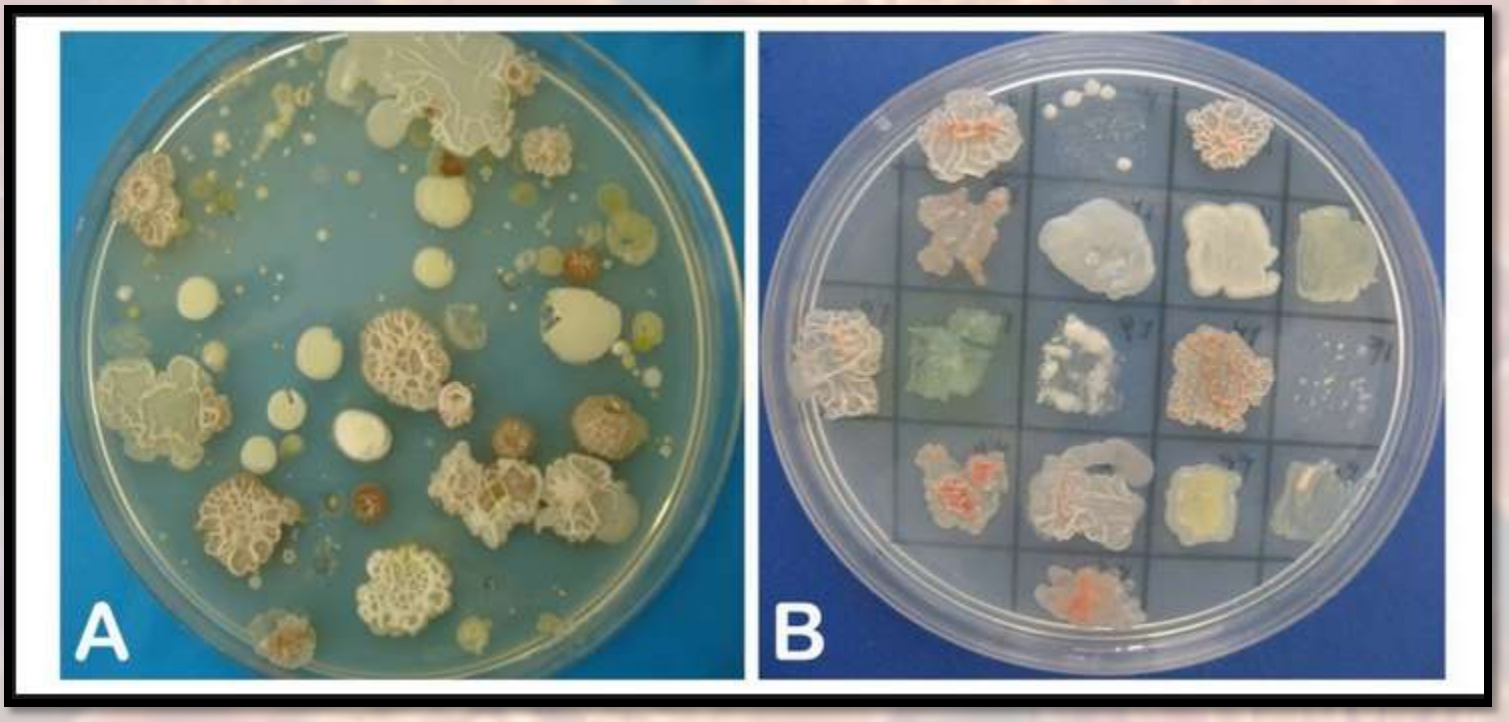


ISOLASI & IDENTIFIKASI MIKROBIA

ISOLASI & IDENTIFIKASI MIKROBIA

A. Isolasi & kultivasi kultur murni
B. Karakterisasi mikroorganisme
C. Penyimpanan kultur murni

A. Isolasi & kultivasi kultur murni (1)

Mikrobia di alam = kultur campuran

Kondisi inkubasi

Macam spesies

ISOLASI

Kultur Murni

Populasi mikrobia yg sama berasal dr sel induk yg sama

Pemilihan media

1.Macam spesies (yg diduga atau yg diinginkan)
2.Asal sampel
3.Kebutuhan nutrisi mo. tsb

Bacterial colonies streaked on media from a Goss' wilt-infected corn leaf that illustrate the methodology for separation and identification of multiple pathogens present in bacterial-infected leaves

http://www.apsnet.org/publications/apsnetfeatures/Pages/bacterium.aspx

1. Preparasi sampel

- Macam sampel
  - Padat, cair, beku, semipadat, dll
- Preparasi :
  - Sampel dihomogenkan (blender, stomacher, dll)
  - Lakukan pengenceran (pepton water 0,1 % atau NaCl 0,85 %)

sampel terdokumentasi informasinya lengkap dan tersimpan memadai

2. Preparasi media

- Macam media isolasi
  - Media padat : Komposisi bervariasi tergantung macam isolat yang diinginkan
  - Media cair : media selektif/ diferensial
  - Media enrichment : untuk memperbanyak jumlah isolat

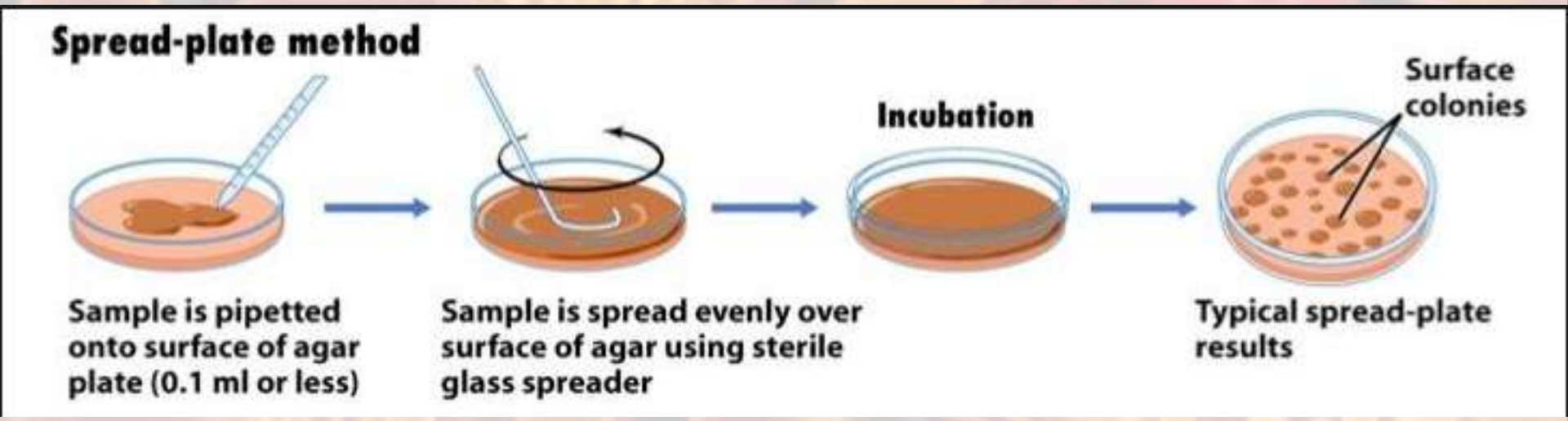
- Media penyimpanan (sementara):
  - Media padat (agar miring)

