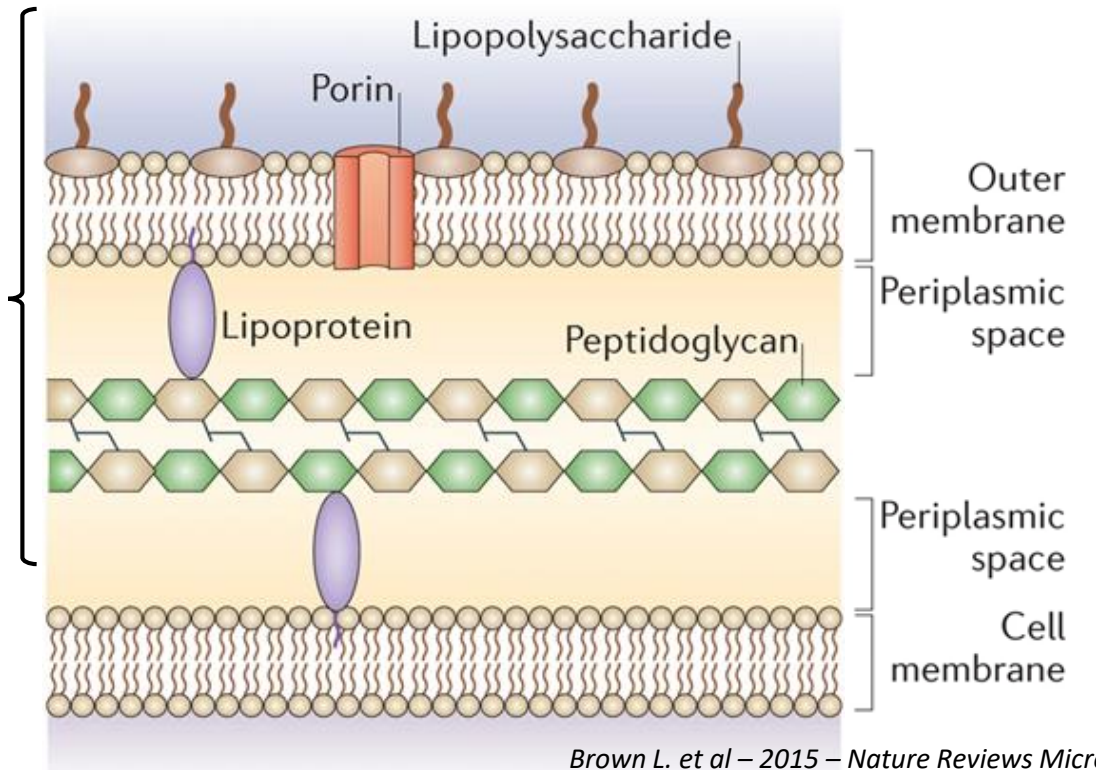
To memorize

- G- bacteria cell wall
 - ✓ Structure
 - ✓ Roles
- Appendages
 - ✓ Fimbriae: adherence, cell invasion, biofilm formation, DNA uptake, microcolony formation
 - ✓ Type IV secretion pili
 - ✓ Flagella
 - Motility
 - Virulence factor

Gram-positive bacteria



Gram-negative bacteria



Brown L. et al – 2015 – Nature Reviews Microbiology