

OSNOVE MIKROBIOLOGIJE

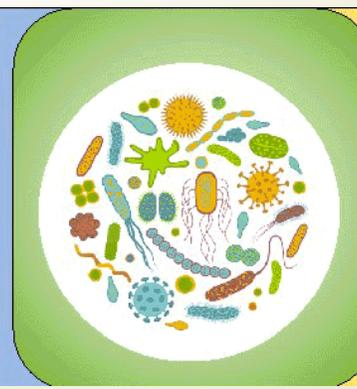
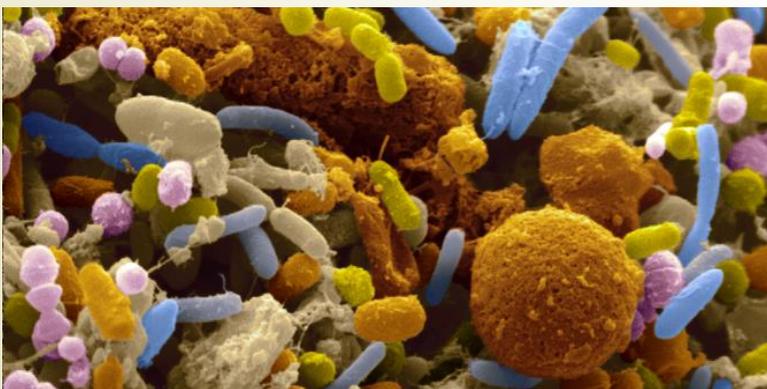
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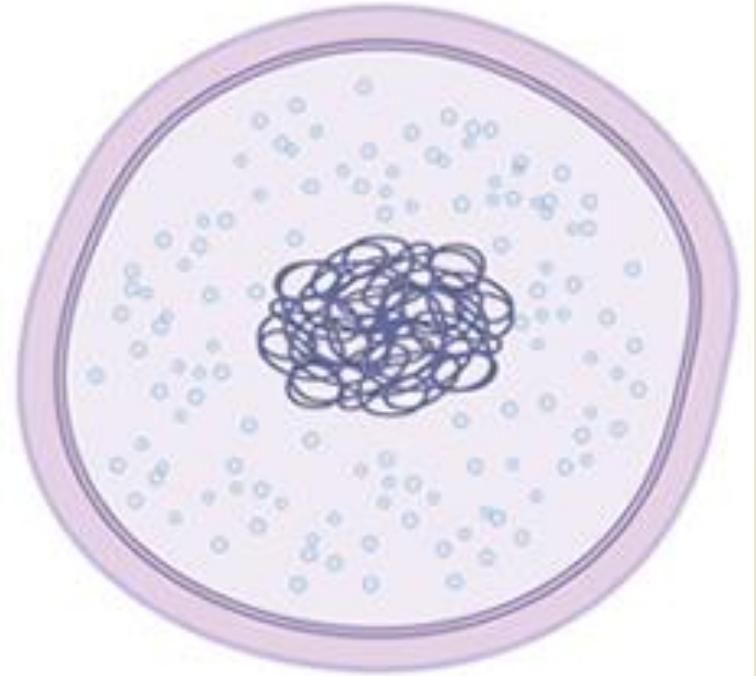
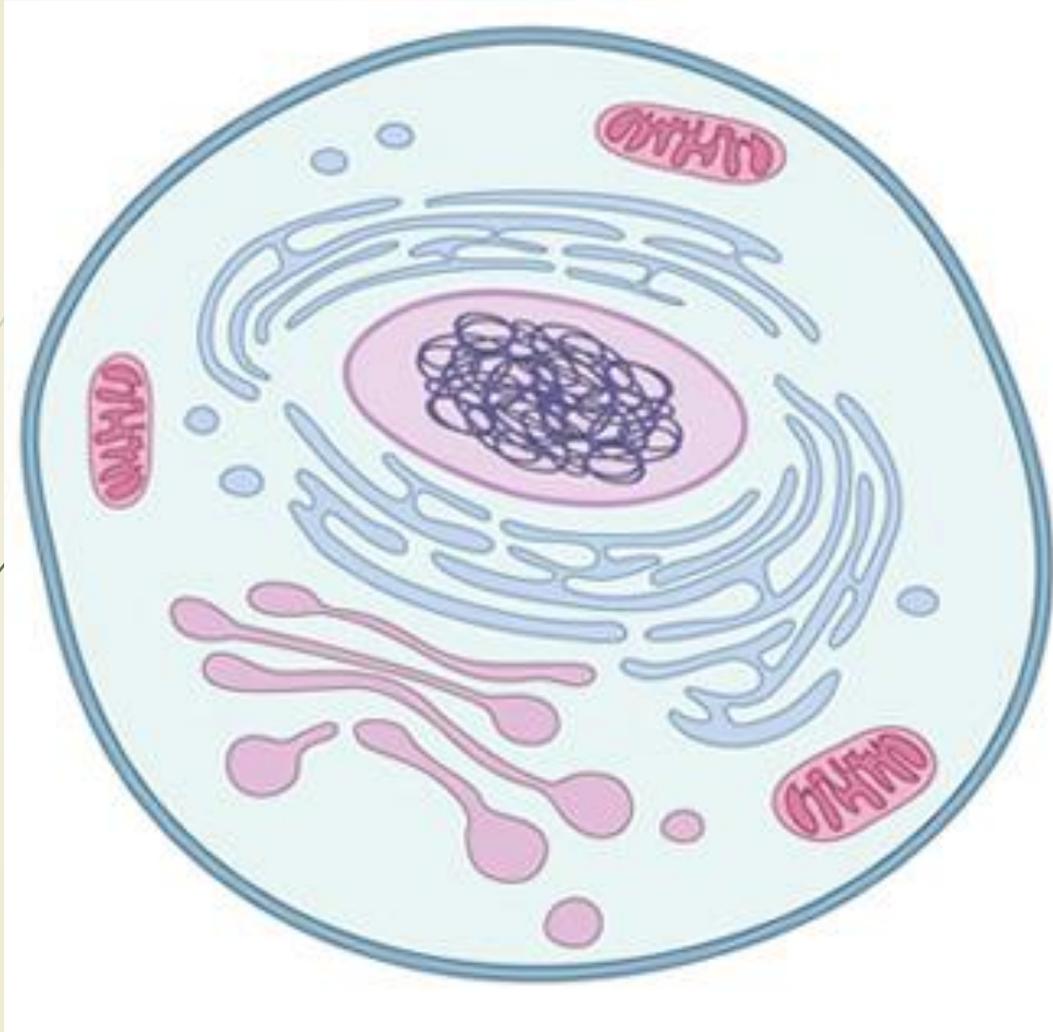
studij biologije i kemije



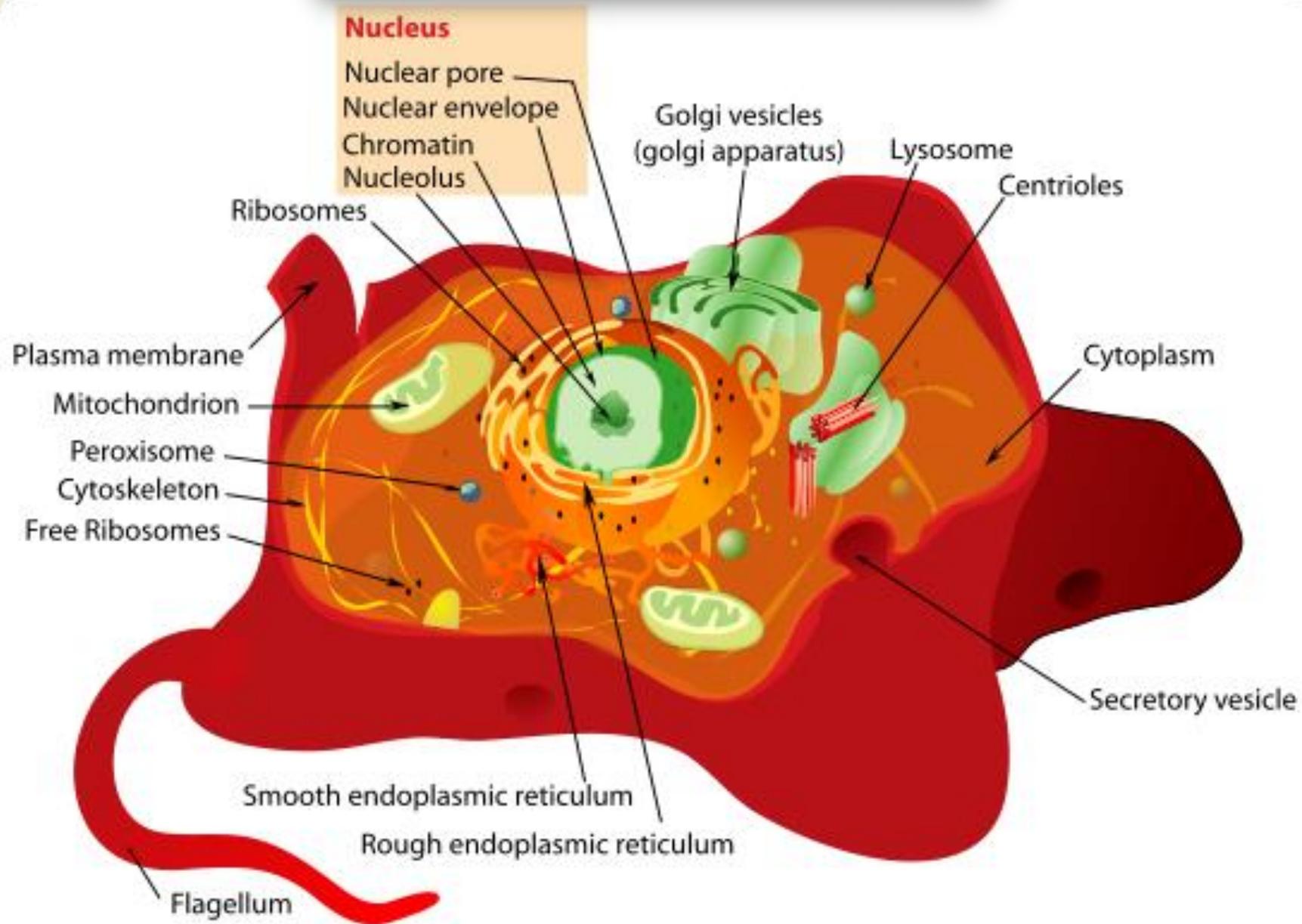


II. PROKARIOTSKA STANICA?: *Bacteria* i *Archaea*

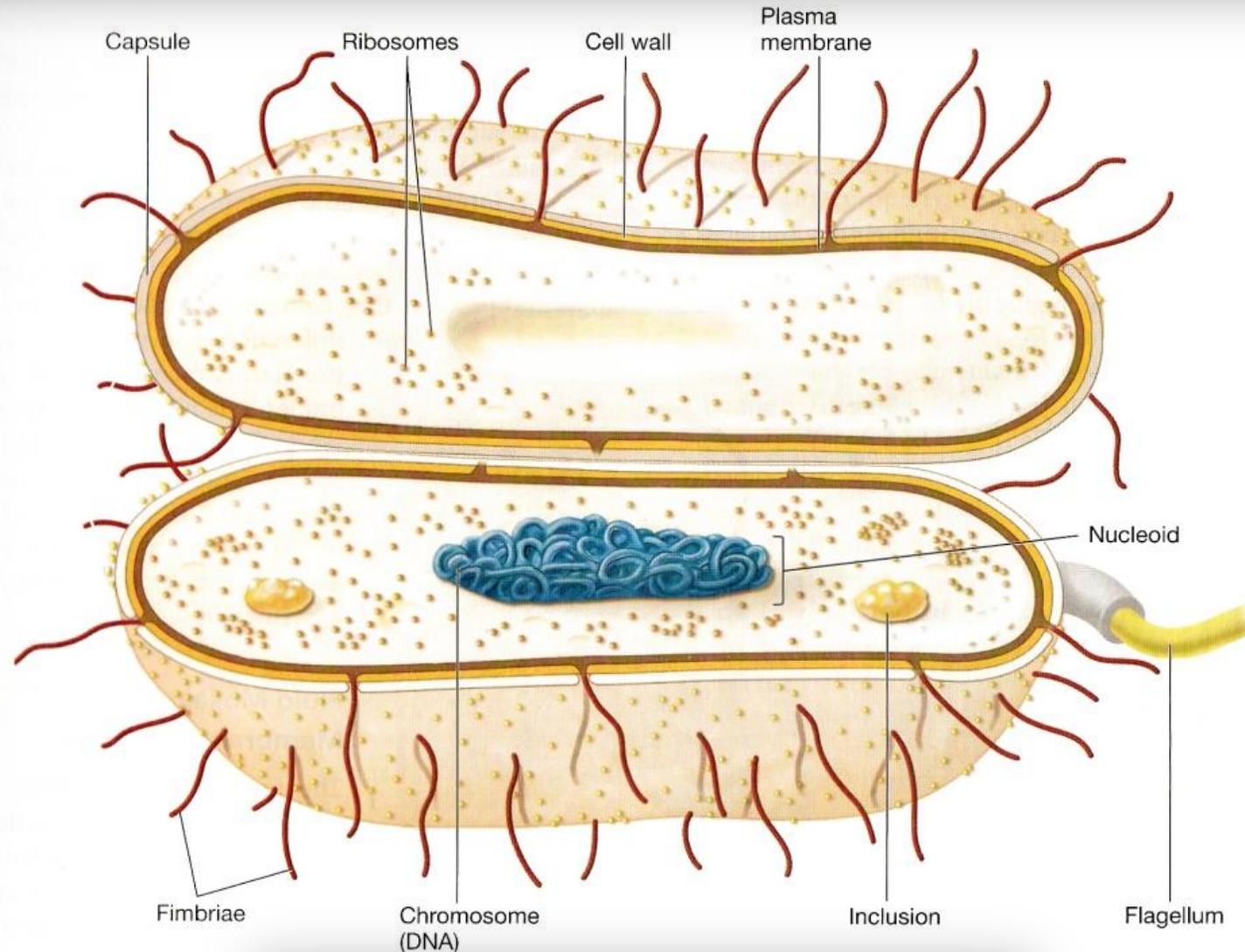
- Organizacija bakterijske stanice i stanice arheja
- Stanične stijenske bakterija i arheja
- Površinske strukture bakterijskih stanica



Eukariotska stanica



Morfologija bakterijske stanice



arheje rijetko imaju kapsulu

“prokaryote controversy”

Table 4.2 Comparison of Procaryotic and Eucaryotic Cells

Property	Procaryotes	Eucaryotes
Organization of Genetic Material		
True membrane-bound nucleus	Absent	Present
DNA complexed with histones	No	Yes
Number of chromosomes	One ^a	More than one
Introns in genes	Rare	Common
Nucleolus	Absent	Present
Mitosis occurs	No	Yes
Genetic Recombination	Partial, unidirectional transfer of DNA	Meiosis and fusion of gametes
Mitochondria	Absent	Present
Chloroplasts	Absent	Present
Plasma Membrane with Sterols	Usually no ^b	Yes
Flagella	Submicroscopic in size; composed of one fiber	Microscopic in size; membrane bound; usually 20 microtubules in 9 + 2 pattern
Endoplasmic Reticulum	Absent	Present
Golgi Apparatus	Absent	Present
Cell Walls	Usually chemically complex with peptidoglycan ^c	Chemically simpler and lacking peptidoglycan
Differences in Simpler Organelles		
Ribosomes	70S	80S (except in mitochondria and chloroplasts)
Lysosomes and peroxisomes	Absent	Present
Microtubules	Absent or rare	Present
Cytoskeleton	May be absent	Present
Differentiation	Rudimentary	Tissues and organs

^aPlasmids may provide additional genetic information.

^bOnly the mycoplasmas and methanotrophs (methane utilizers) contain sterols. The mycoplasmas cannot synthesize sterols and require them preformed. Many procaryotes contain hopanoids.

^cThe mycoplasmas and Archaea do not have peptidoglycan cell walls.