3. Metoda inokulasi

a. Spread plate
b. Pour plate
c. Streak plate
d. Direct plating

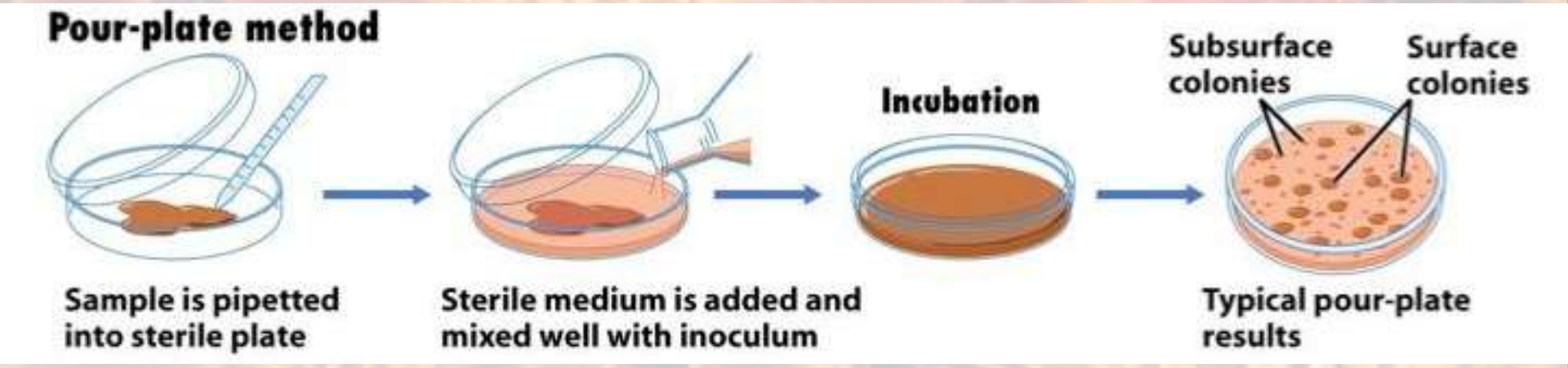
Spread plate

- meratakan inokulum (sampel) pada permukaan media agar dengan driglaski



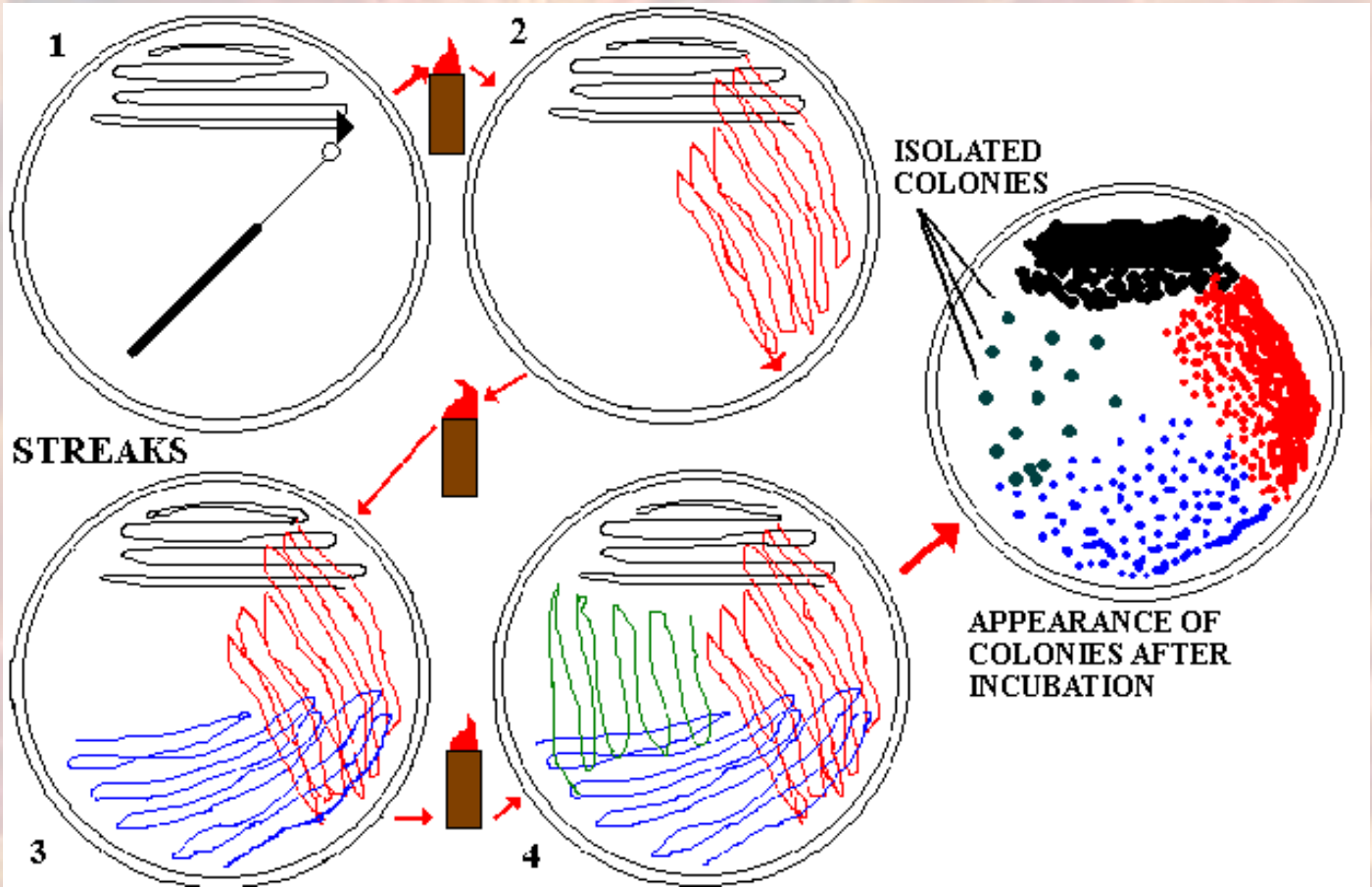
Pour plate

- inokulum dicampur ke dalam media agar yang masih mencair (45 °C), kemudian dituangkan ke dalam petri



Streak plate

- Inokulum digoreskan pada permukaan medium agar dengan ose



Direct plating

- Sampel (padat) langsung ditanamkan pada permukaan medium agar

- Koloni tumbuh di permukaan media → streak & spread plate.
- Koloni tumbuh di permukaan bahan → direct plating
- Koloni tumbuh dalam media → pour plate

Isolasi berdasarkan perbedaan kenampakan, warna, bentuk koloni dg streak plate pd media penyimpan (agar miring)