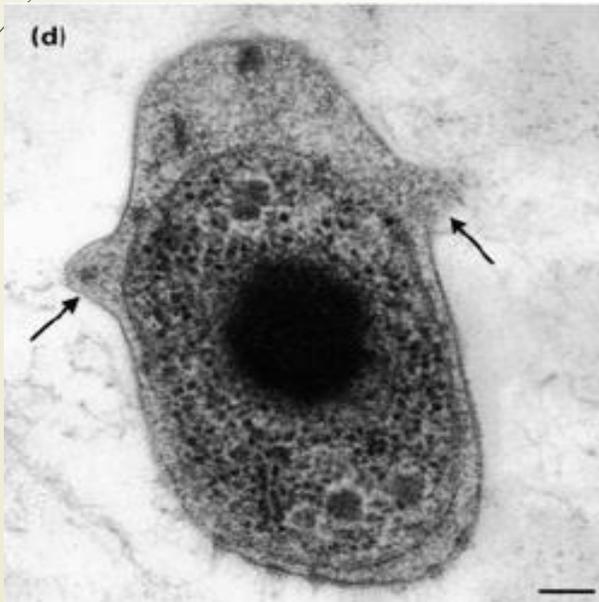
“prokaryote controversy”

Table 4.1 Comparison of Bacterial and Archaeal Cells

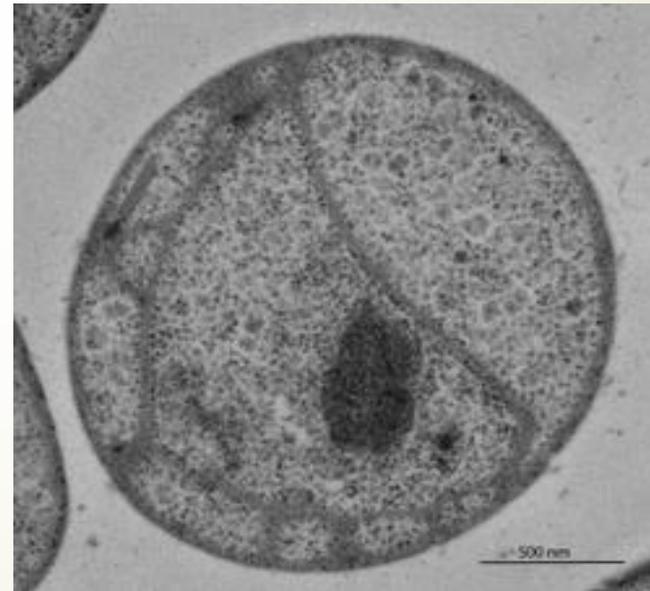
Property	Bacteria	Archaea
Plasma membrane lipids	Ester-linked phospholipids	Glycerol diethers, glycerol tetraethers
Cell wall constituents	Peptidoglycan present in nearly all; some lack cell walls.	Highly diverse. Most commonly an S-layer; some include polysaccharides or protein or both; some lack cell walls. Peptidoglycan is always absent.
Inclusions present	Yes	Yes
Ribosome size	70S	70S
Chromosome structure	Circular, double-stranded (ds) DNA	Circular dsDNA
Plasmids present	Yes, circular and linear dsDNA	Yes, circular dsDNA
Capsules or slime layers	Common	Rare
Fimbriae, pili	Common	Pili common, including archaella
Motility structure	Flagellum	Archaellum
Cytoskeleton	Multiple homologues of tubulin and actin	Multiple homologues of tubulin and actin
Extracellular vesicles	Yes	Yes

“prokaryote controversy”

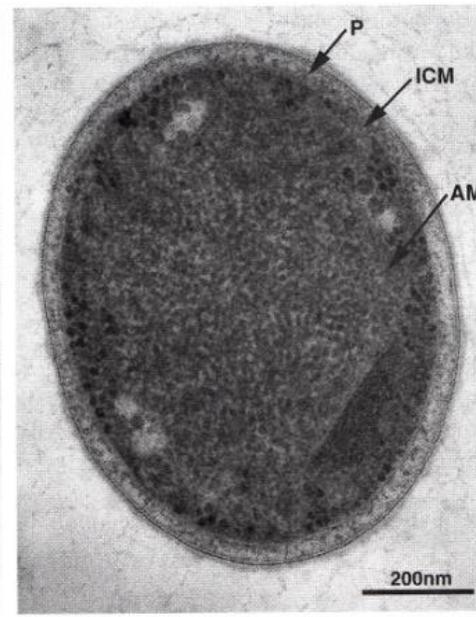
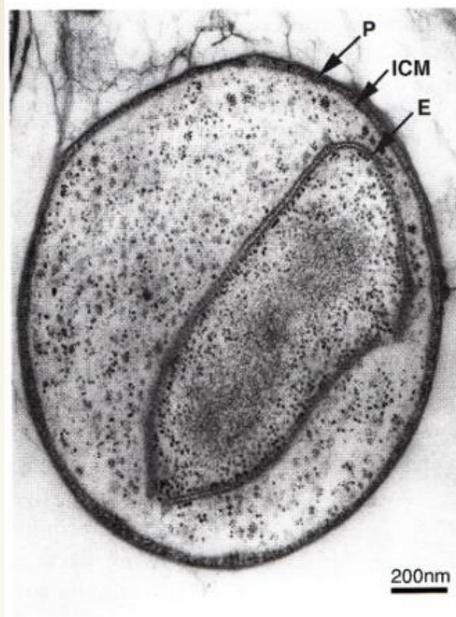
- postojanje citoskeletona često i u bakterija i arheja
- phylum *Planctomycetes* – bakterije koje nalazimo u moru i tlu
- dijele se pupanjem, a neke vrste imaju **genetički materijal unutar membrane** te **pirelulosome** i organele **anamoksome** koji posjeduju membrane (reakcije anamoks – oksidacija NH_4^+ do N_2)



Pirelellula staleyi

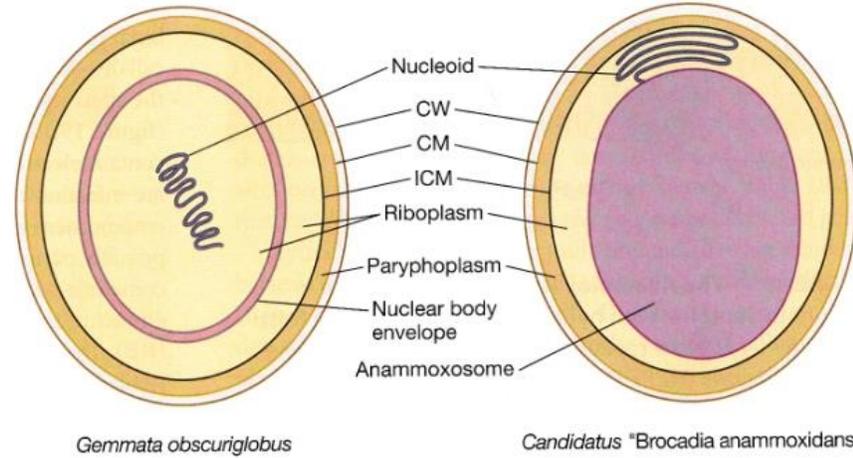


Gemmata obscuriglobus



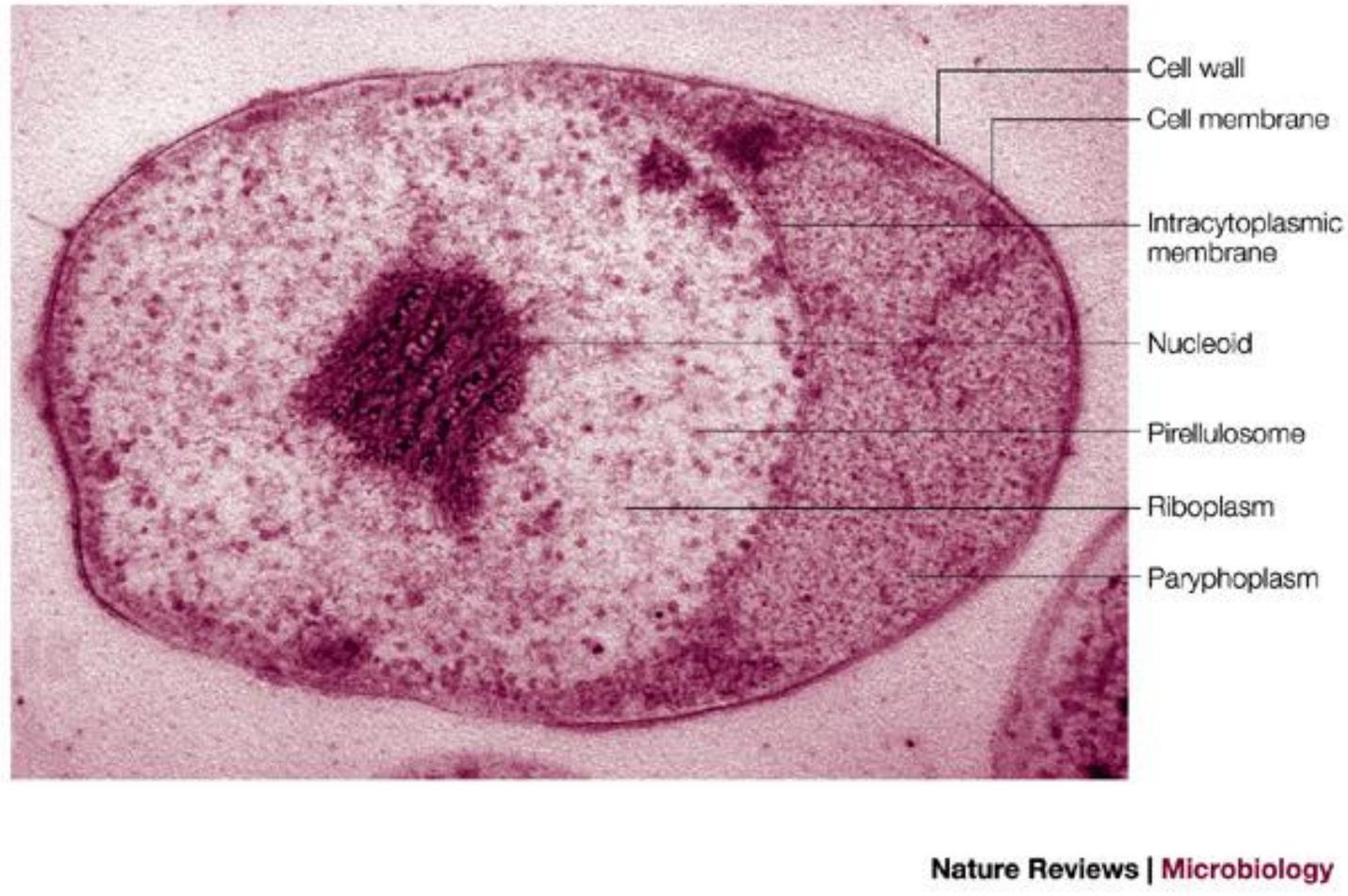
(a)

(b)



(c)

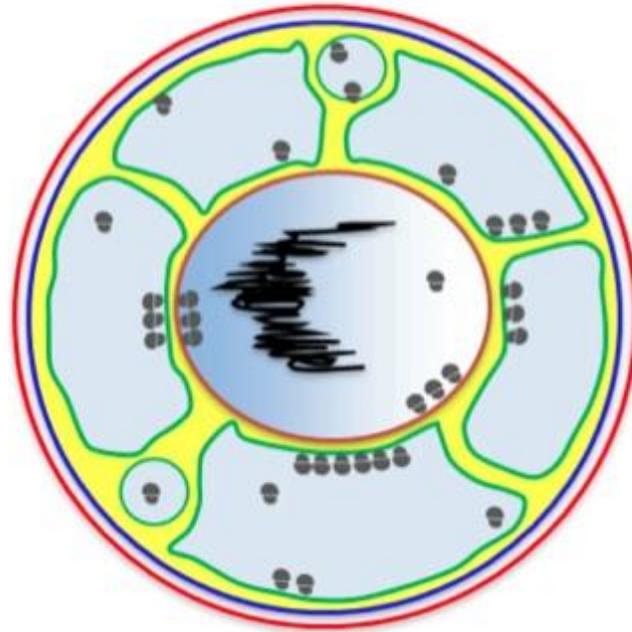
FIGURE 19.10 Planctomycete Cellular Compartmentalization. (a) An electron micrograph of *Gemmata obscuriglobus* showing the nuclear body envelope (E), the intracytoplasmic membrane (ICM), and the paraphoplasm (P). (b) An electron micrograph of the anaerobic ammonia-oxidizing planctomycete *Candidatus "Brocadia anammoxidans"*. The anammoxosome is labeled AM. (c) Schematic drawings corresponding to (a) and (b): cell wall (CW), cytoplasmic membrane (CM).



Pirellula marina

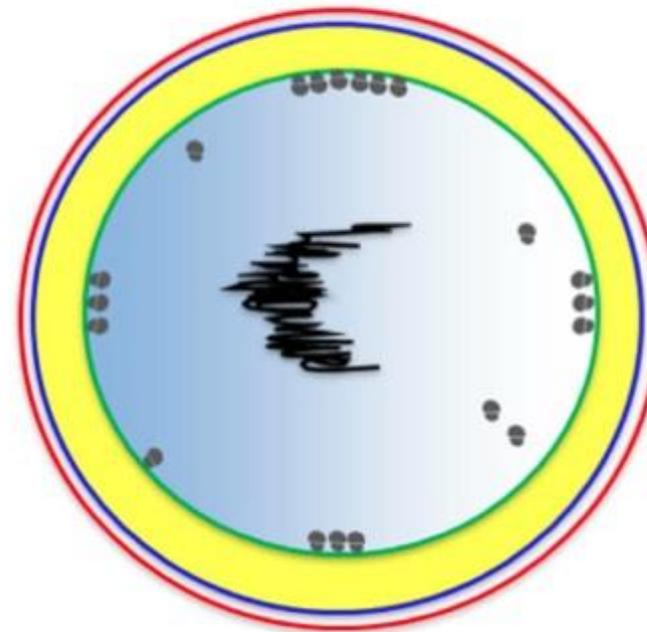
- **pirelulosom** ili riboplasma – sadrži ribosome
- **parifoplazma** – periferna citoplazma bez ribosoma

A



Gemmata obscuriglobus

B



Planctomycete cell
(pirellula type)

Figure 3. Proposed cell organisations of planctomycetes. A) The cell plan for *G. obscuriglobus* proposed in the current publication. This cell plan mostly follows the established view in past publications (as in [4]) including ribosome-less paraphoplasm and intracytoplasmic membrane (ICM), except for presentation of riboplasm, which now appears as multiple vesicles surrounding the nuclear body. Cell wall is indicated in red; cytoplasmic membrane - in dark blue; paraphoplasm - in yellow; riboplasm and nuclear body interior - in light blue; intracytoplasmic membrane - in green; inner nuclear body membrane - in brown; nucleoid DNA - in black; ribosomes - grey circles. **B)** The cell plan for *Pirellula*, which is considered as "simplest" among planctomycetes. A major internal compartment defined by an intracytoplasmic membrane (internal to cytoplasmic membrane bounding the protoplast) encloses a naked nucleoid. Unlike *G. obscuriglobus*, this bacterium thus does not contain a membrane-bounded nuclear body within the major internal pirellulosome compartment. Designation of the structures the same as for (A).
doi:10.1371/journal.pone.0091344.g003

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PLOS ONE

Structural Studies of Planctomycete *Gemmata obscuriglobus* Support Cell Compartmentalisation in a Bacterium

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- postojanje staničnih odjeljaka – kompartmentalizacija stanice

- predložen novi mehanizam stanične diobe kod vrste *Gemmata obscuriglobus*

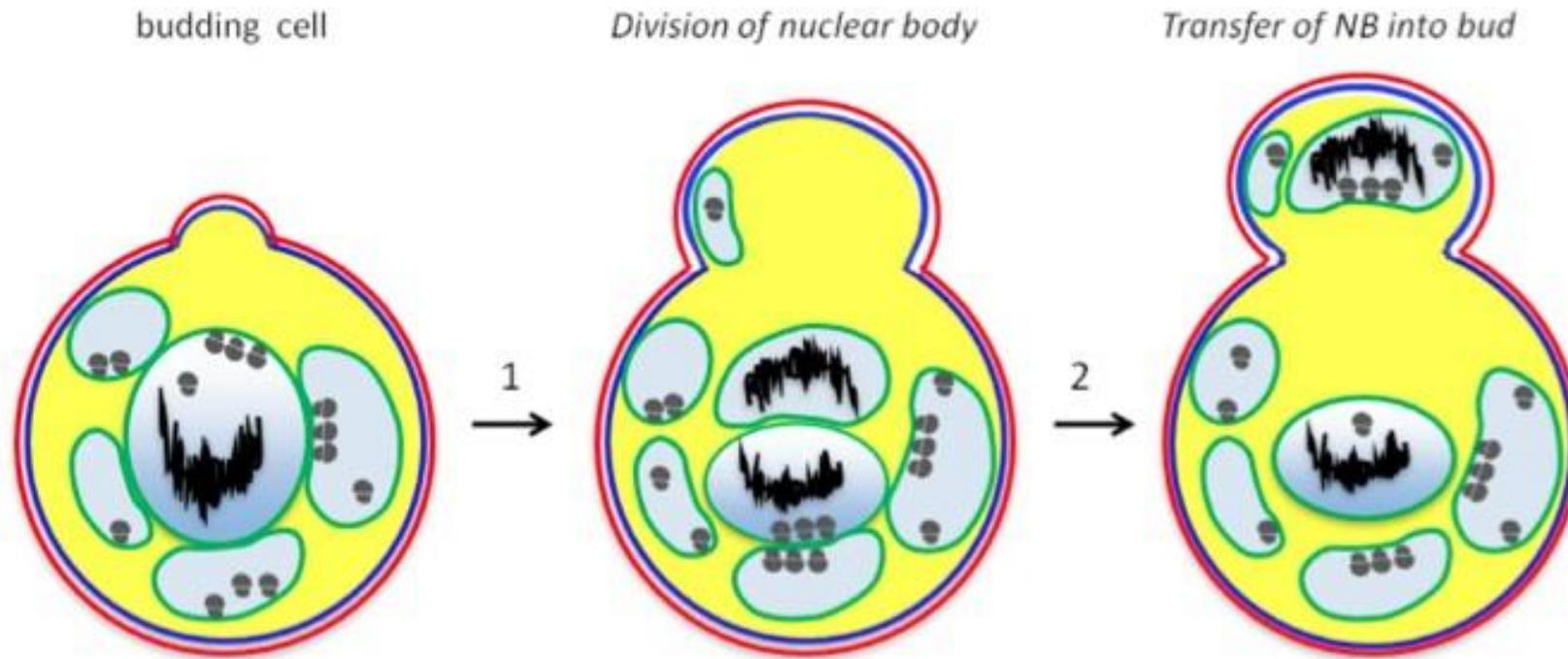


Figure 4. A model for mechanism of cell division of *G. obscuriglobus* cells. Step 1, the bud appears as a hump on the surface of a cell (Figures S5 and S6 in File S1). The nuclear body is divided, before or during the formation of a bud, forming two fully enveloped structures, as shown in step 2. Finally, one of the nuclear bodies migrates into a newly formed cell (step 3). Other riboplasm vesicles not containing nucleoid DNA are also transferred into the newly formed cell (Figure S6B in File S1). Cell wall is indicated in red; plasma membrane – blue; ICM – green; paryphoplasm – yellow; riboplasm – light blue; nucleoid – black; ribosomes – grey circles.
doi:10.1371/journal.pone.0091344.g004

Zajedničke strukture bakterija i arheja i njihove funkcije

Plazma-membrana

- selektivno-propusna barijera, mehanička zaštita stanice, transport hranjivih i otpadnih tvari, mjesto odvijanja mnogih metaboličkih procesa (respiracija, fotosinteza), detekcija okolišnih čimbenika za kemotaksiju

Ribosomi

- sinteza proteina

Uklopine (inkluzije)

- skladištenje ugljika, fosfata i ostalih tvari

Nukleoid

- lokalizacija genetičkog materijala (DNA)

Periplazmatski prostor

- sadrži enzime za hidrolizu i vezne proteine za procesiranje i uzimanje hranjivih tvari

Stanična stijenka

- daje bakterijama oblik i služi zaštiti od stanične lize

Kapsule

- otpornost na fagocitozu, prihvaćanje za površine

Fimbrije i pili

- vezanje na površine, bakterijsko parenje

Flagele

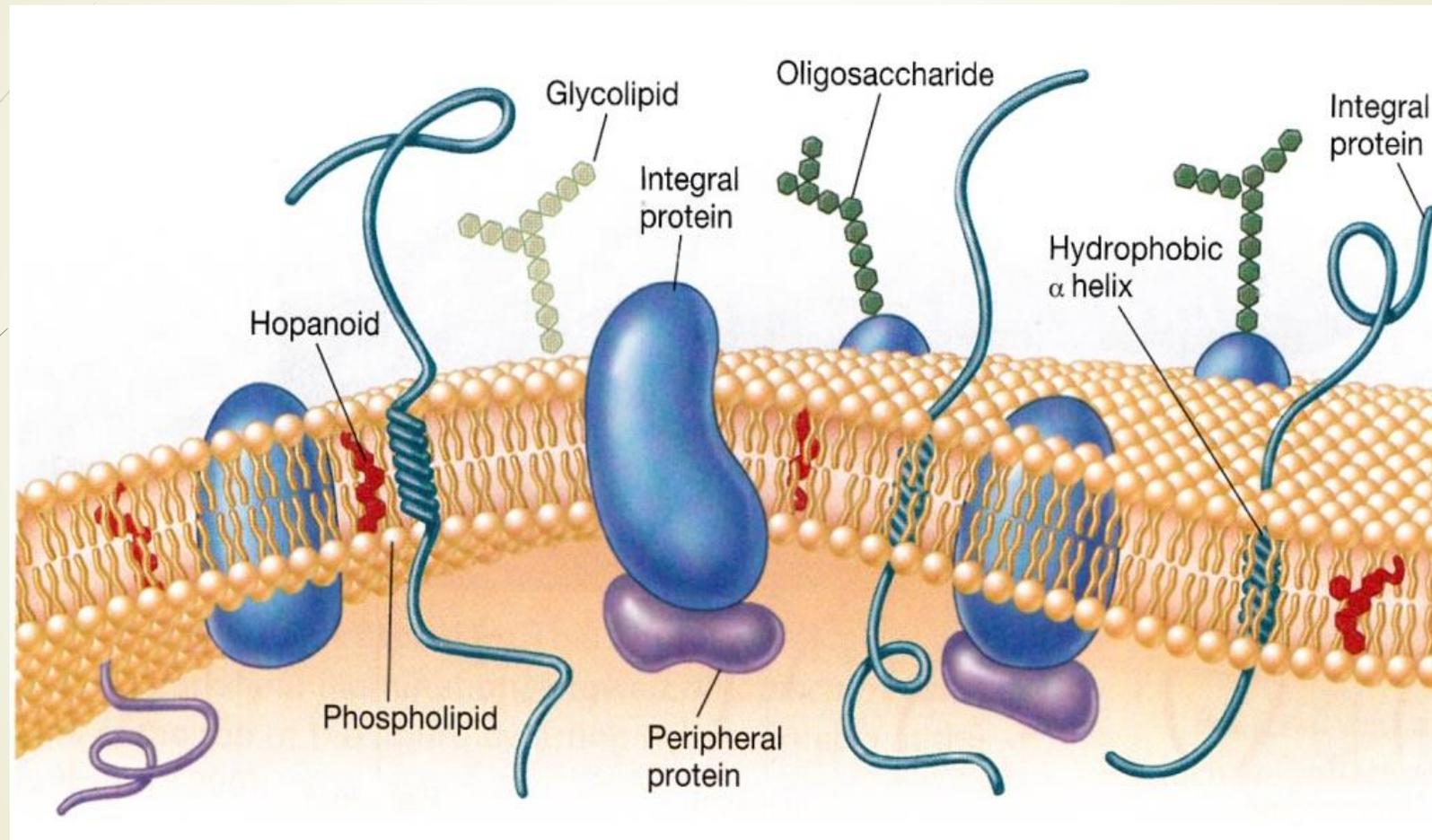
- pokretanje

Endospore

- opstanak u nepovoljinim okolišnim uvjetima; samo kod bakterija

Stanična ovojnica bakterija

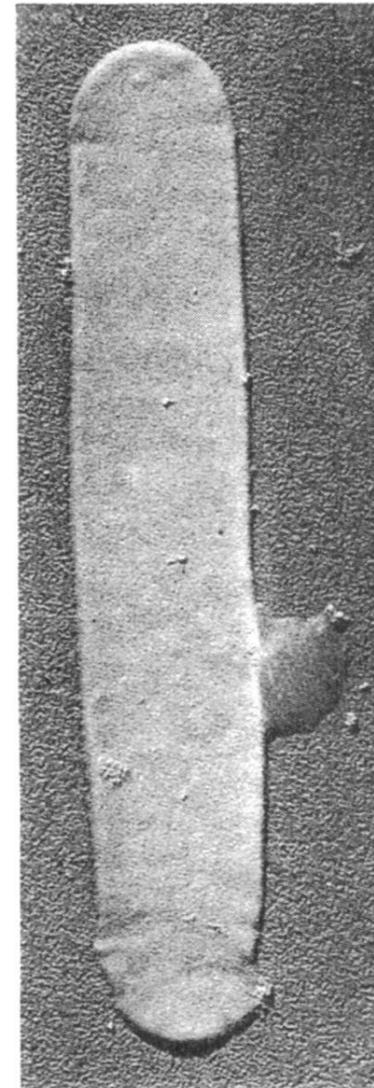
- plazma-membrana + stanična stijenka + dodatni sloj(evi)



Struktura bakterijske membrane – fluidni mozaični model

Stijenke bakterijskih stanica

- **stanična stijenka** – obično kruti sloj koji se nalazi smješten izvan stanične membrane
- osigurava osebujan oblik, mehaničku potporu te zaštitu bakterijske stanice (stanica bez stijenke puca u hipotoničnoj otopini)
- nemaju je bakterije iz razreda **Mollicutes** (mikoplazme, fitoplazme, spiroplazme), dok su kod koljena **Planctomyces** specifične građe i ne sadrže peptidoglikan (murein)
- stijenka s pridruženim tvarima može biti odgovorna za otrovnost (virulenciju) bakterija
- može štiti stanicu od toksičnih tvari
- mjesto djelovanja nekih antibiotika – sprječavaju sintezu stanične stijenke

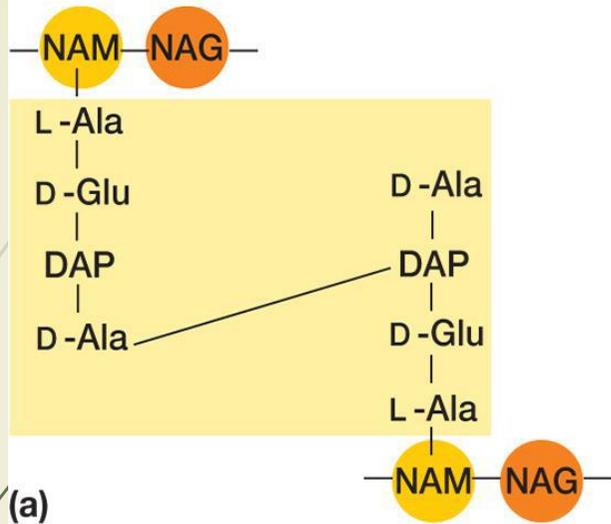


Slika 32.1.

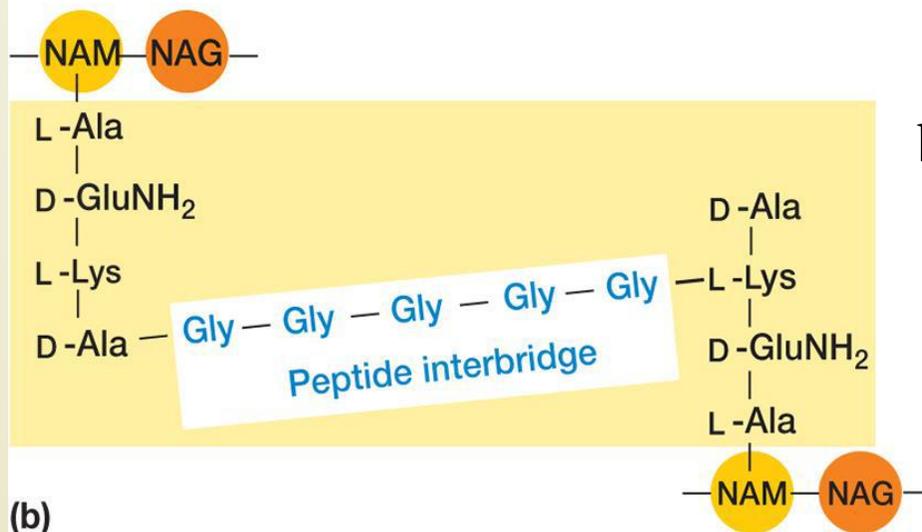
Elektronska mikrofotografija izdvojene stanične stijenke vrste *Bacillus licheniformis* (ljubaznošću dra Nathana Sharona).

- lanci peptidoglikana unakrsno su povezani
- različito kod Gram-pozitivnih i Gram-negativnih bakterija

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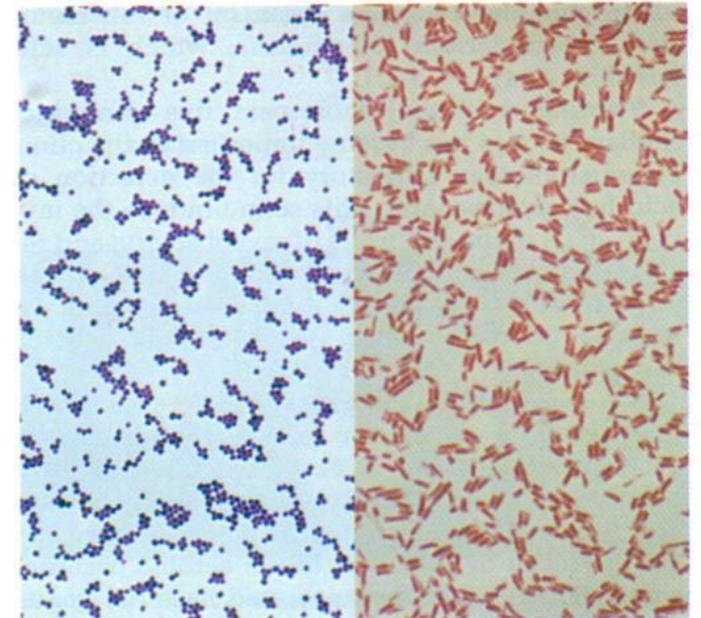
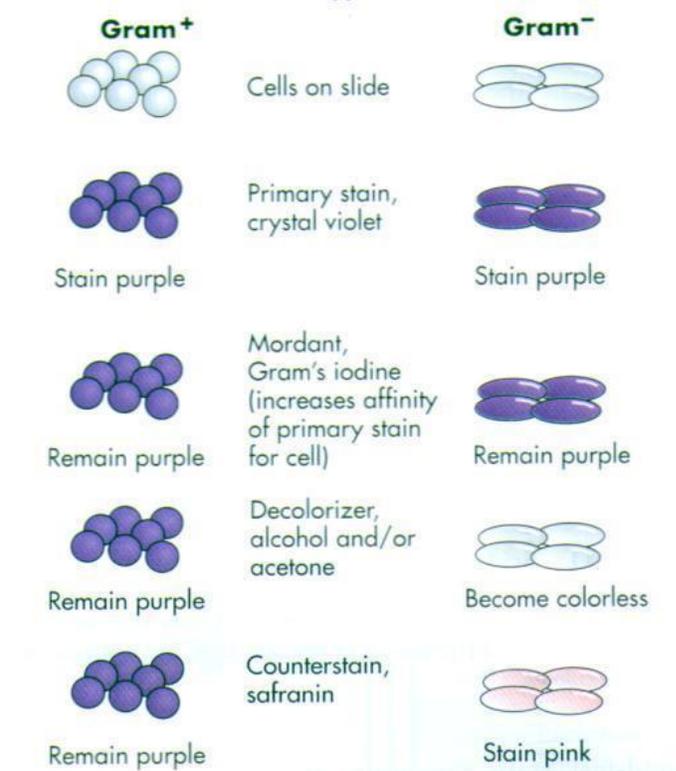
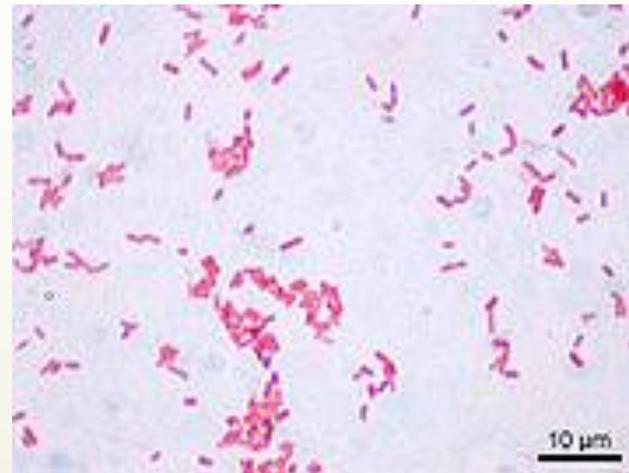
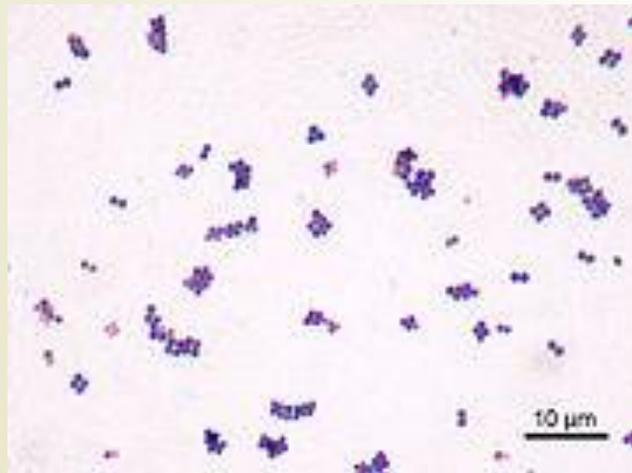
- a) Gram-negativne bakterije (*Echerichia coli*)
 - izravno povezani
 (D-Ala – diaminopimerinska kiselina)



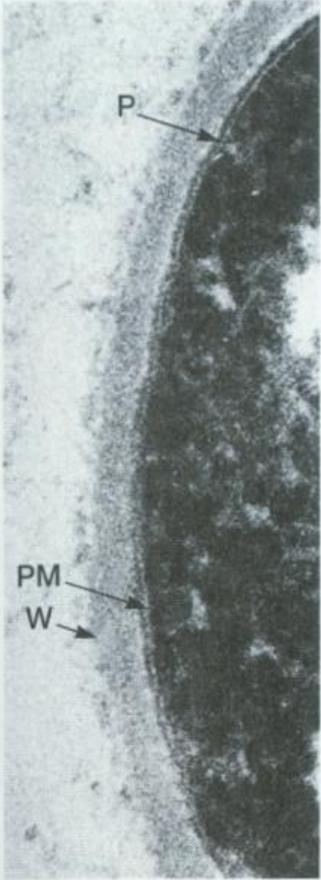
- b) Gram-pozitivne bakterije (*Staphylococcus aureus*)
 - povezani pentaglicinskim mostom

➤ Hans Christian Gram, 1884. – bojanje po Gramu

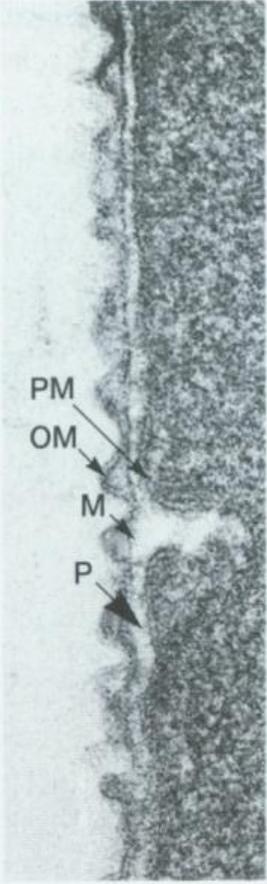
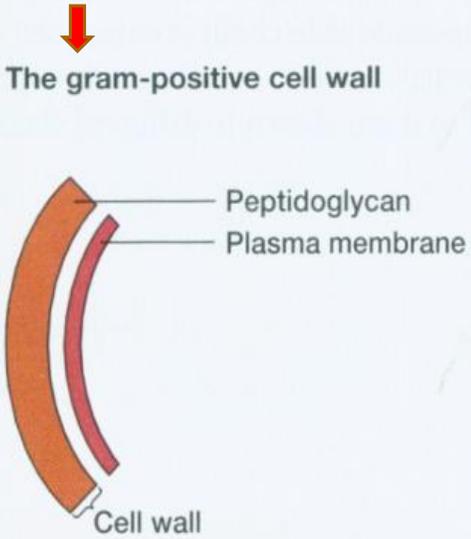
➤ bojanje po Gramu – reakcija je uvjetovana različitom građom staničnih stijenki dvaju bakterijskih razreda: **Gram-pozitivnih i Gram-negativnih** bakterija