4. Metoda isolasi = metoda aseptik

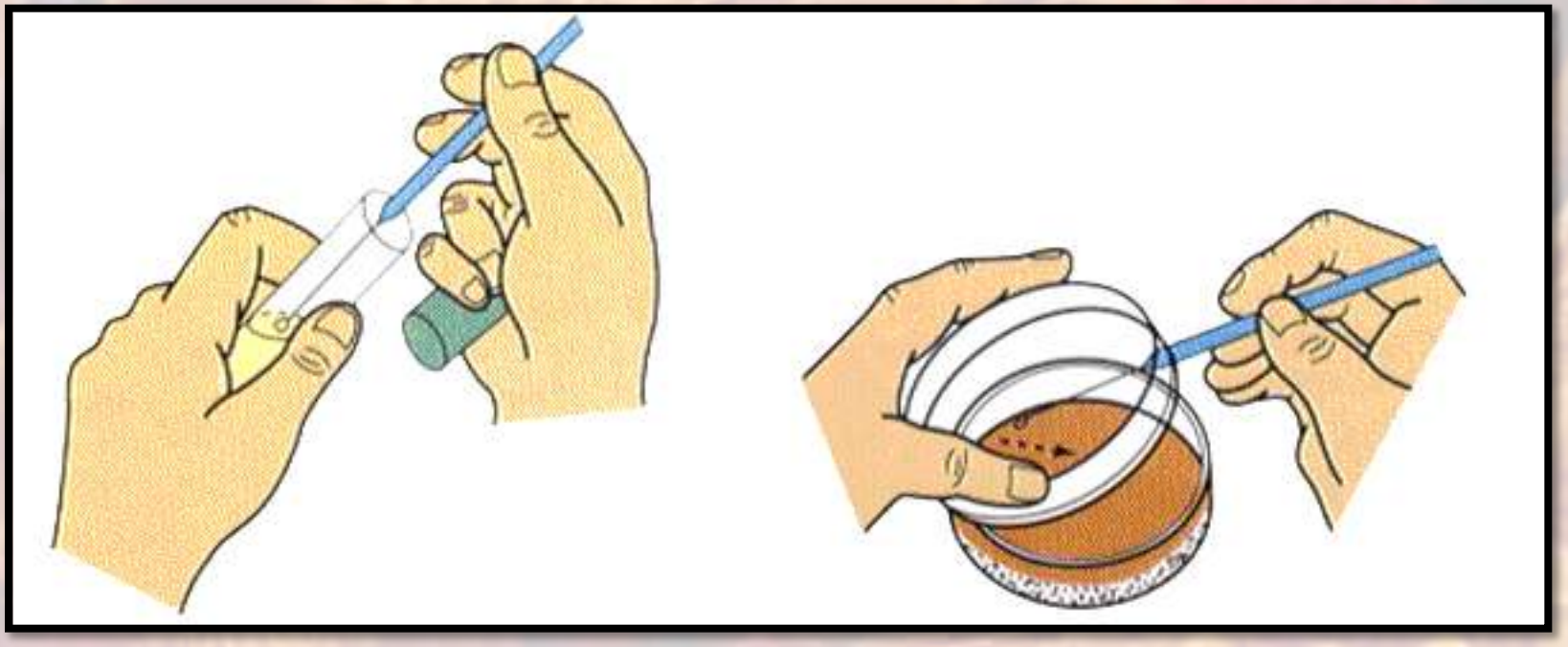
- Yaitu teknik untuk menjaga agar tidak terjadi kontaminasi selama penanganan kultur

  Caranya :

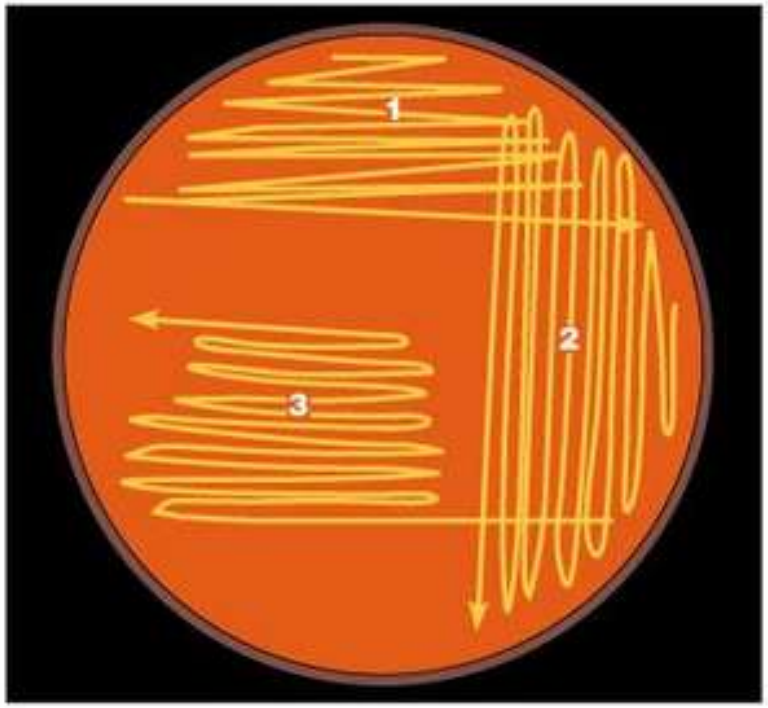
  - Lakukan pemindahan kultur pada ruangan dan meja yang bersih
  - Pemindahan kultur dengan ose, harus dilakukan pemanasan ose diatas api
  - Sebelum dan sesudah pemindahan, ujung wadah (plate / gelas reaksi) di panaskan diatas api

Metoda kultur murni

Isolasi kultur murni dengan *plating* (*streak plate*)



Cara menggores pada teknik kultur murni



(a) The direction of streaking is indicated by arrows. Streak series 1 is made from the original bacterial culture. The inoculating loop is sterilized following each streak series. In series 2 and 3, the loop picks up bacteria from the previous series, diluting the number of cells each time. There are numerous variants of such patterns.

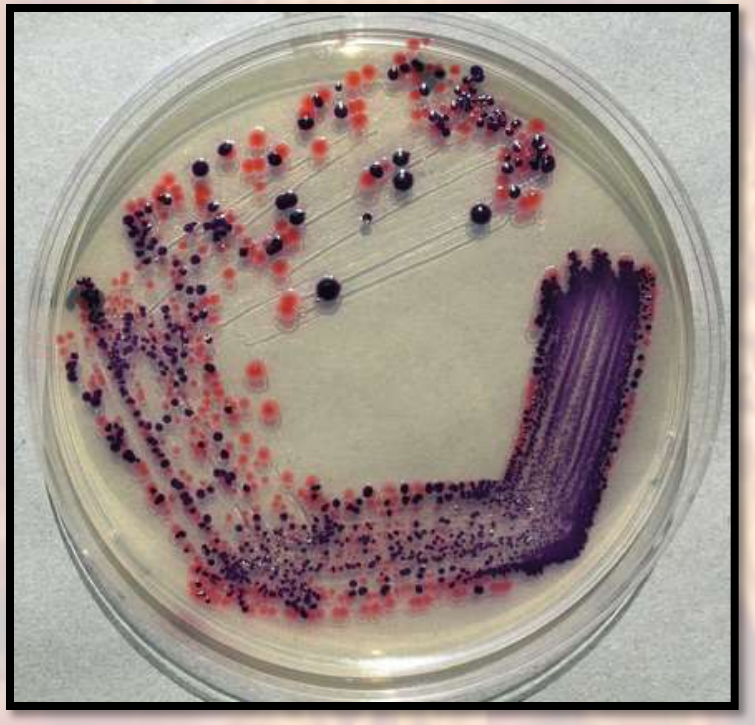
(b) In series 3 of this example, notice that well-isolated colonies of bacteria of two different types, red and white, have been obtained.