Grada stanične stijenke



Gram-pozitivna bakterija



Gram-negativna bakterija

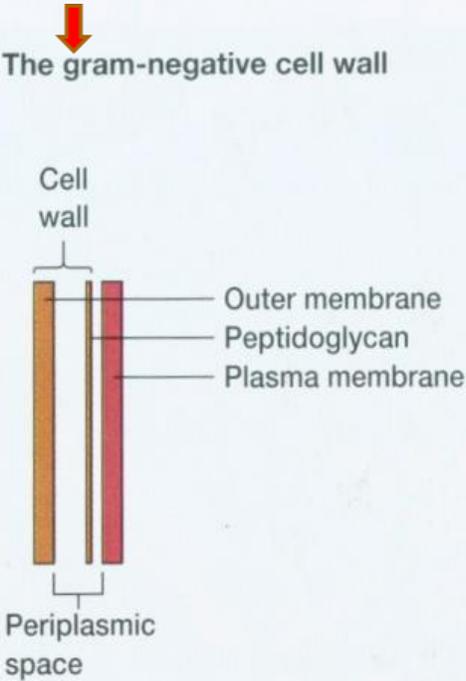
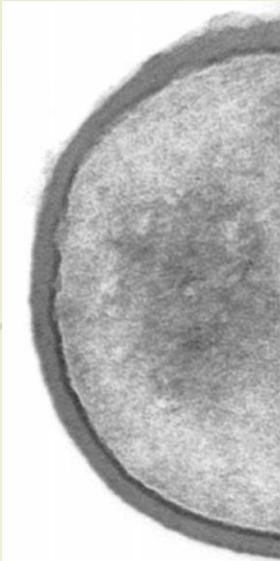
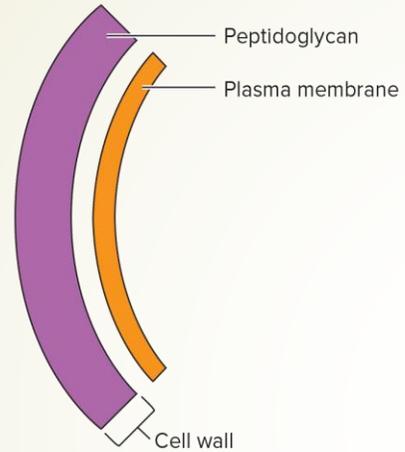


Figure 3.15 Gram-Positive and Gram-Negative Cell Walls. The gram-positive envelope is from *Bacillus licheniformis* (left), and the gram-negative micrograph is of *Aquaspirillum serpens* (right). M; peptidoglycan or murein layer; OM, outer membrane; PM, plasma membrane; P, periplasmic space; W, gram-positive peptidoglycan wall.

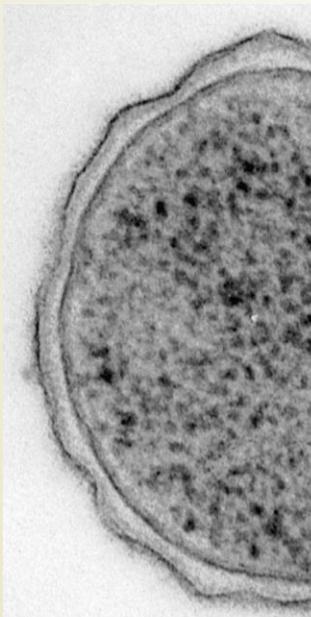
Grada stanične stijenke



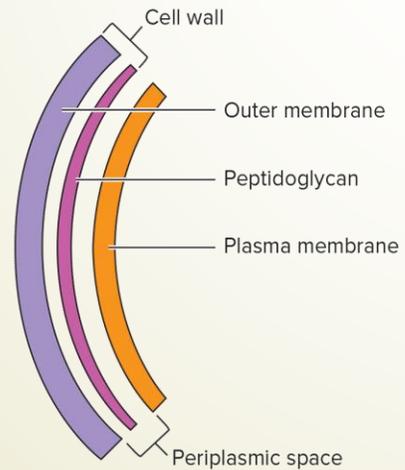
The typical Gram-positive cell envelope



Gram-pozitivne bakterije
- **monodermi**



The typical Gram-negative cell envelope

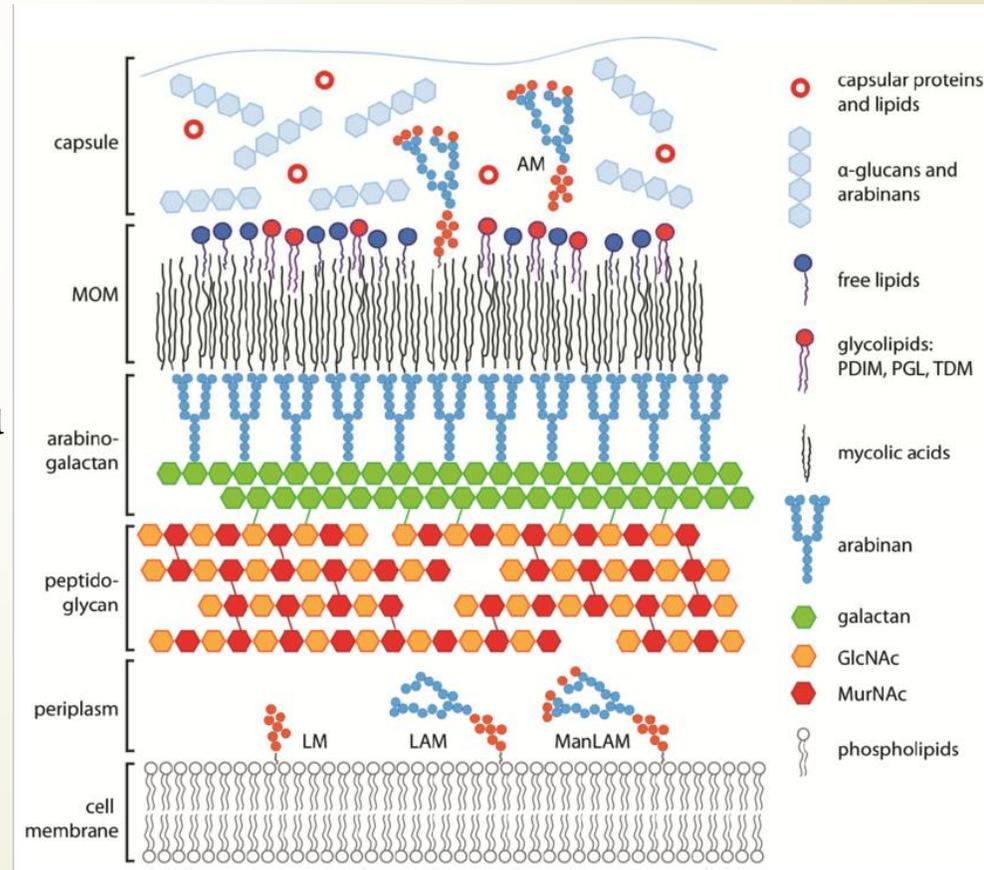


Gram-negativne bakterije
- **didermi**

Grada stanične stijenke

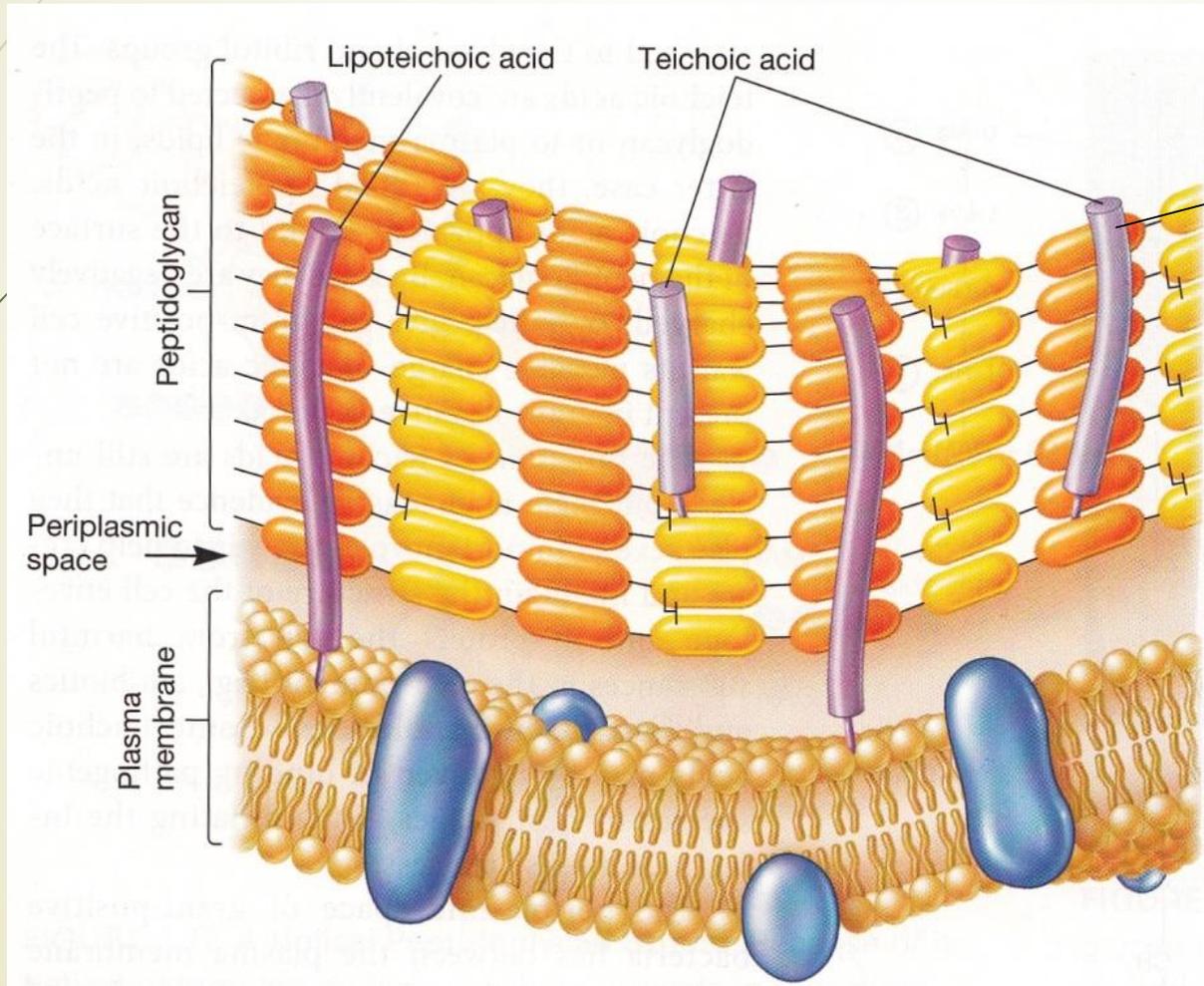
Gram-neutralne bakterije; Gram – „duhovi” (Gram – „ghosts”)

- stanične stijenke imaju svojstva i Gram + i Gram – bakterija
- Mycobacteria (Mycobacteriaceae) – preko 200 patogenih bakterija
- *Mycobacterium tuberculosis*
- debela, hidrofobna stanična stijeka bogata **mikoličnom kiselinom**
- **mikolična kiselina** – specifična dugolančana masna kiselina prisutna samo u mikobakterijama
- daje otpornost na kemijske agense, dehidraciju i daje zaštitu od makrofaga (imuni sustav domaćina)

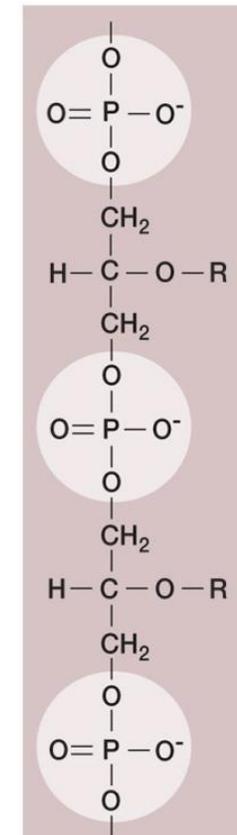


Stanične stijenke Gram-pozitivnih bakterija

- 20-80 nm debeli peptidoglikanski sloj
- sadrži teichojevu kiselinu koje nema kod Gram-negativnih bakterija – funkcija nije sasvim razjašnjena – održavanje strukture i negativnog naboja stanične stijenke
- rodovi *Bacillus*, *Streptococcus*, *Staphylococcus*, *Clostridium*...

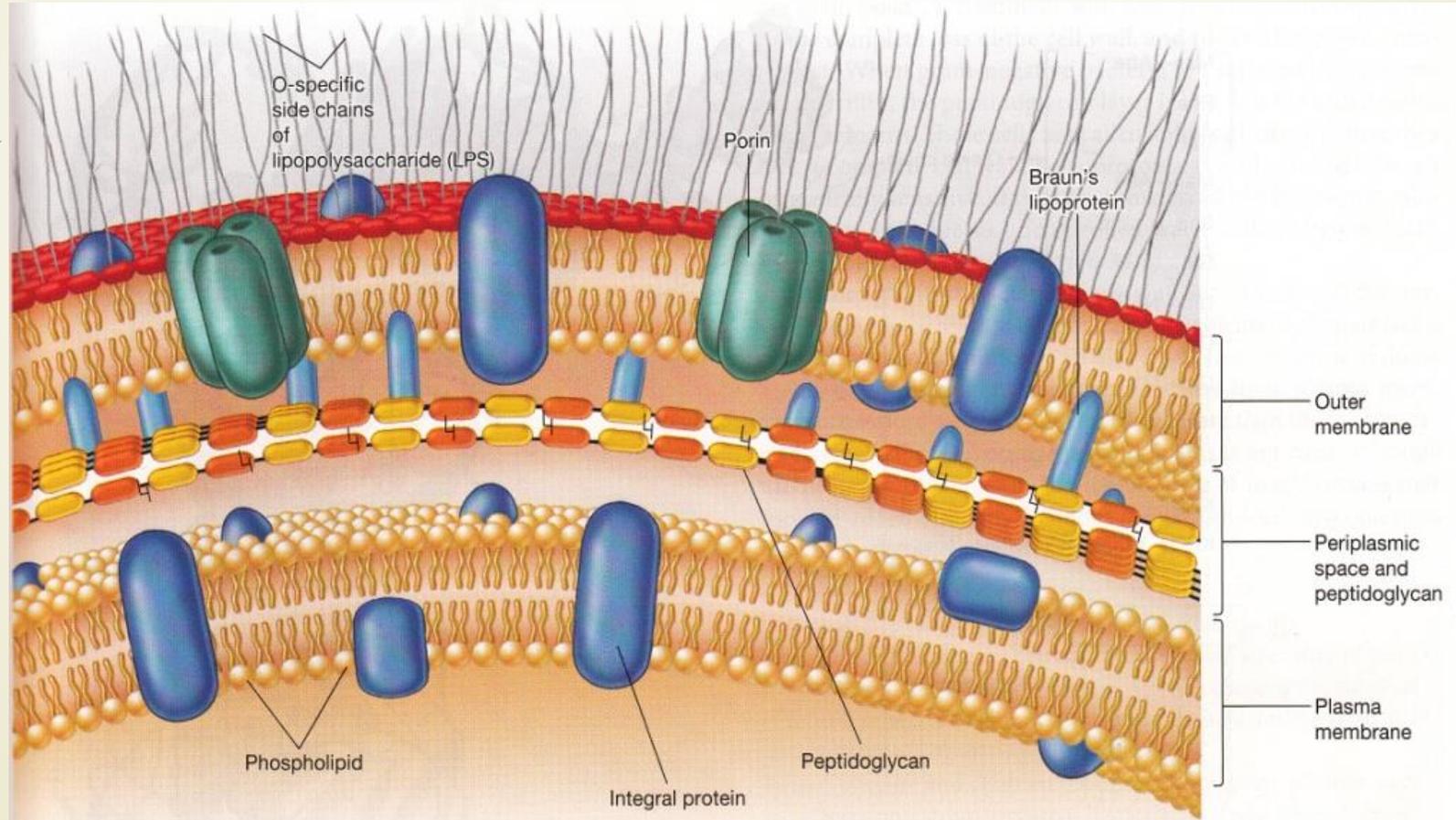


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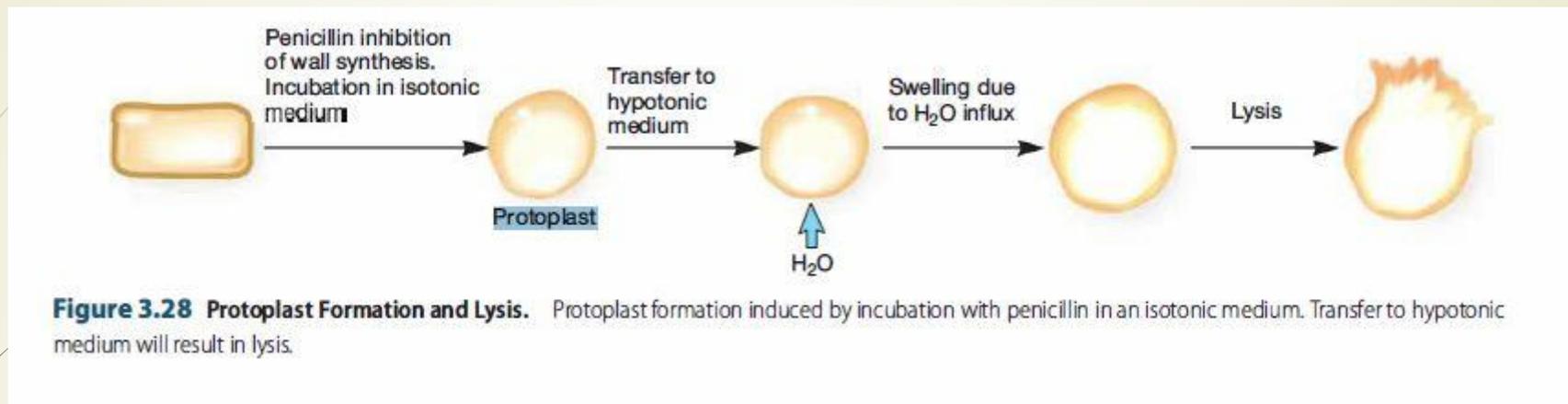
Stanične stijenke Gram-negativnih bakterija

- nešto složenija građa
- **2-8 nm** debeli peptidoglikanski sloj okružen **7-8-nm** debelom vanjskom membranom
- periplazmatski prostor – može činiti i do 40% ukupnog staničnog volumena
- vanjska membrana – lipopolisaharidi (LPS), Braunov lipoprotein, porin
- mnoge patogene bakterije su Gram-negativne bakterije
- *Escherichia coli*, rodovi *Salmonella*, *Shigella*, *Klebsiella*, *Neisseria*, *Helicobacter*, *Haemophilus*



Antibiotik penicilin blokira sintezu stanične stijenke

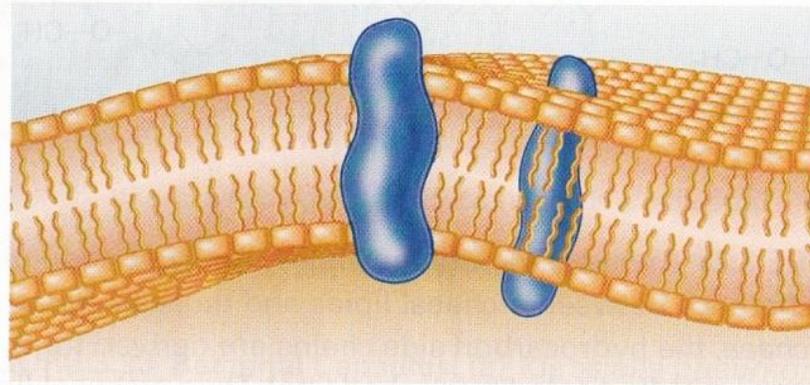
- penicilin sprječava sintezu peptidoglikana (transpeptidaciju)



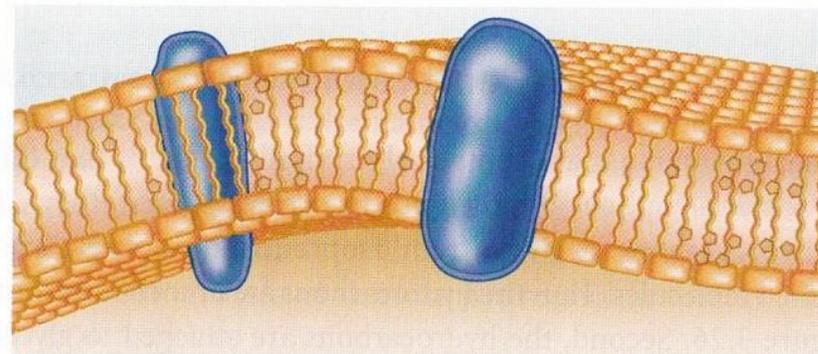
- stanična **osmoza** – difuzija vode kroz plazma-membranu iz hipotonične otopine (okoliš) u hipertoničnu otopinu (citoplazma)
- **stanična stijenka štiti bakteriju od prsnuća – lize**
- **lizozim** – enzim koji razara staničnu stijenku bakterija – ne specifična antimikrobna zaštita i prva linija obrane – slina, suze, majčino mlijeko, bjelanjak jajeta
- **protoplast** – stanica Gram-pozitivne bakterije s uklonjenom staničnom stijenkom
- **sferoplast** – stanica Gram-negativne bakterije s djelomično uklonjenom staničnom stijenkom
- neke bakterije **nisu osjetljive na penicilin jer nemaju staničnu stijenku** – razred *Mollicutes* (mikoplazme, ureaplazme, fitoplazme.....)

Stanična ovojnica arheja

- plazma-membrane arheja sastoje se uglavnom od lipida koje se razlikuju od bakterijskih i eukariotskih lipida



(a) Bilayer of C_{20} diethers



(b) Monolayer of C_{40} tetraethers

Stanične stijenke arheja

- arheje ne karakteriziramo kao Gram pozitivne ili Gram-negativne
- ne sadrže peptidoglikan
- S-sloj debljine 20 – 40 nm sastavljen od proteina ili glikoproteina
- **pseudomurein** – molekula slična peptidoglikanu mureinu

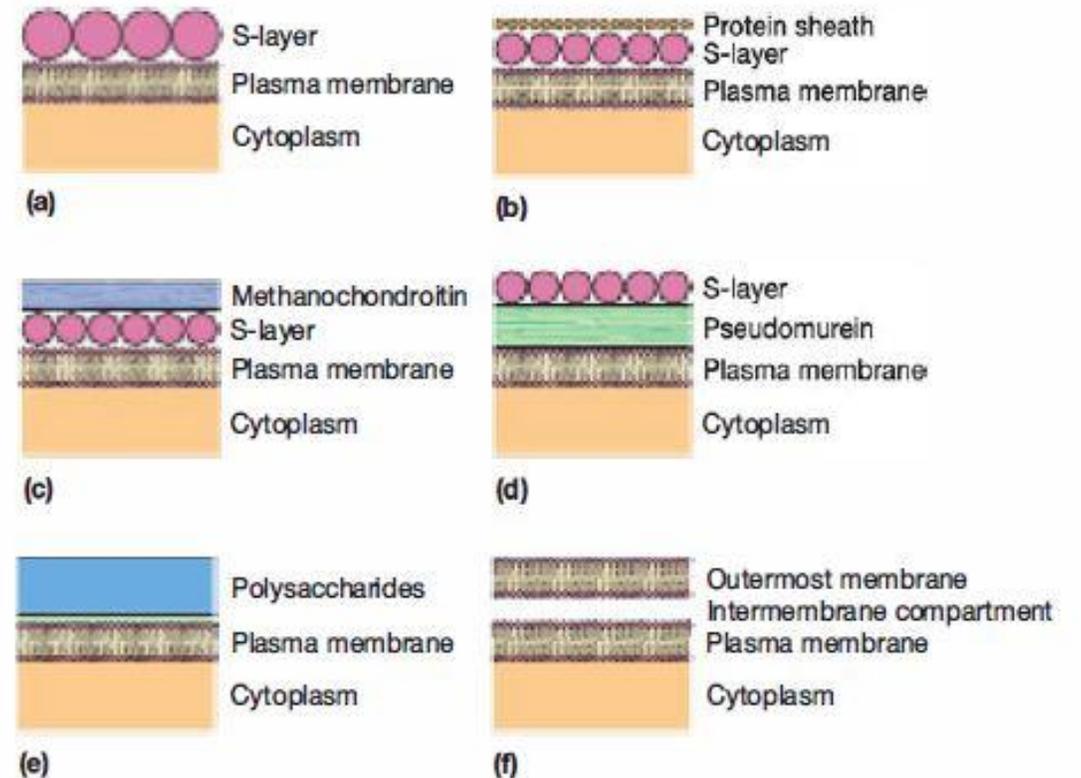


Figure 4.6 Archaeal Cell Envelopes. (a) *Methanococcus*, *Halobacterium*, *Pyrodictium*, *Sulfolobus*, and *Thermoproteus* cell envelopes. (b) *Methanospirillum* cell envelope. (c) *Methanosarcina* cell envelope. (d) *Methanothermus* and *Methanopyrus* cell envelopes. (e) *Methanobacterium*, *Methanosphaera*, *Methanobrevibacter*, *Halococcus*, and *Natronococcus* cell envelopes. For *Methanosphaera*, the polysaccharide layer is composed of pseudomurein. (f) *Ignicoccus* cell envelope. The outermost membrane contains protein complexes that form pores.

POVRŠINSKE STRUKTURE BAKTERIJSKIH STANICA

Čahura ili kapsula; glikokaliks

- tanja ili deblja sluzava naslaga na površini bakterijskih stanica
- kemijski sastav je različit u različitim vrsta bakterija: **polisaharidi**, **polipeptidi** ili jedno i drugo (karakterizacija čahure, npr. u reakciji sa specifičnim antitijelima, doprinosi identifikaciji bakterija)
- karakter čahure određuje izgled površine bakterijskih kolonija

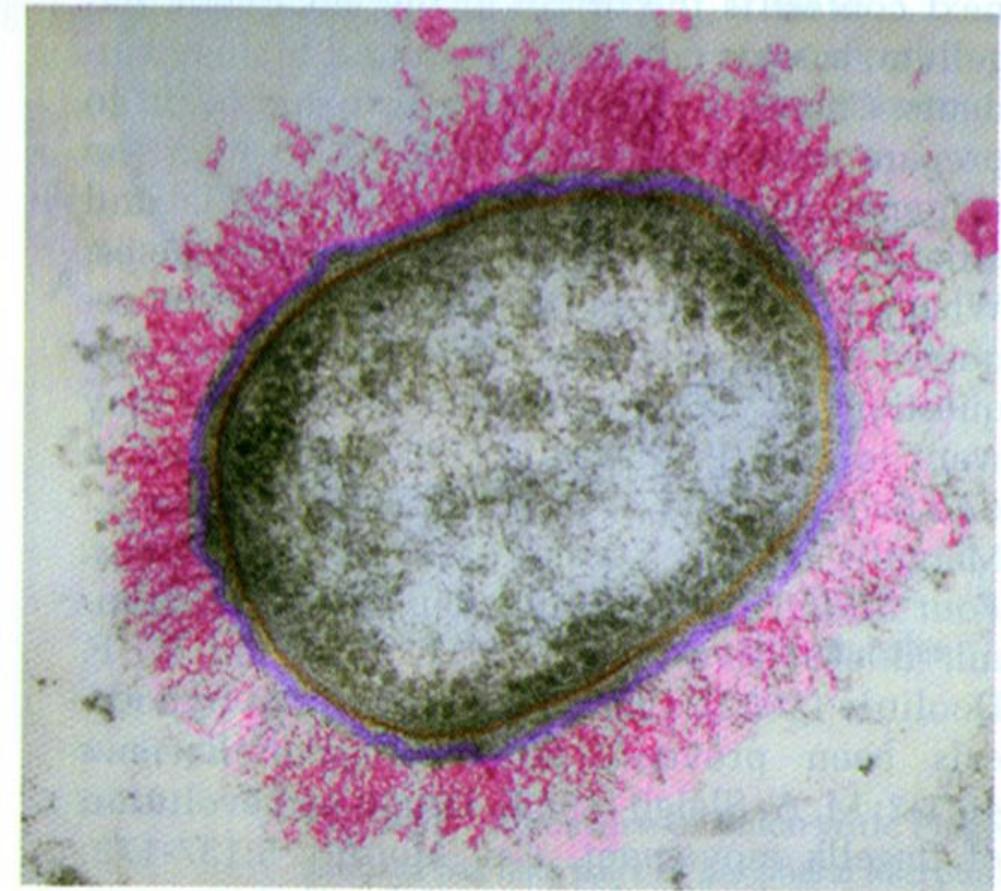
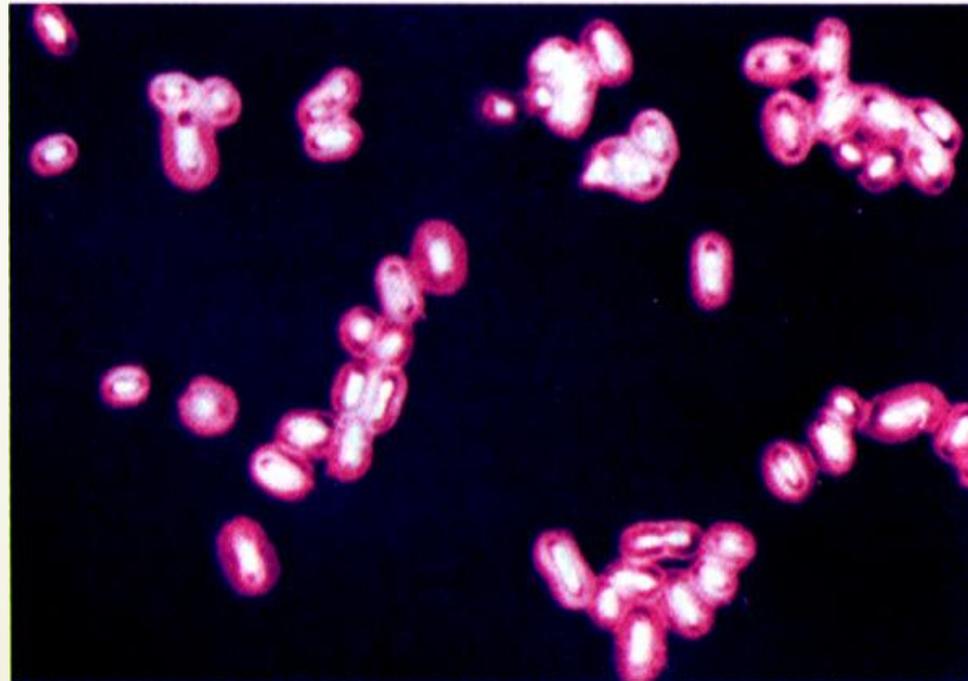


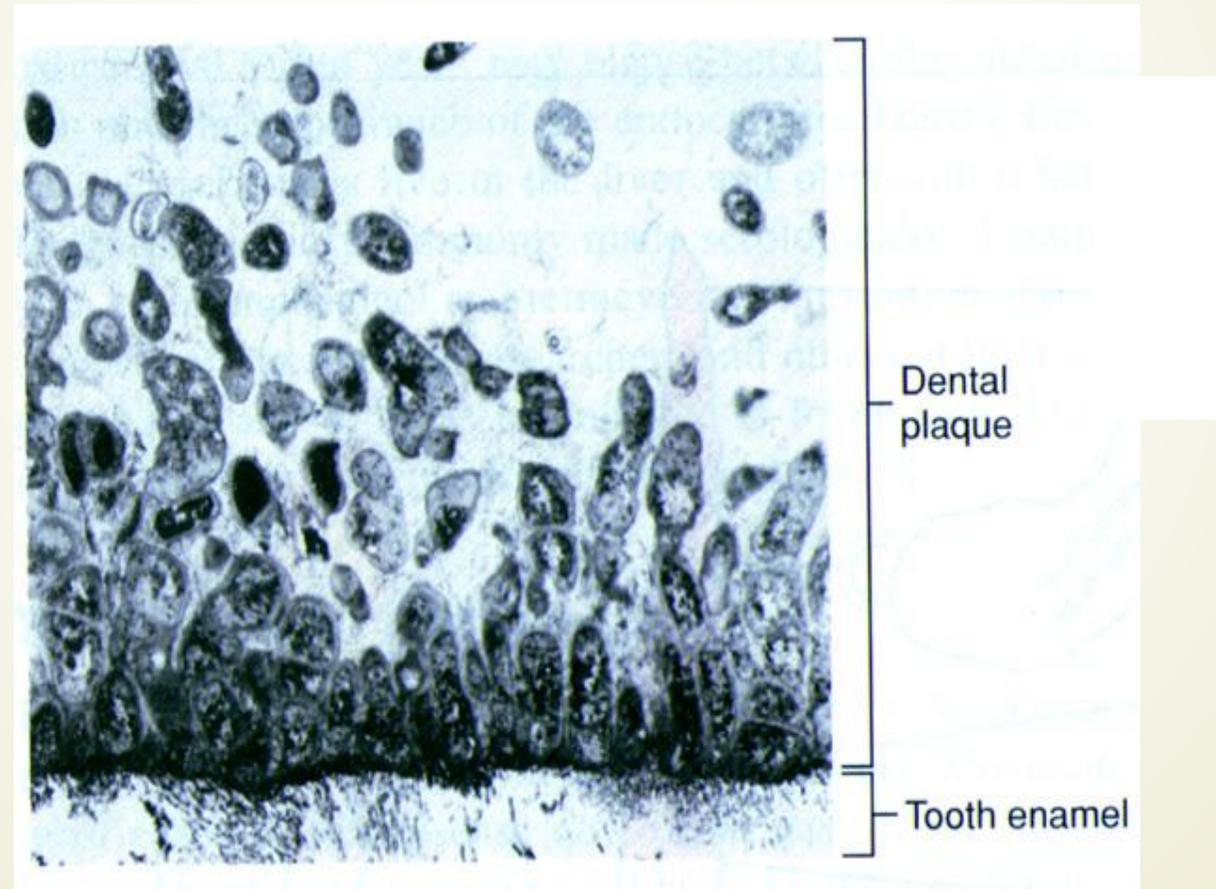
Fig. 3-53 Glycocalyx. Colorized micrograph of the glycocalyx (red) of a Gram-negative bacterium. (59,000 \times .)