Mikrobia yang berbeda mempunyai kenampakan koloni yang berbeda

Penyimpanan isolat

- Penyimpanan sementara → suhu 4-10 °C (refrigerator)
- Penyimpanan lama → kering beku (liofilisasi), (-196) °C (N2 cair) atau freezer (-70)sd (-120) °C

5. Kultivasi



- Koloni yang tumbuh tergantung
  1. Macam media
  2. Kondisi inkubasi (suhu, oksigen, dll)

     ➔ tergantung jenis isolat yang diinginkan

- Lama inkubasi tergantung macam mikrobia

http://intranet.tdmu.edu.ua/data/kafedra/internal/micbio/classes_stud/en/stomat/ptn/Microbiology,%20virology%20and%20immunology/2/Lesson_03_The%20main%20methods%20and%20principles%20of%20pure%20cultures%20isolation.htm

Cara mempelajari mikrobia

1. **Pengamatan langsung**
   Mikroskop, macamnya :
   - **Light microscope**
   - **Bright field [warna, pengecatan]**
   - **Dark field**
   - **Phase contrast**
   - **Fluorescence**
   - **Electron microscope**
2. **Pengamatan kultur**
   - **Sifat pertumbuhan [aerobik?]**
   - **Kebutuhan metabolit**
3. **Molekuler**
   - **Test genetik**
   - **Hibridisasi Southern blot**
   - **PCR/analisa sekuens**
   - **Analisa protein (SDS-PAGE)**
4. **Diaknosa tidak langsung & pertumbuhan *in vivo***
   - **Serologi & inokulasi pada binatang**

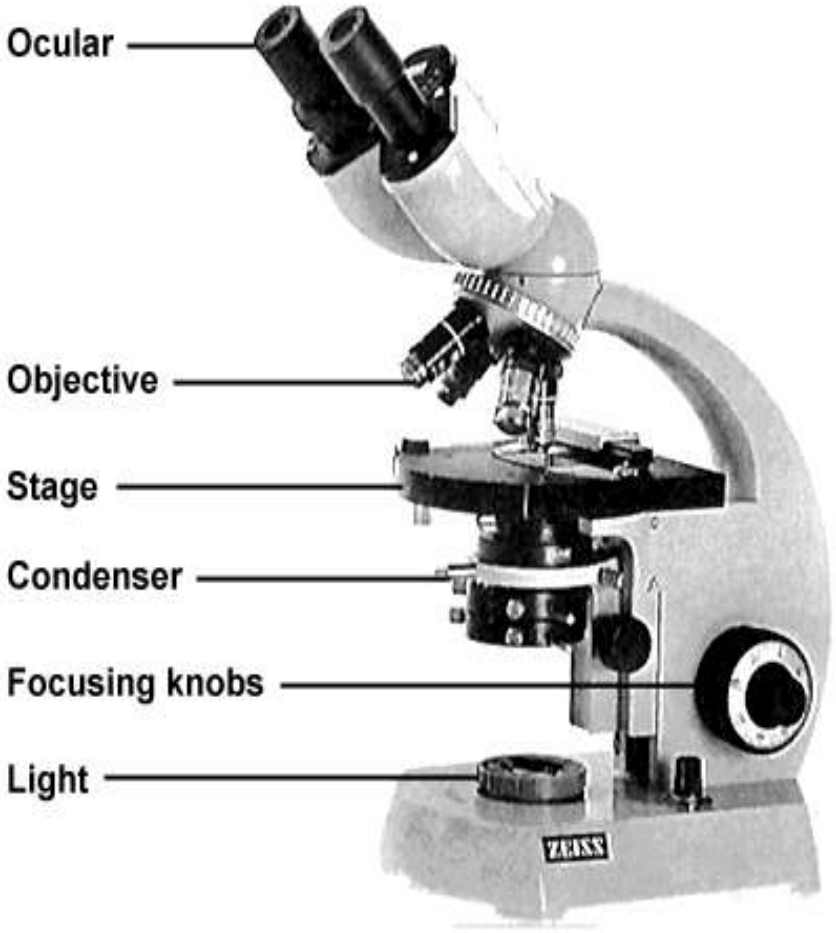
Mikroskop : sebagai alat pengamatan

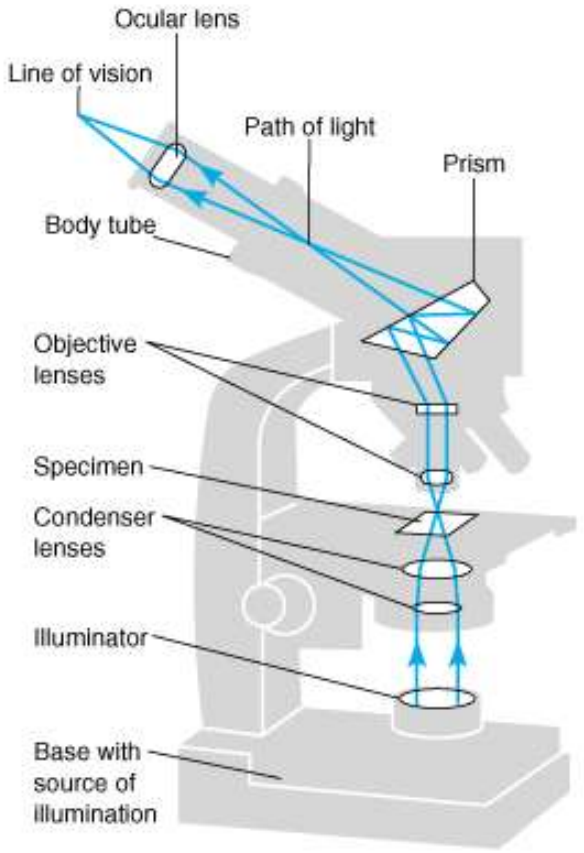
1. Light microscope (optic)
2. Electron microscope

Light microscope (optic)

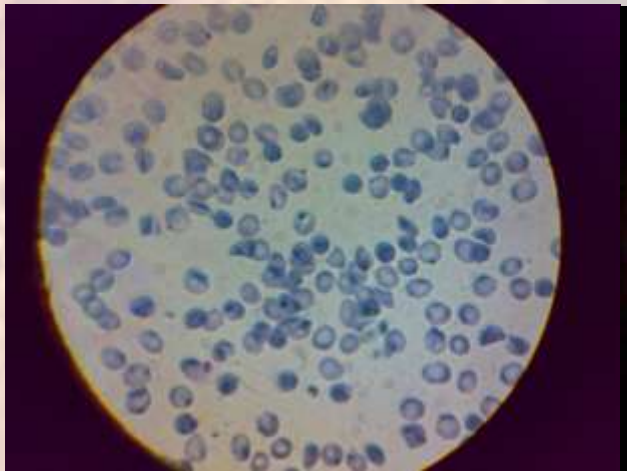
- menggunakan sinar visible
- resolving power (daya pisah/kemampuan memisahkan 2 titik yg berdekatan) = 0,25 um (partikel yg ukuranya <0,25 um tak dapat dibedakan)
- perbesaran sampai 1000 kali
- 3 lensa: lensa kondensor, lensa obyektif, lensa okuler

Tipe pembesaran	Obyektif	Okuler	Total
Lemah	10x	10x	100x
Sedang	40x	10x	400x
Kuat	100x	10x	1000x