- štiti stanicu od isušivanja, patogene bakterije zaštićuje od fagocitoze, omogućuje pričvršćenje na različite površine (i time preživljavanje u određenom okolišu), bakterije čini otpornijima na napad bakteriofaga
- mutante nekih bakterija, nesposobne stvarati čahuru, **ne mogu prouzročiti razvoj bolesti** (*Streptococcus pneumoniae*, *Clostridium perfringens*, *Bacillus anthracis*, *Haemophilus influenzae*)



Klebsiella pneumoniae

- neke bakterije stvaraju izrazito sluzavu kapsulu ako hranjiva podloga sadrži saharozu (*Leuconostoc mesenteroides*, *Streptococcus mutans*)
- *Streptococcus mutans* – veza između formiranja kapsule i predispozicija za nastanak karijesa



- **zubni plak** – biofilm - zajednica mikroba čije su stanice povezane međusobno i pričvršćene na neku biogenu ili abiogenu podlogu
- oko 1000 bakterijskih vrsta uključeno u stvaranje zubnog plaka
- rodovi *Streptococcus*, *Actinomyces*, *Lactobacillus*...
- vrsta *Streptococcus dentisani* – izolirana iz osoba bez pojave karijesa – baktericidno djelovanje na ostale bakterije plaka – potencijalni probiotik (**ACDC** klaster gena)

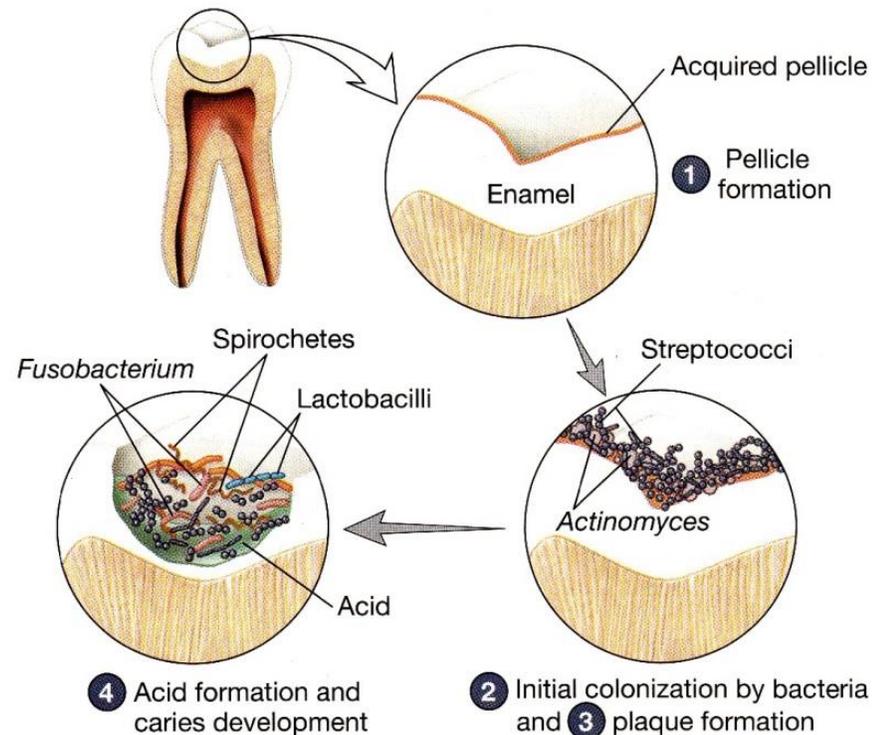


FIGURE 38.34 Stages in Plaque Development and Cariogenesis. A drawing of a microscopic view of pellicle and plaque formation, acidification, and destruction of tooth enamel.

- Formiranje biofilma - *quorum sensing*

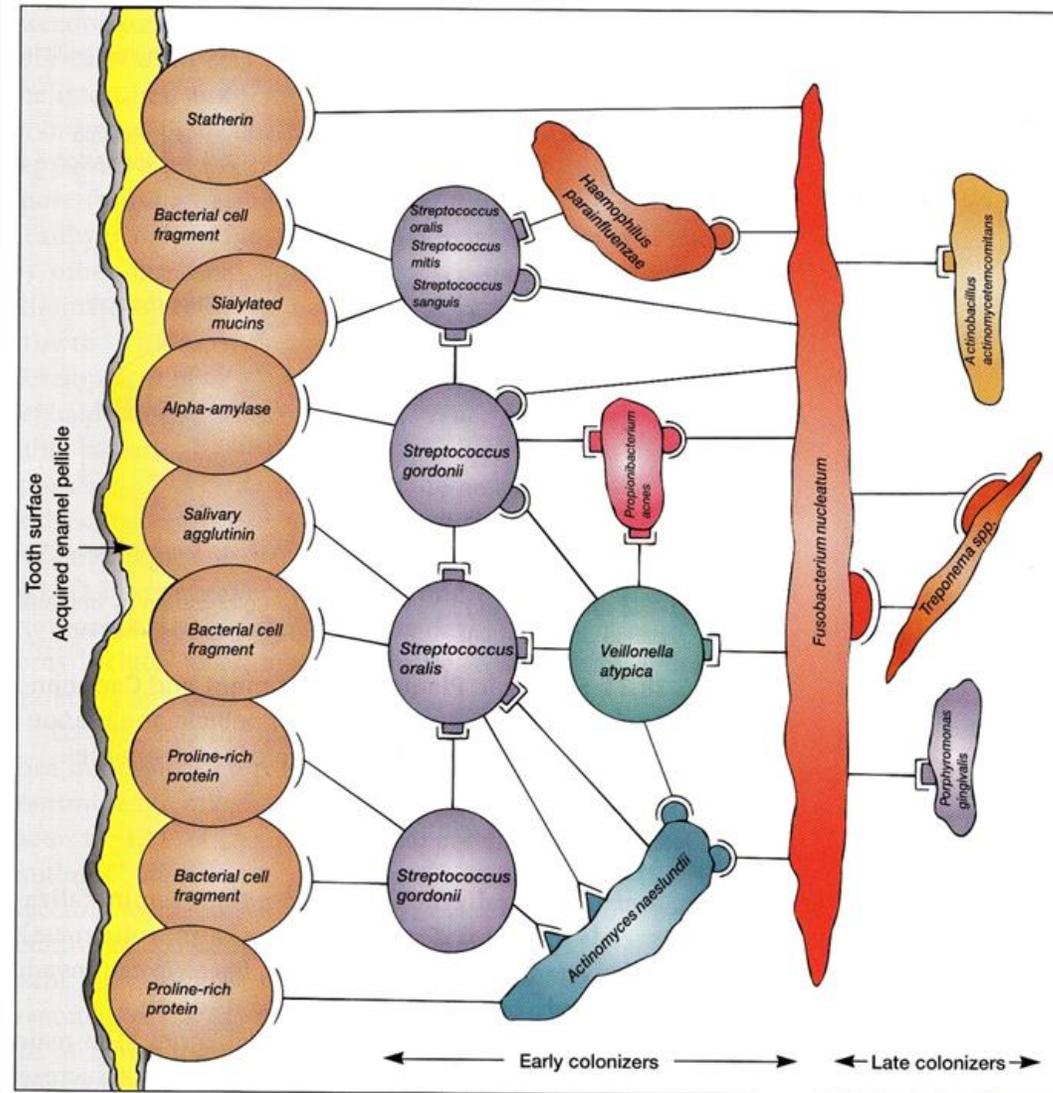
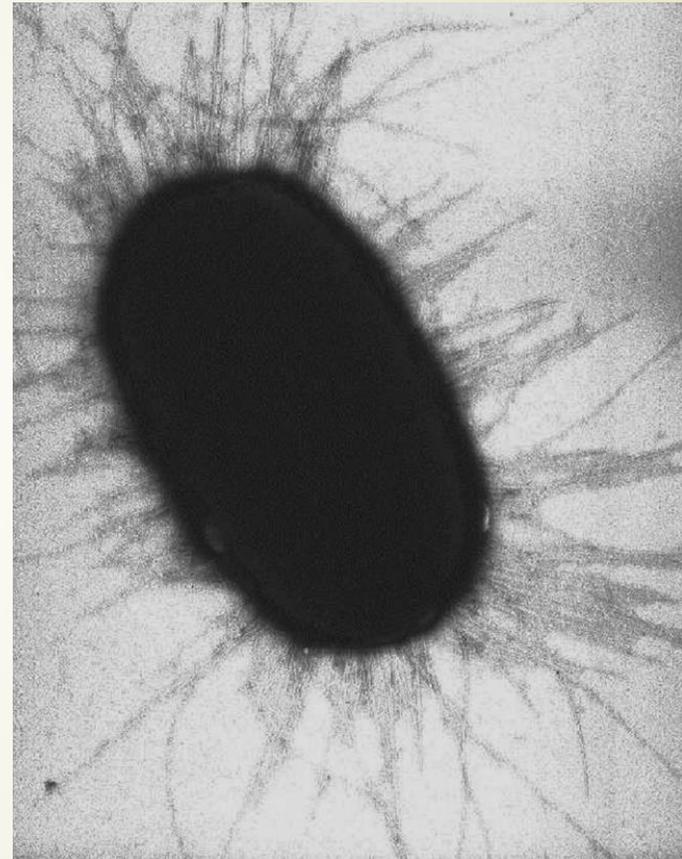


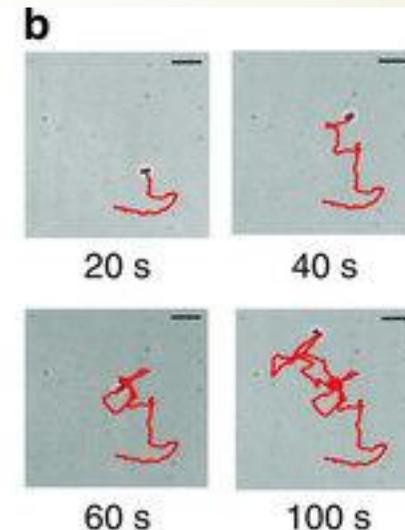
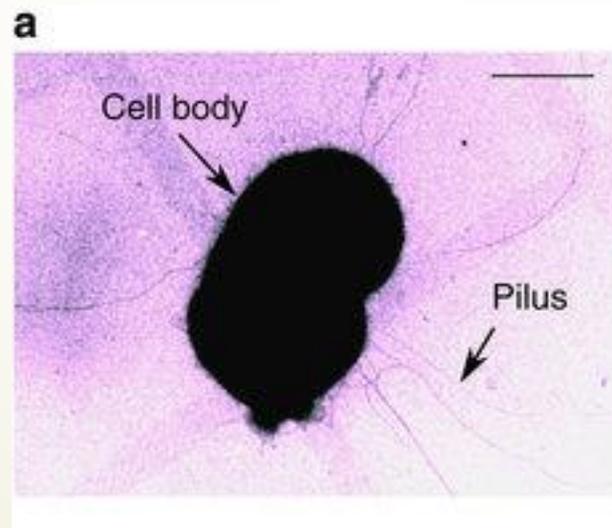
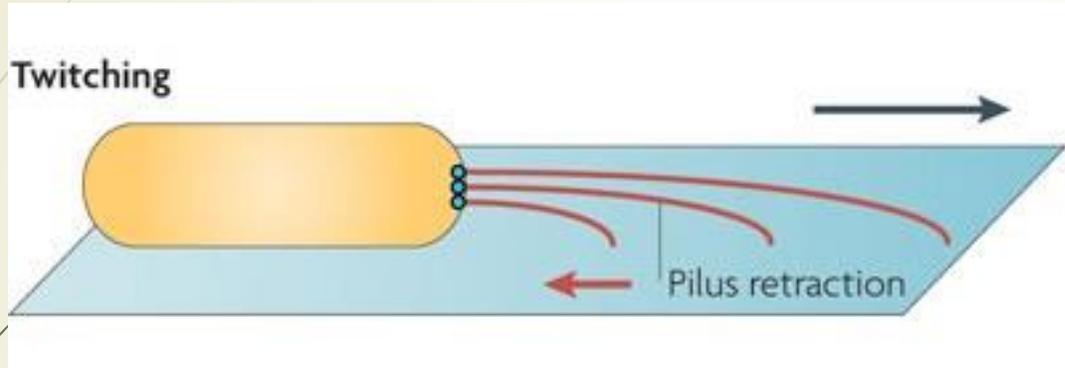
FIGURE 38.35 The Formation of Dental Plaque on a Freshly Cleaned Tooth Surface. The proposed temporal relationship of bacterial accumulation and multigeneric coaggregation during the formation of dental plaque on the acquired enamel pellicle. Early tooth surface colonizers coaggregate with each other, and late colonizers coaggregate with each other. With a few exceptions, early colonizers do not recognize late colonizers. After the tooth surface is covered with the earliest colonizers, each newly added bacterium becomes a new surface for recognition by unattached bacteria.

FIMBRIJE (lat. *fimbria* – vlakno) I PILI (lat. *pilus* – dlaka)

- manje nitaste tvorbe prisutne u većem broju na površini stanice (češće u Gram-negativnih bakterija)
- **fimbrije** – kraće, **3-10 nm**; **pili** – dulji, **9-10 nm**
- građeni su poput bičeva, ali od druge vrste proteina (**fibrilin**, odnosno **pilin**)
- fimbrije i “obični” pili služe prihvaćanju na površinu drugih stanica (npr. na membranu stanica sluznice)
- **spolni** ili **seksualni pili** omogućuju bakterijsku konjugaciju



- **pili tipa IV** – odgovorni za gibanje trzanjem (*twitching motility*) – *Pseudomonas aeruginosa*, *Myxococcus xanthus*, *Neisseria gonorrhoeae*



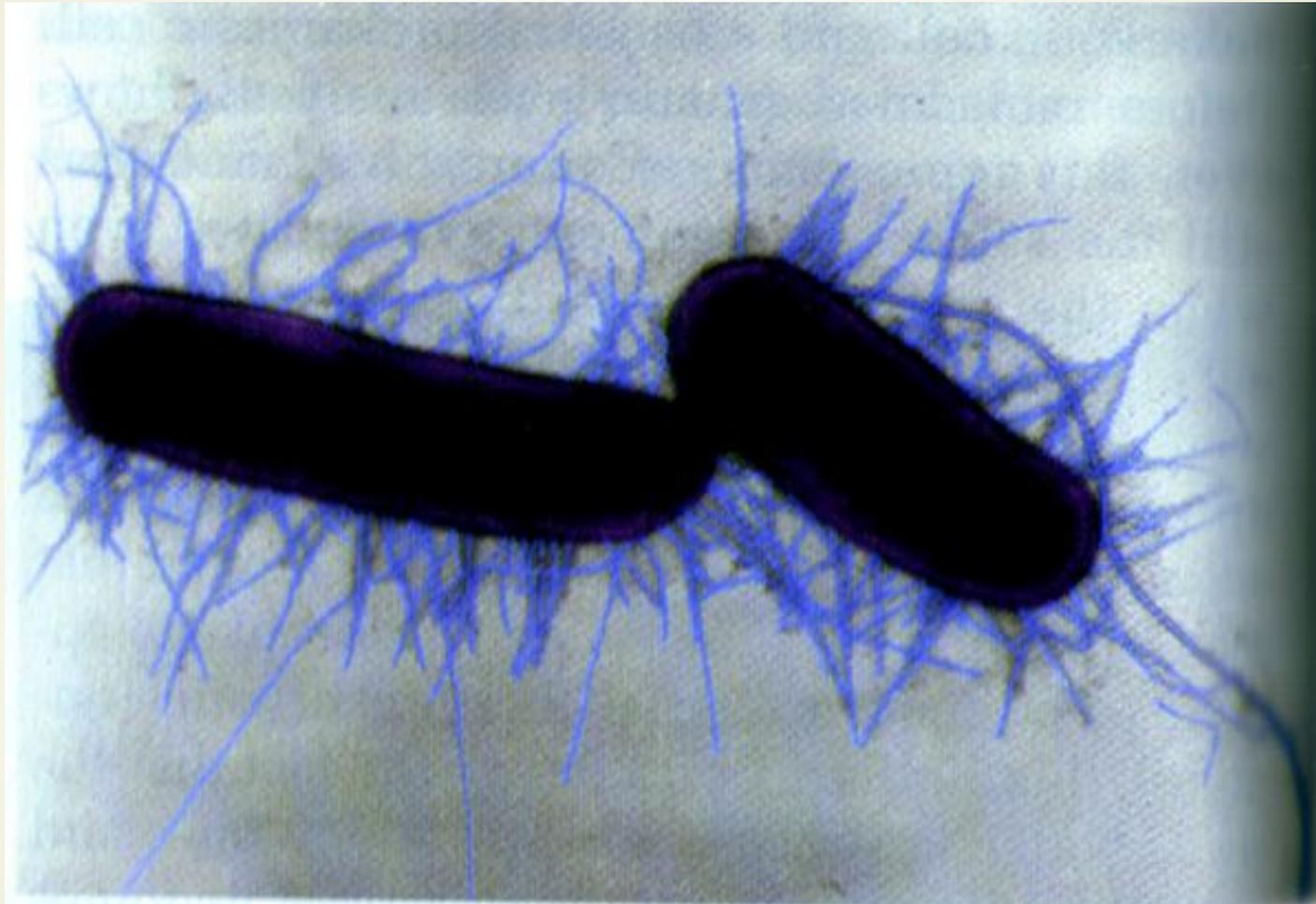
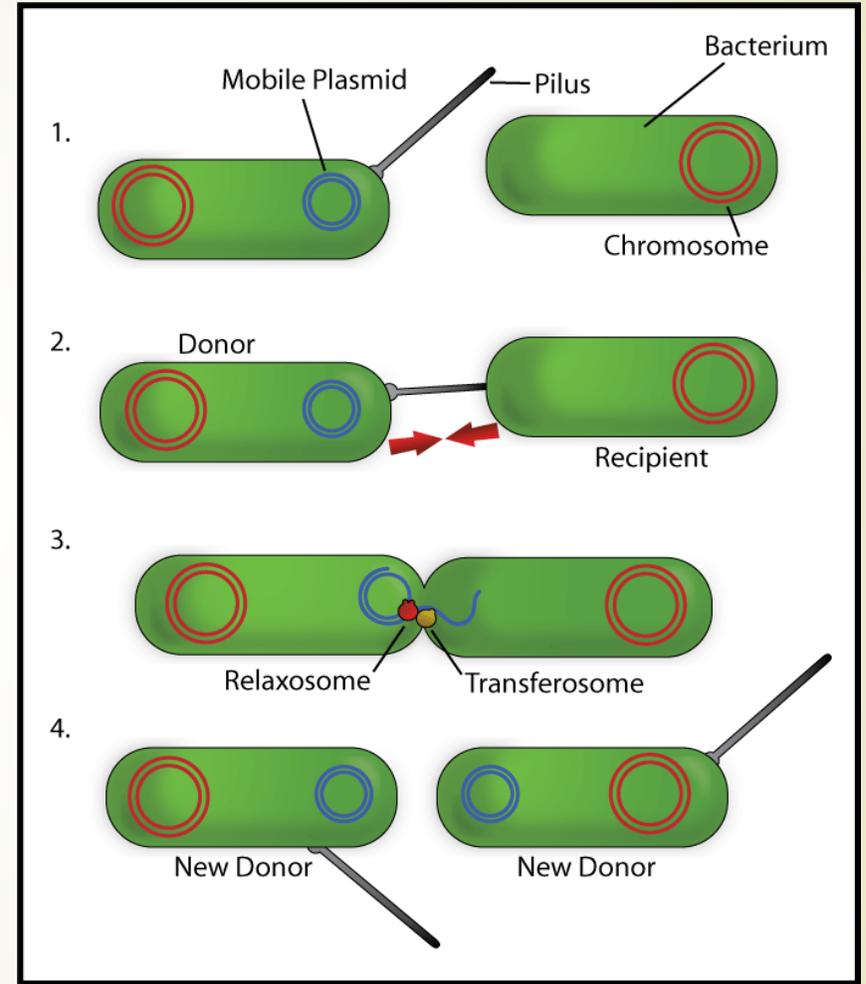
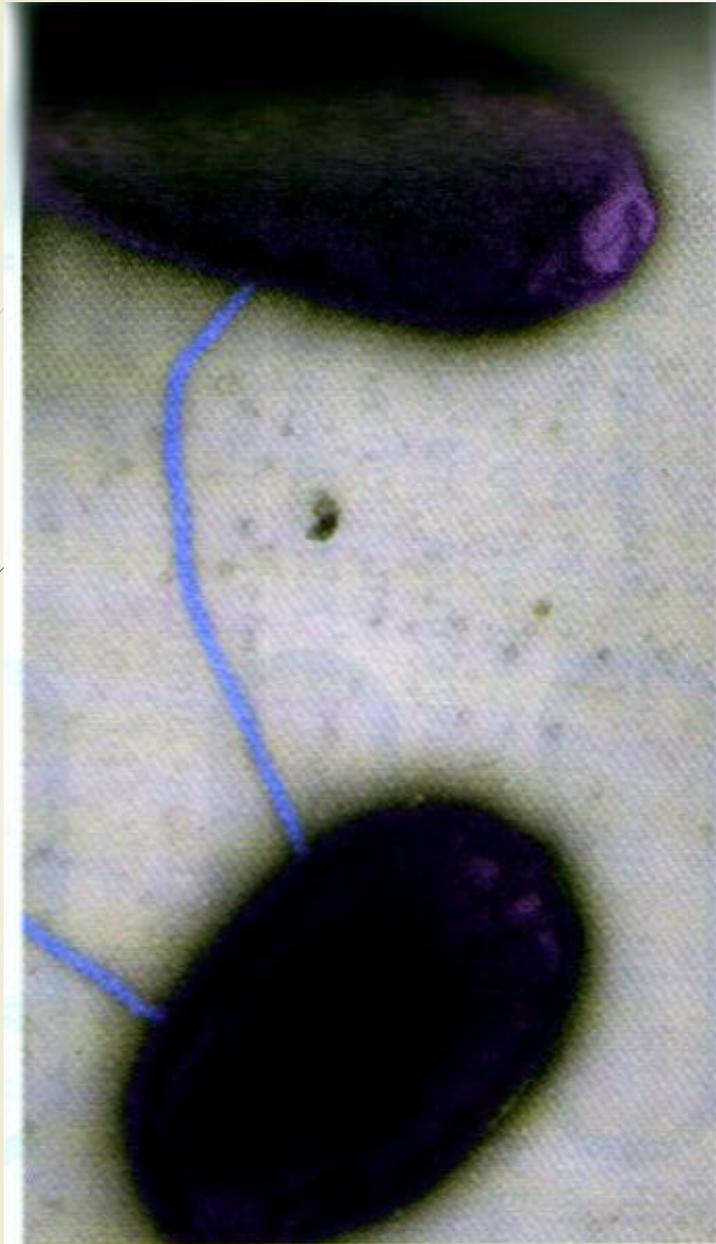
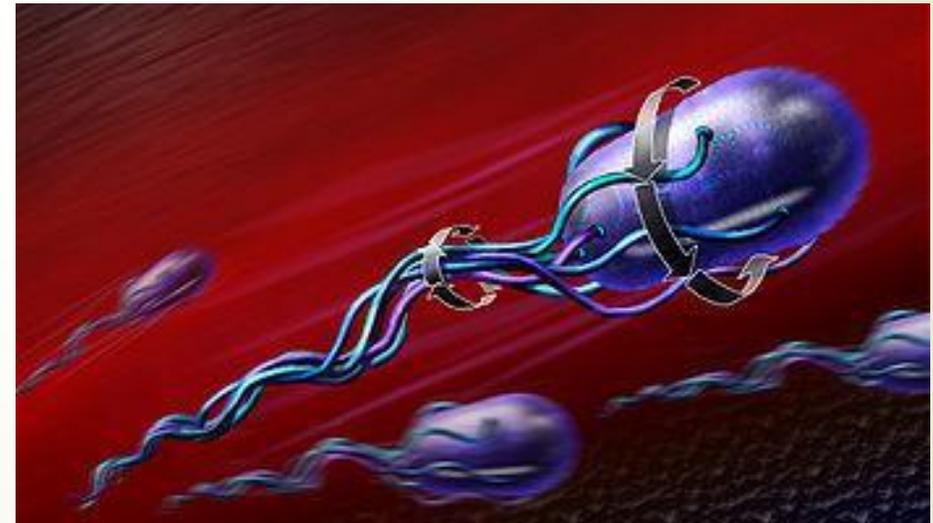
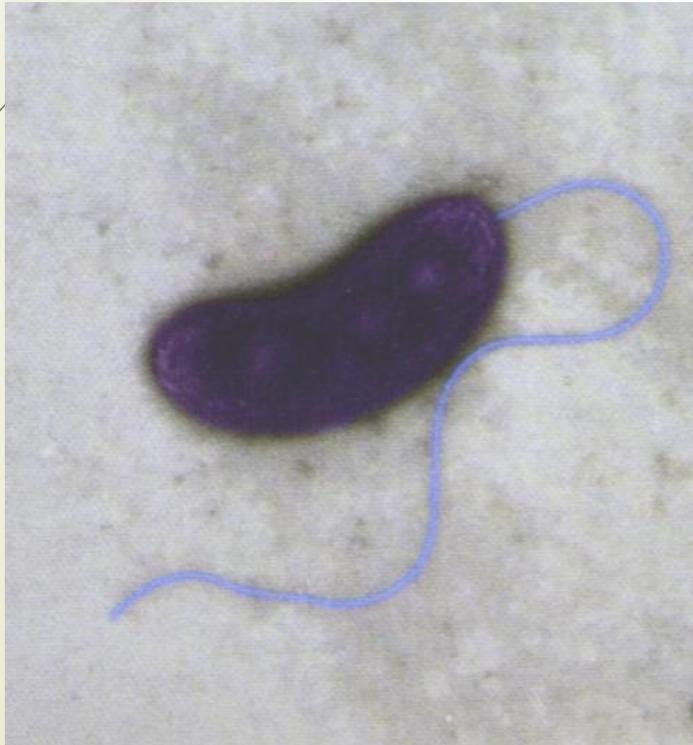


Fig. 3-54 Pili and Fimbriae.



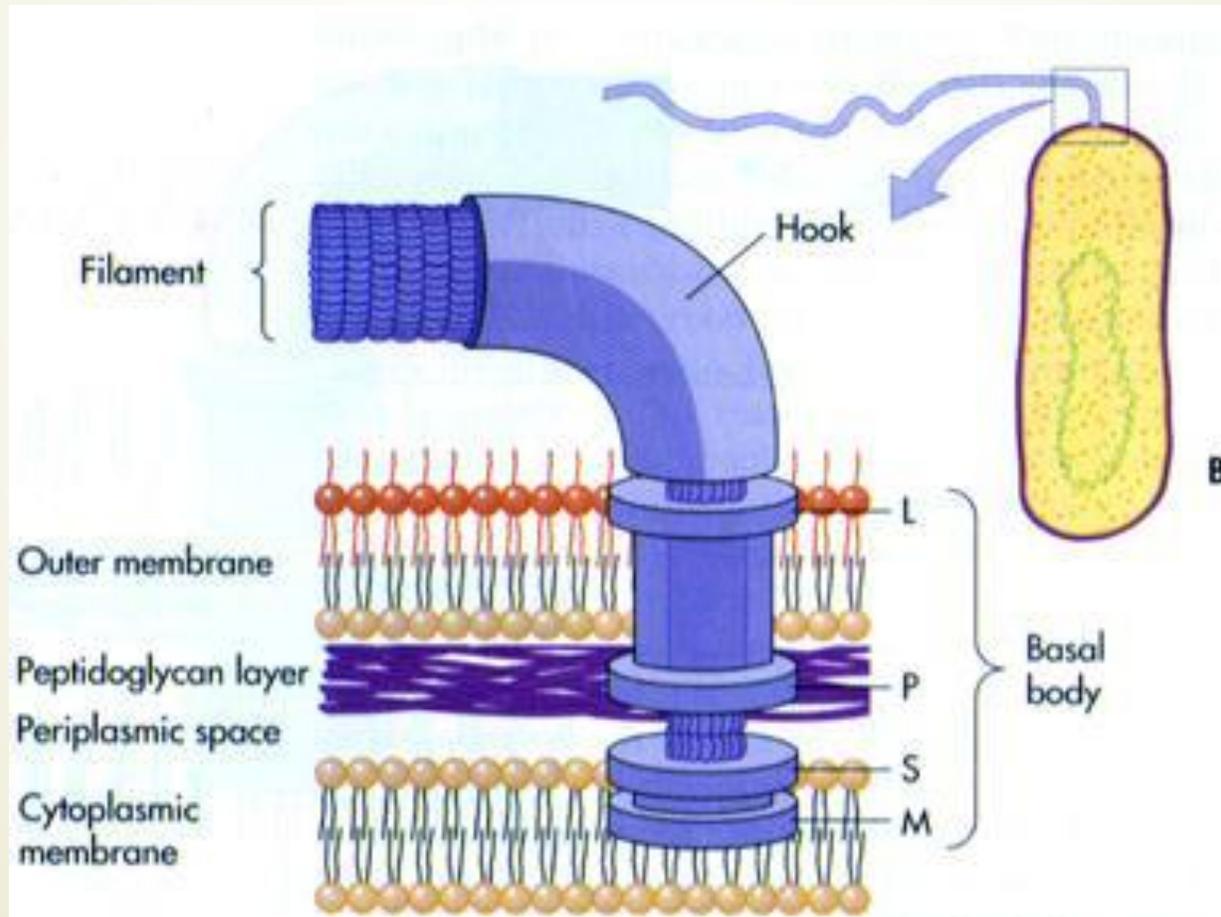
Bičevi ili flagele

- filamentozne tvorbe na površini bakterijskih stanica promjera oko 20 nm, te duljine 15 do 20 μm
- uočavanje bičeva: elektronskim mikroskopom, svjetlosnim mikroskopom nakon impregnacije srebrnim solima



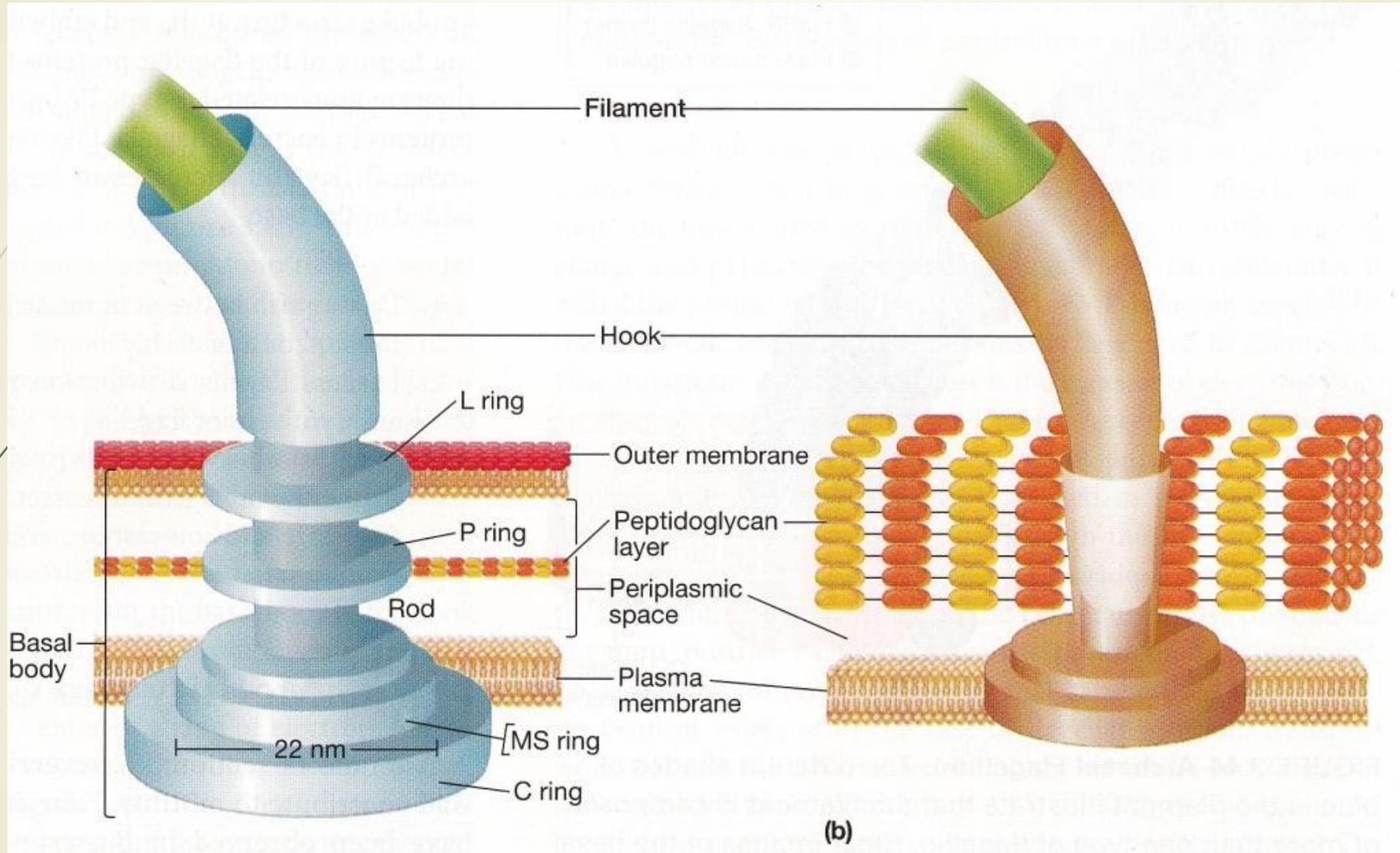
Građa biča

- bazalno tijelo učvršćuje bič na staničnu stijenku i plazma-membranu
- filament je izgrađen od mnogo lanaca **proteina flagelina** isprepletenih oko središnje šupljine (stalnog je promjera, nije obavijen membranom)



Gram-negativna bakterija

Gram-pozitivna bakterija



građa biča arheja

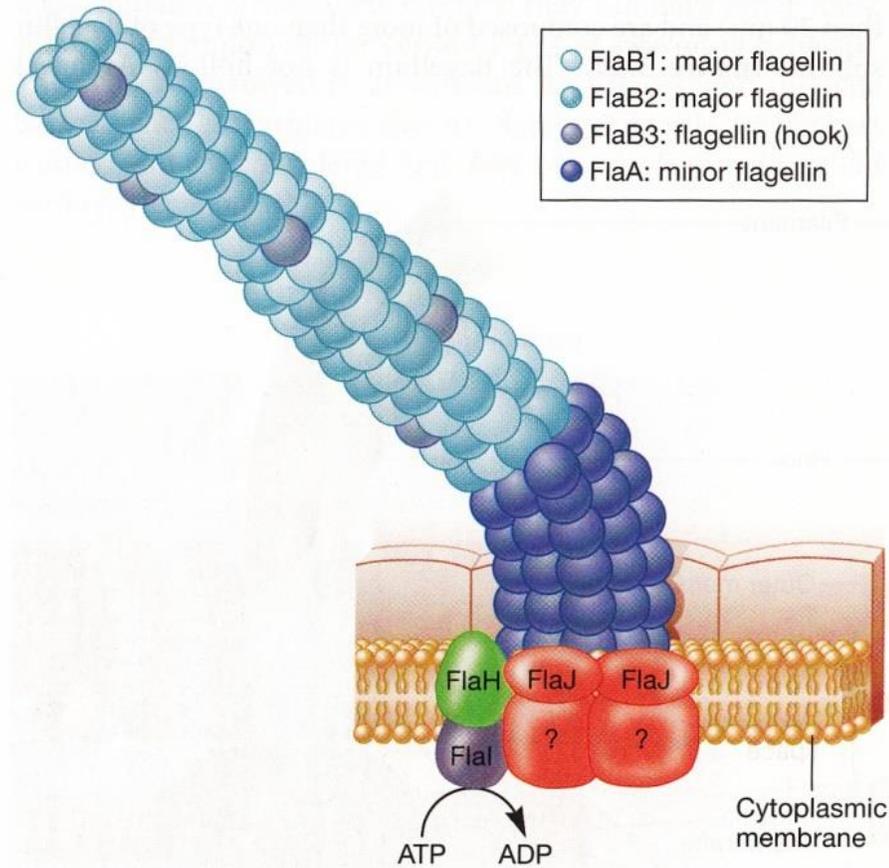


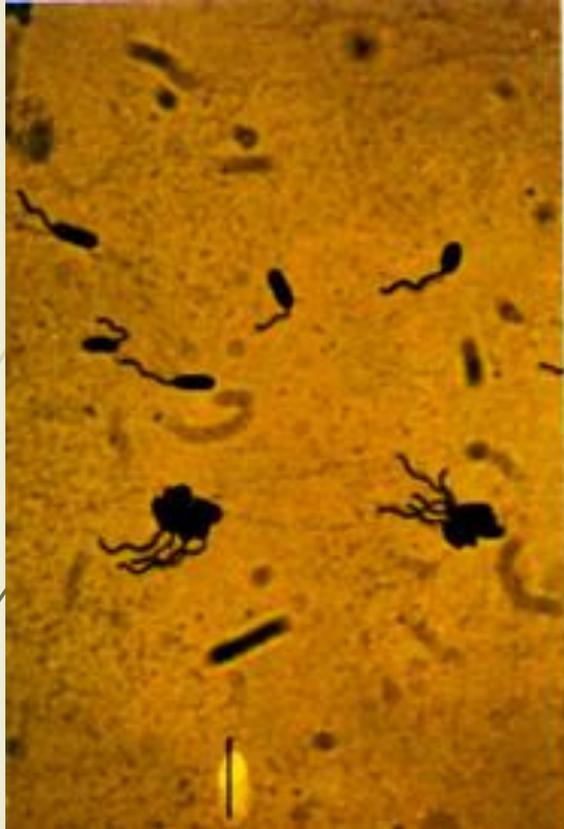
FIGURE 3.44 Archaeal Flagellum. The different shades of blue in the filament illustrate that the filament is composed of more than one type of flagellin. Clear images of the basal body have not been obtained, although some electron micrographs show a knob at the cell end of the flagellum, as illustrated here.