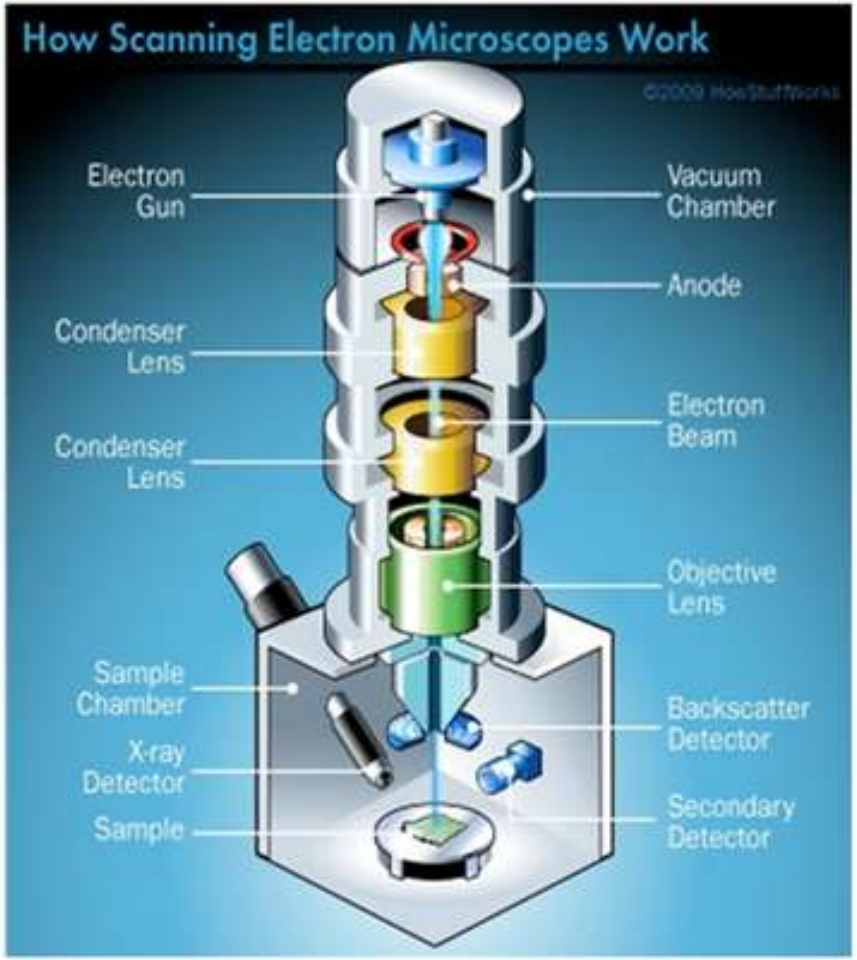


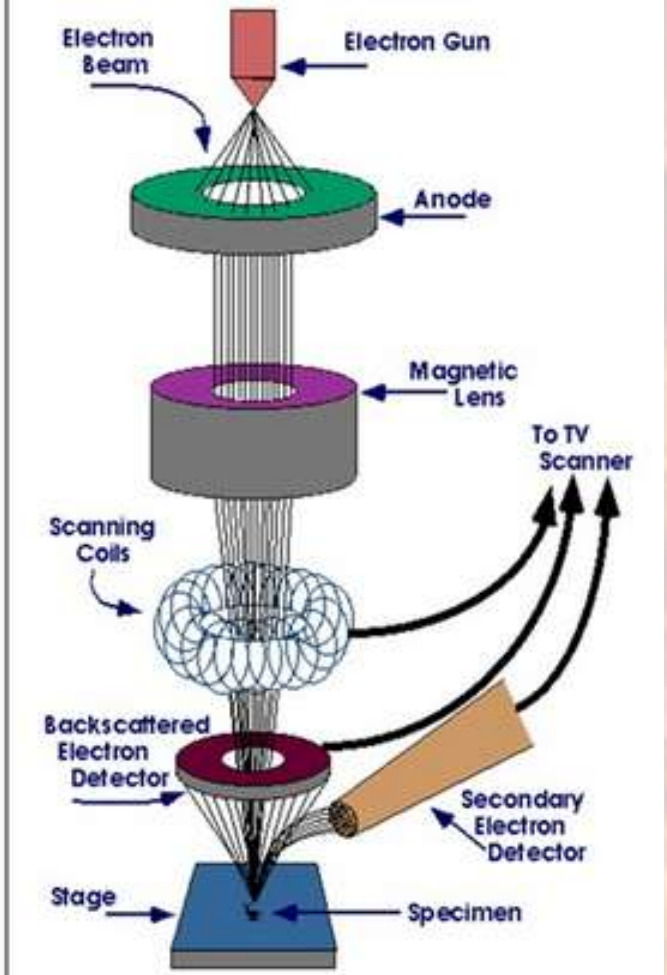
(b) The path of light (bottom to top)

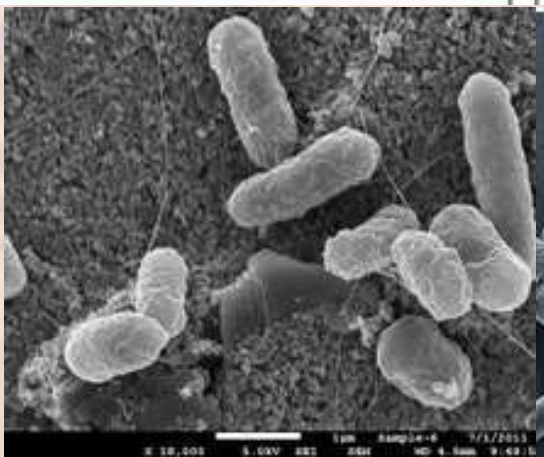


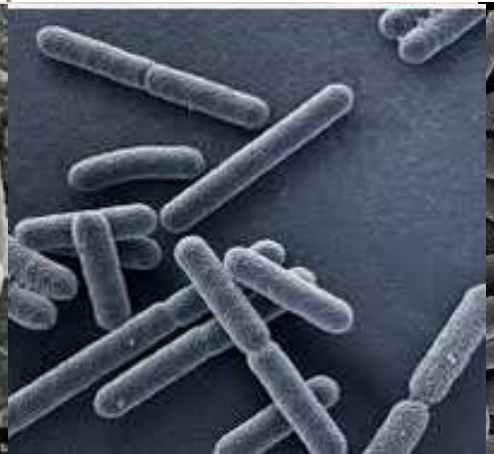
Electron microscope

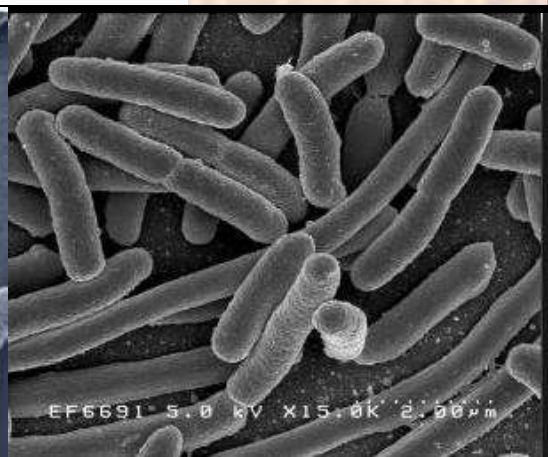
- menggunakan sinar dan elektron beam
- resolving power = 0,003 um
- Pada panjang gelombang yang sangat pendek 0,005-0,0003 nm
- perbesaran 200.000 – 400.000


How Scanning Electron Microscopes Work
Electron Gun
Vacuum Chamber
Anode
Condenser Lens
Electron Beam
Condenser Lens
Objective Lens
Sample Chamber
Backscatter Detector
X-ray Detector
Secondary Detector
Sample


Electron Beam
Electron Gun
Anode
Magnetic Lens
To TV Scanner
Scanning Coils
Backscattered Electron Detector
Secondary Electron Detector
Stage
Specimen