Prema rasporedu i broju bičeva razlikujemo četiri tipa bakterijskih stanica:

- **monotrih** – jedan bič
- **lofotrih** – nakupina bičeva s jedne ili obje strane stanice
- **amfitrih** – po jedan bič na svakom polu stanice
- **peritrih** – više bičeva raspoređenih jednoliko po cijeloj površini bakterijske stanice

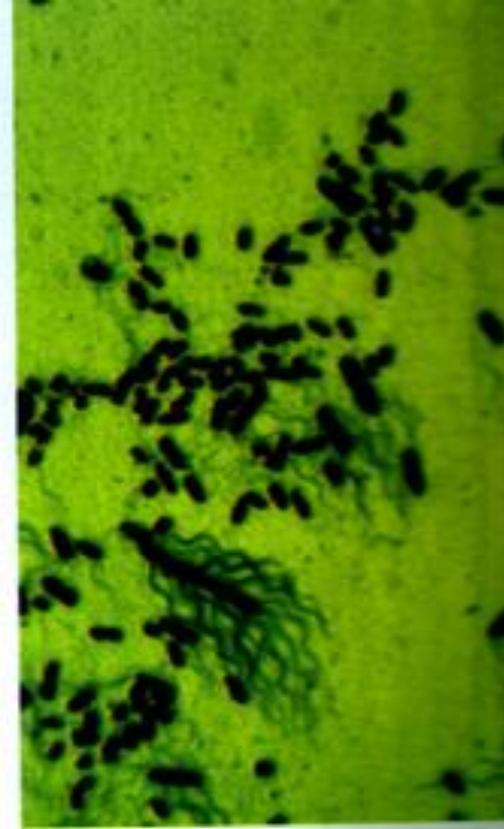
- raspored i broj bičeva obilježje su pojedinih bakterijskih vrsta



monotrih
(*Pseudomonas*)

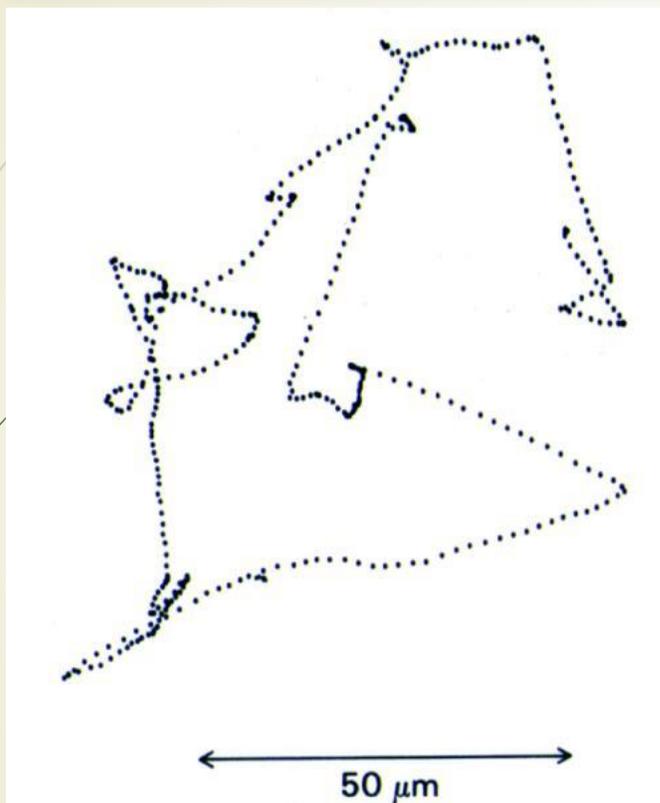


lofotrih
(*Spirillum*)



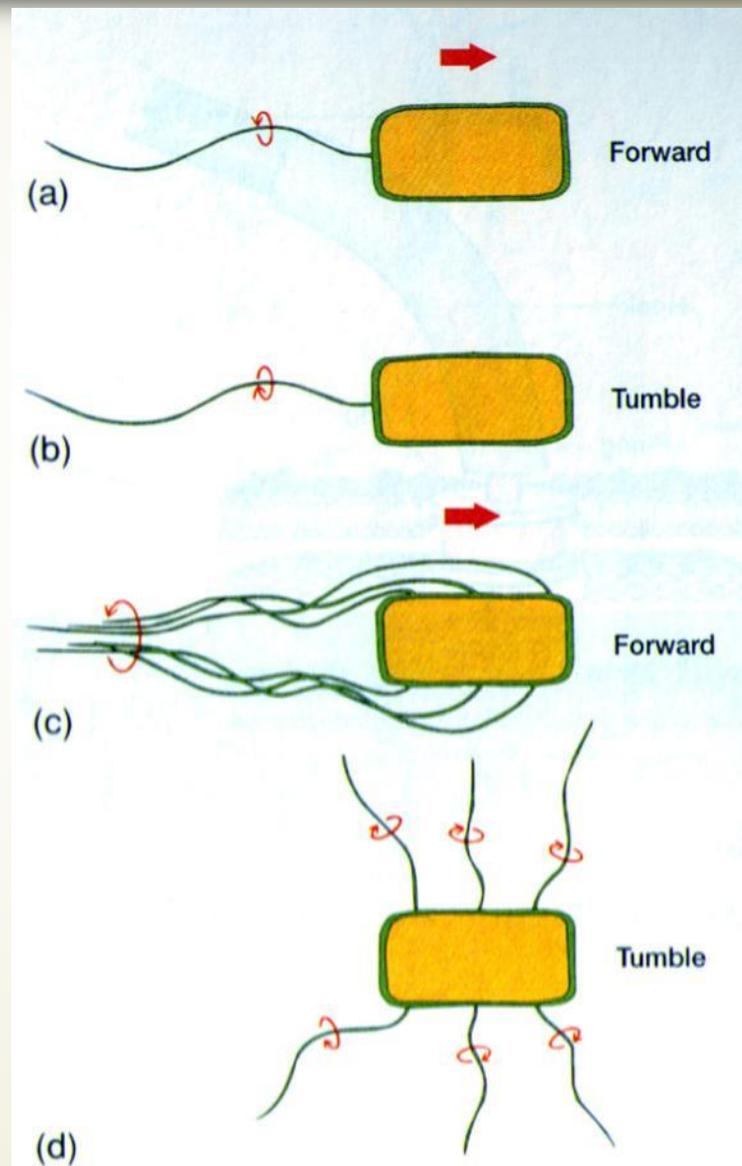
peritrih
(*Proteus vulgaris*)

Bičevi služe **pokretanju** bakterijskih stanica: rotacijom bazalnog tijela okreće se poluukočeni filament i potiskuje stanicu kroz tekući medij



Slika 37.41.
Snimka traga bakterije *E. coli* dobivenog mikroskopom koji automatski slijedi kretanje bakterije u tri dimenzije. Vremenski razmaci snimljenih točaka iznose 80 ms (iz: H.C. Berg, *Nature* 254/1975/:390).

Brzina gibanja
20-90 μm/sekundi





Slika I-24. Štapićasta bakterija *Salmonella typhi* [*Salmonella tifi*] tijekom diobe, s otprilike 12 bičeva (dulje tvorbe) i više od 100 fimbrija (snimljeno elektronskim mikroskopom, povećano 16 000 puta)

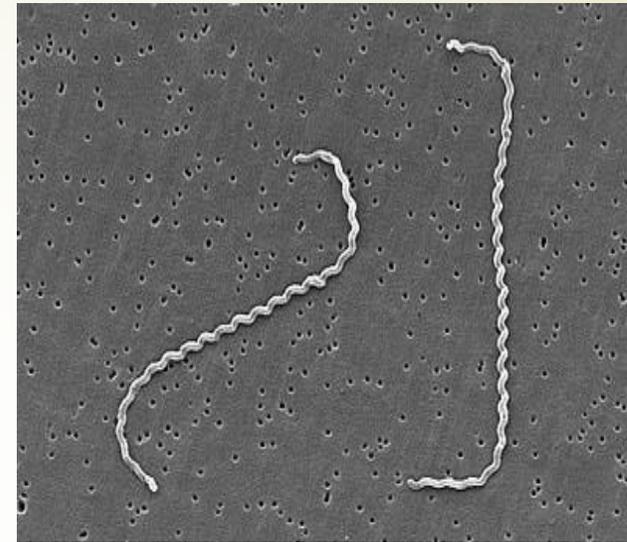
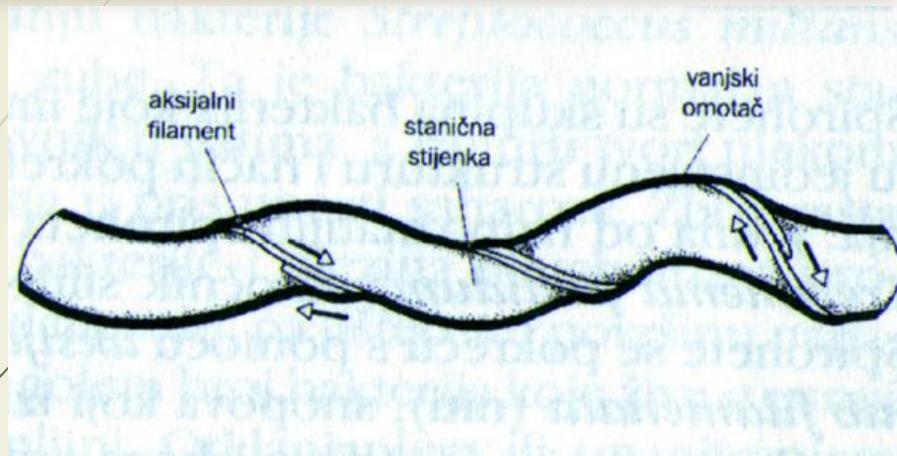
<https://www.youtube.com/watch?v=4hexn-DtSt4>

<https://www.youtube.com/watch?v=BgDdkTKjM0>

<https://www.youtube.com/watch?v=MNR48hUd-Hw>

Ostali oblici gibanja bakterija različiti od flagelarnog

AKSIJALNI FILAMENTI

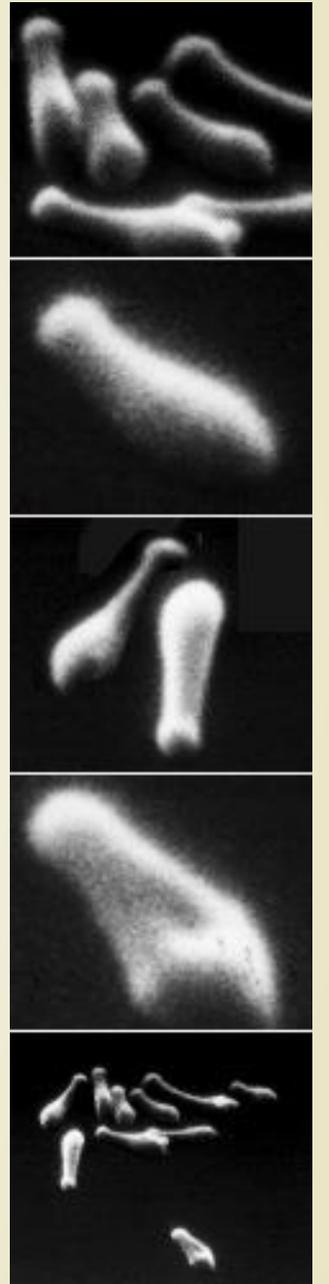
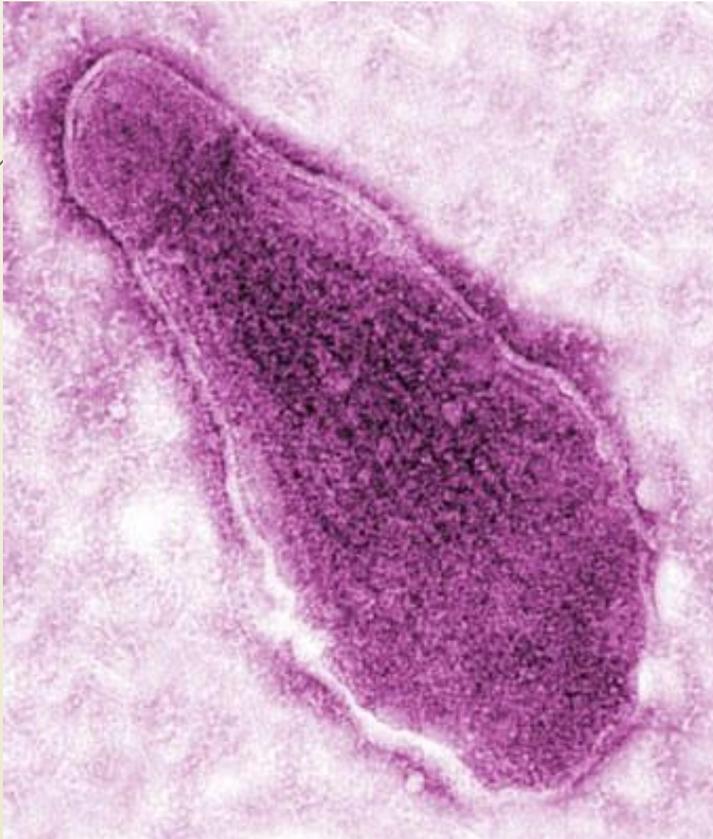


Leptospira interrogans

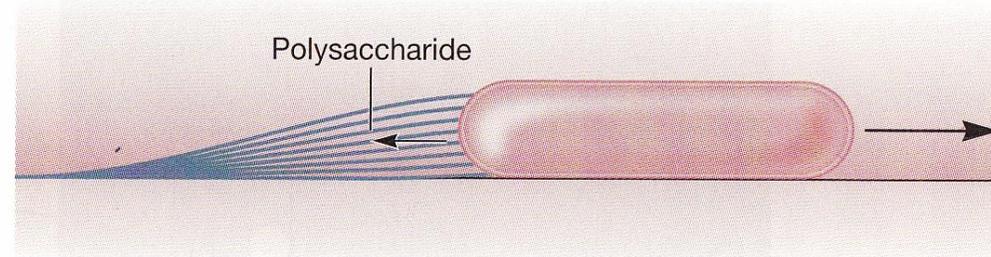
- nitaste tvorbe koje služe pokretanju **spiroheta**
- pružaju se duž bakterijske stanice, između peptidoglikanskog sloja stijenke i vanjske ovojnice
- struktura im nalikuje strukturi biča
- rotacija filameta uzrokuje rotaciju ukočene spiralne stanice u suprotnom smjeru pa se bakterija pokreće poput svrdla kroz viskozni medij

GIBANJE KLIZANJEM (“GLIDING MOTILITY”)

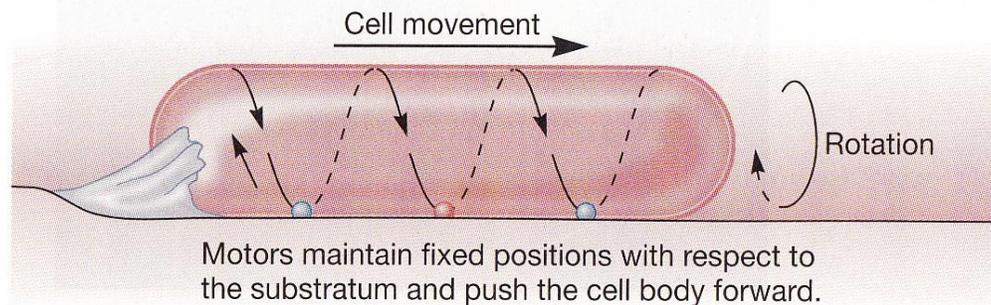
- nema vidljivih vanjskih struktura koje omogućuju ovaj tip gibanja
- mehanizam nije u potpunosti poznat
- neke **cijanobakterije, miksobakterije, citofage i neke mikoplazme** (*Mycoplasma mobile*)



- mogući mehanizmi putem sekrecije polisaharida



(a)



(b)

FIGURE 3.48 Possible Mechanisms for the Adventurous Motility of *Myxococcus xanthus*. (a) Polysaccharide secretion model. (b) Focal adhesion model.

GIBANJE TRZANJEM (“TWITCHING MOTILITY”)

- istežanje i povlačenje **pila tipa IV**
- *Pseudomonas aeruginosa*, *Myxococcus xanthus*, *Neisseria gonorrhoeae*

<http://www.youtube.com/watch?v=yGMSQNBDq48>