EF6691 5.0 kV X15.0K 2.00µm

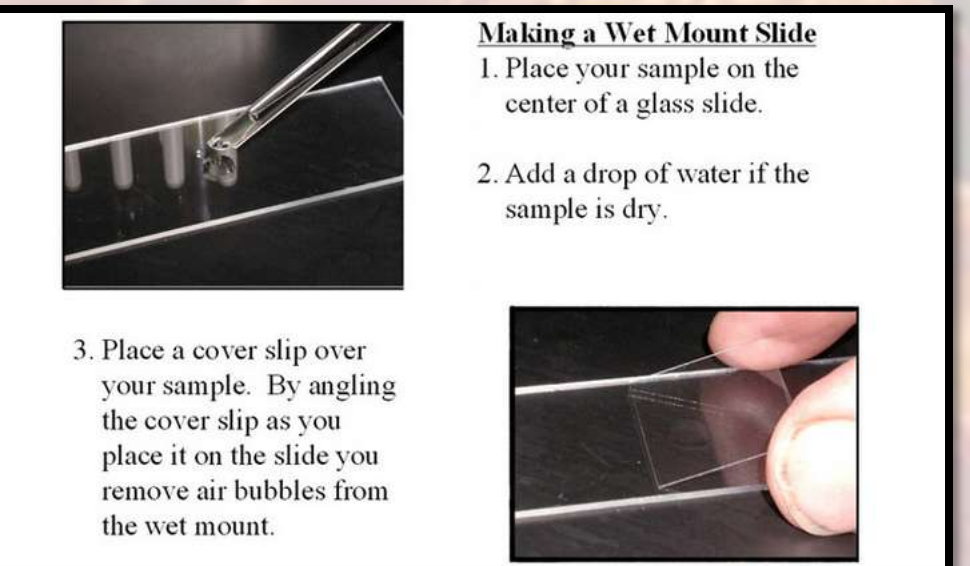
Persiapan mikrobia untuk pengamatan mikroskopik

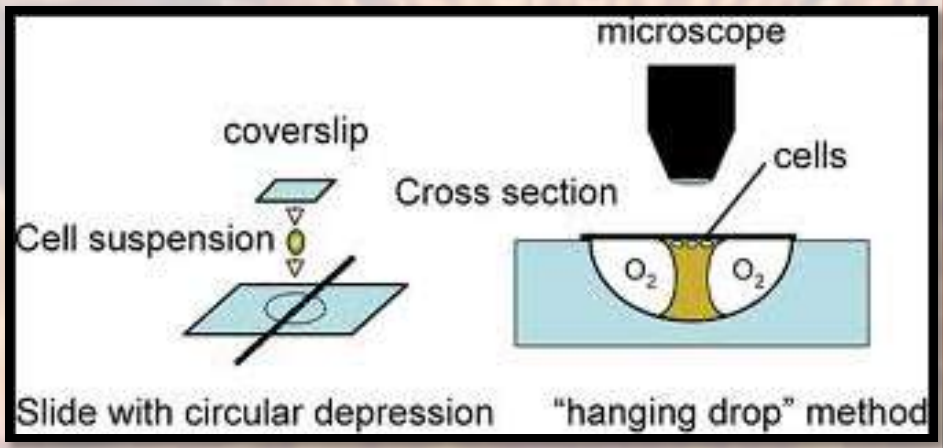
Preparat mikrobia :

1. Wet mount dan hanging drop

   Wet mount: suspendi mo. Diletakkan pd gelas benda & menutupnya dg gelas penutup

   Hanging drop: sama seperti wm. Tapi gelas benda punya cekungan di tengahnya





2. Pengecatan :

Suspensi sel diletakkan di gelas benda → dikeringkan →fiksasi→diberi cat

Tujuan:

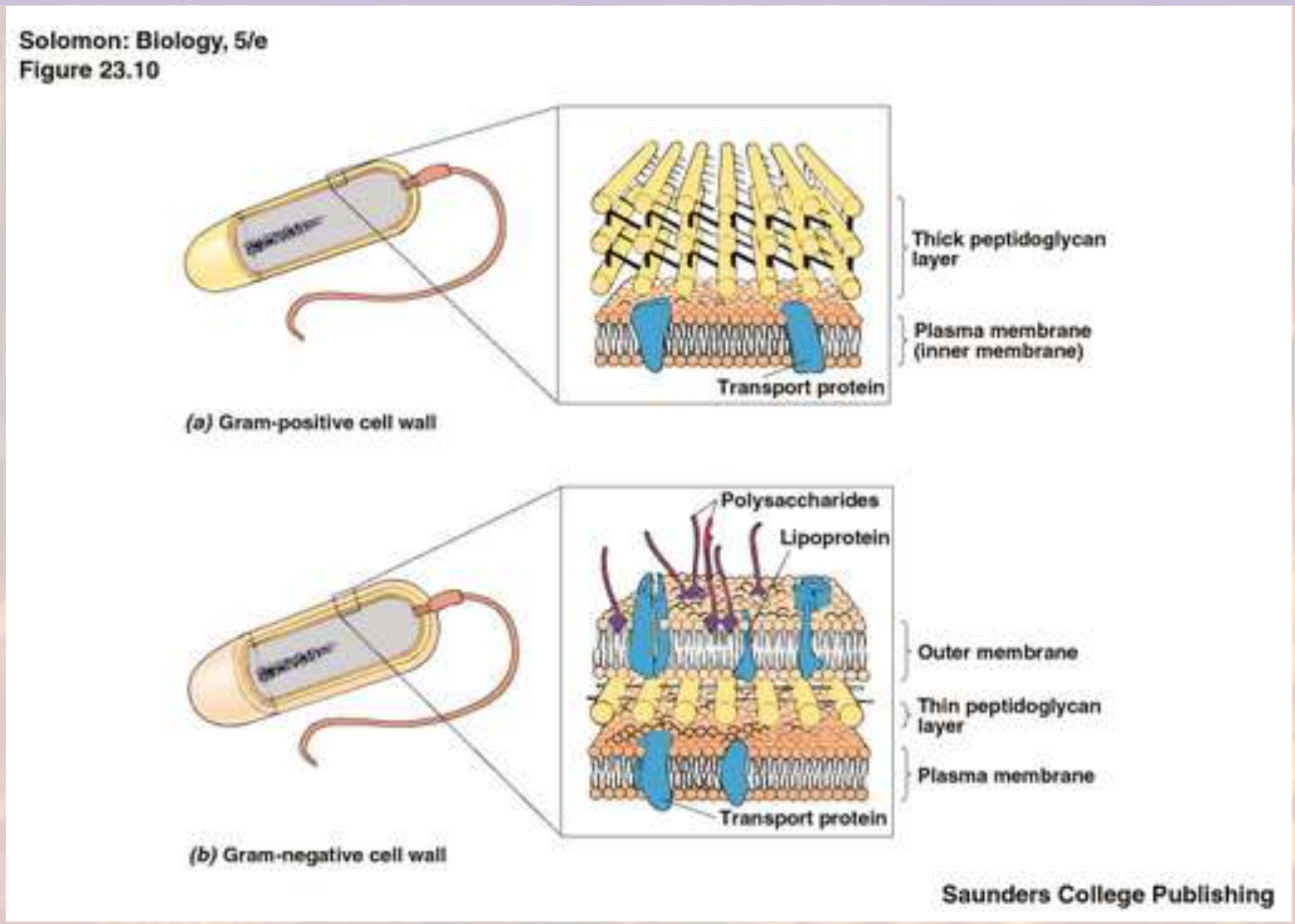
a. Melihat struktur sel
b. Mengidentifikasi struktur dalam sel
c. Mengidentifikasi mo.

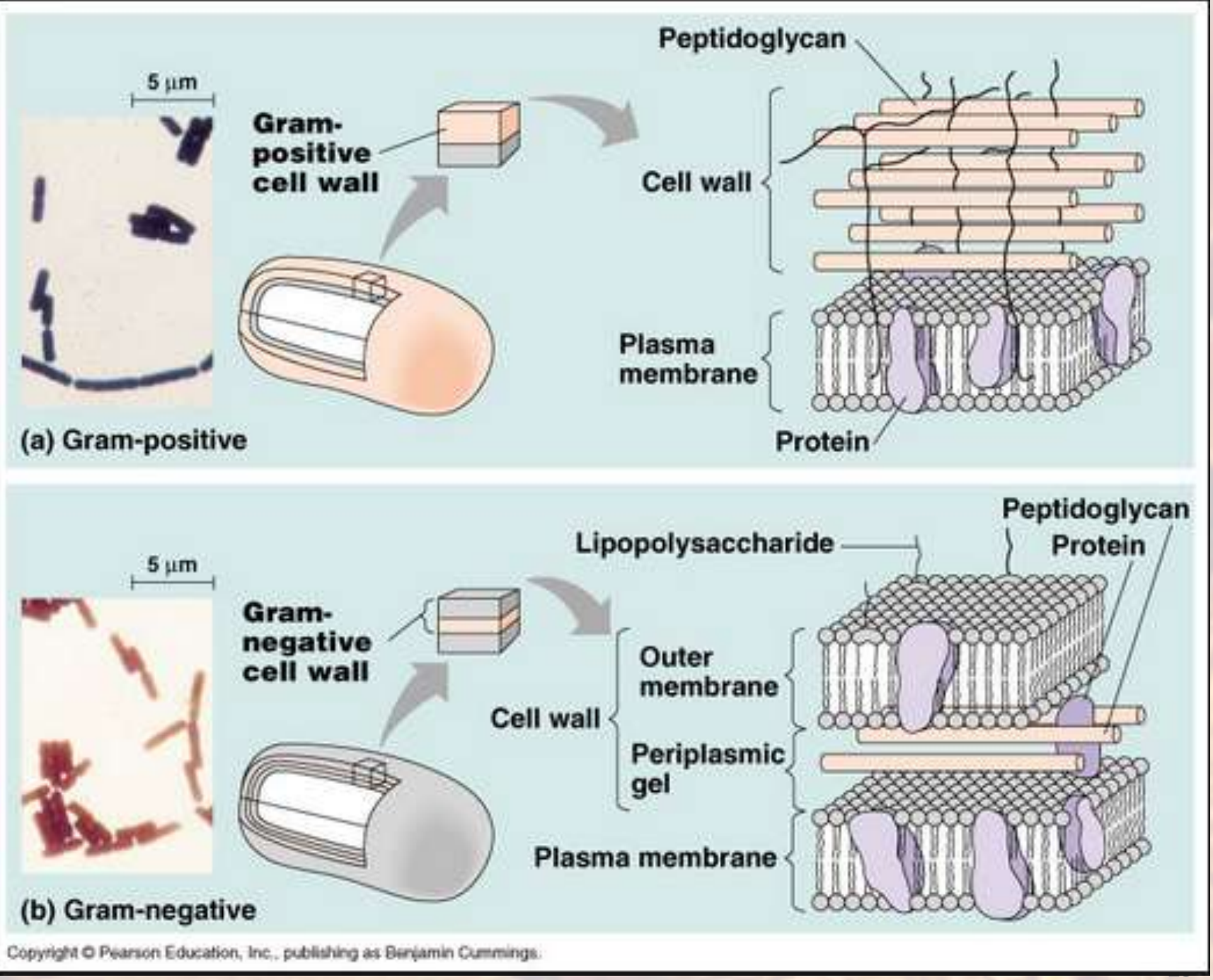
Macam pengecatan:

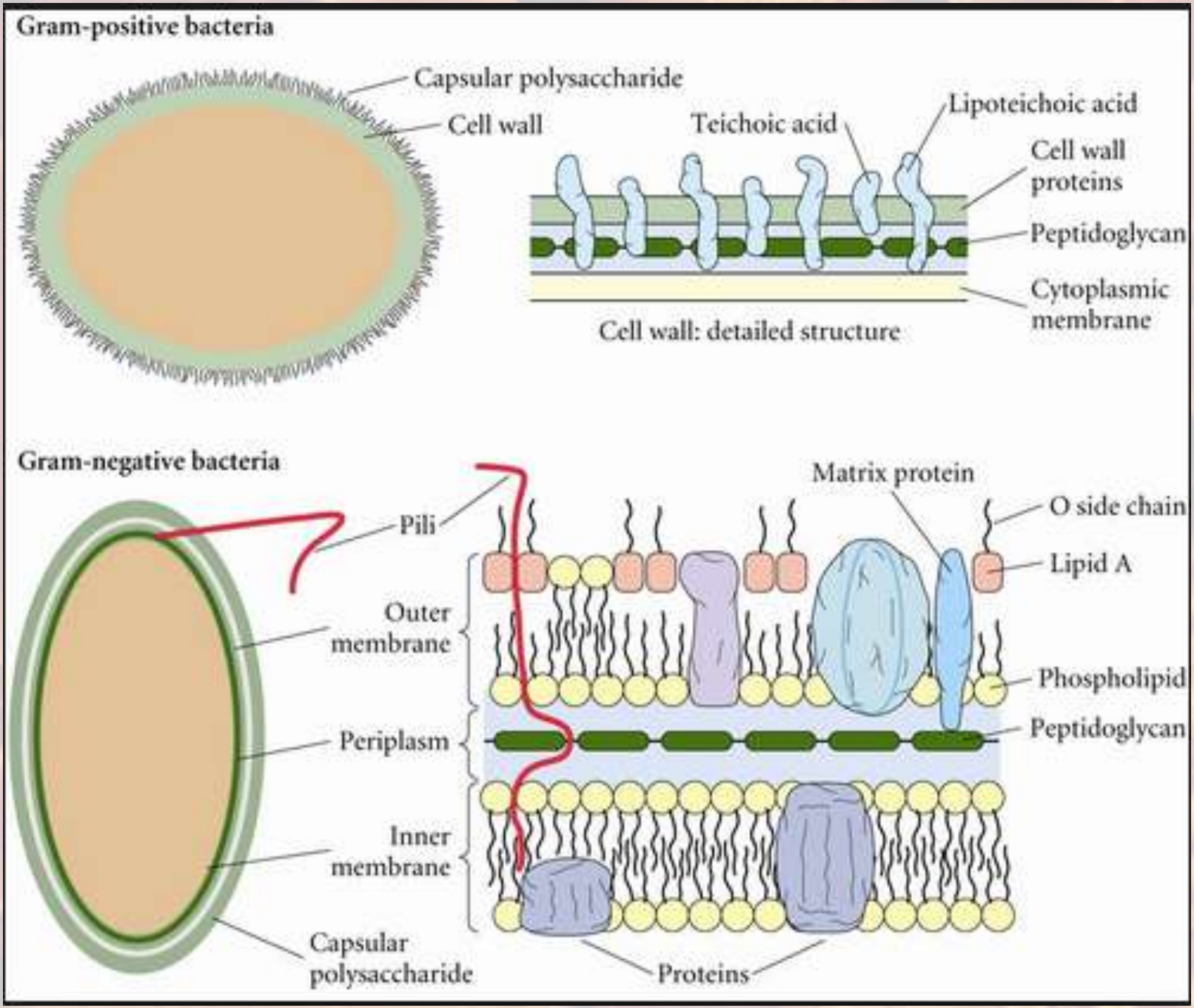
a. Sederhana
b. Diferensial

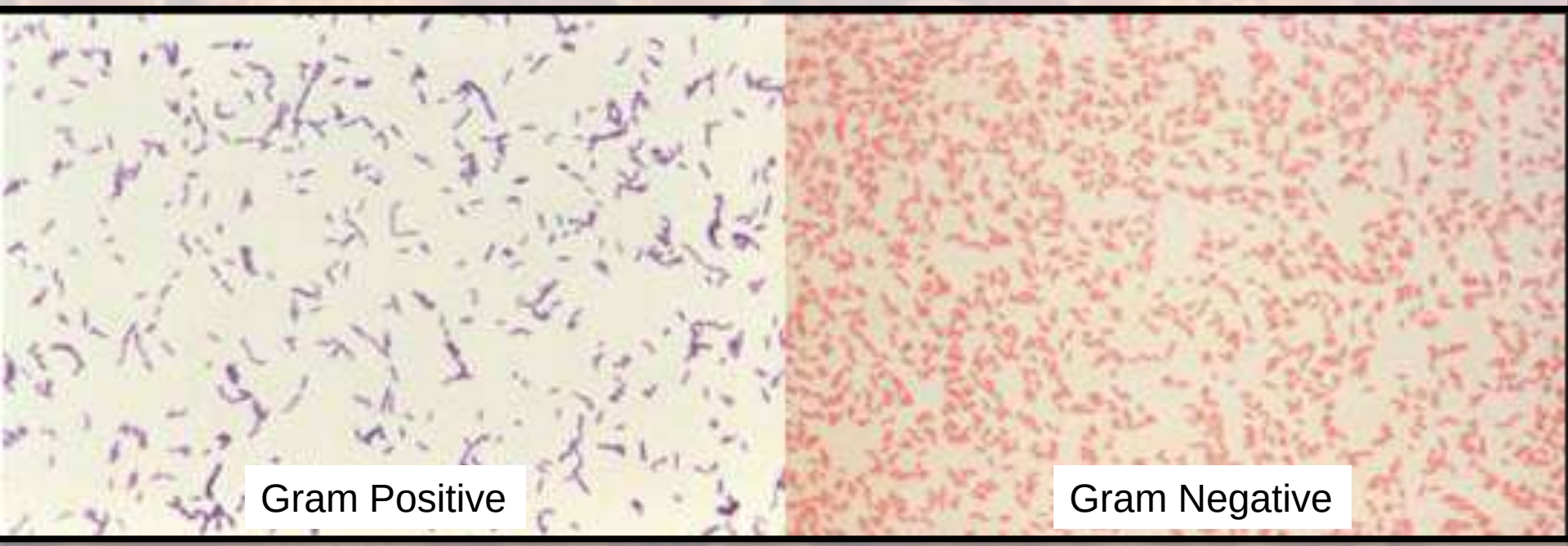
- **Sederhana:**
  Pengecatan dengan 1 macam cat
- **Diferensial :**
  Pengecatan dengan 2 atau lebih cat
  untuk membedakan sifat biokimia dan struktur sel,
  misalnya :
  - Gram : membedakan komponen penyusun dinding sel (ketebalan peptidoglikan)
  - Endospora : deteksi adanya spora,
  - Capsule : untuk deteksi capsule (**K antigen)**
  - Acid fast stain: berguna untuk identifikasi *Mycobacterium*.
  - Flagela : untuk deteksi ada/tidaknya flagela dan pengaturannya
  - Giemsa dsb

Pengecatan Gram





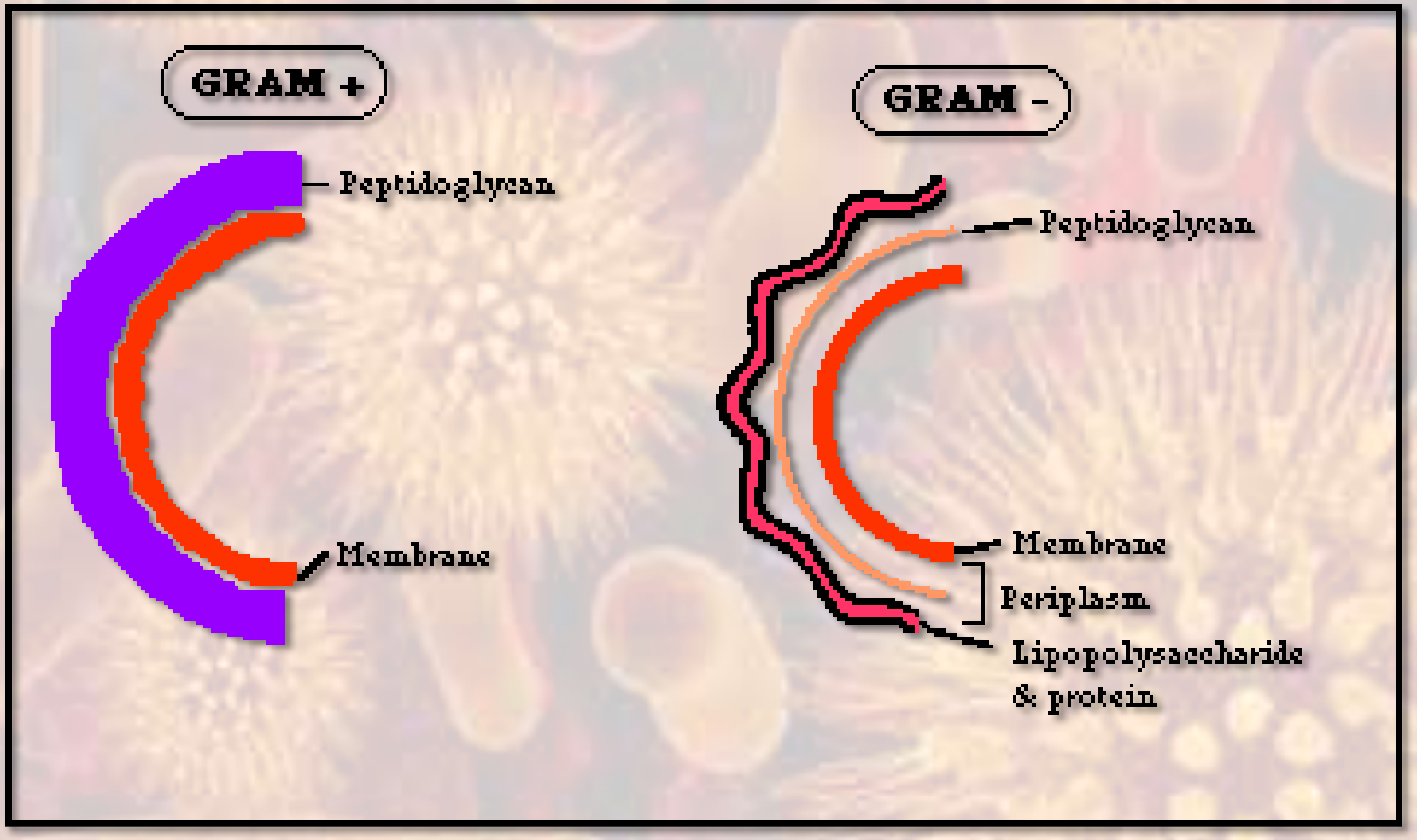

Gram-positive bacteria
Capsular polysaccharide
Cell wall
Teichoic acid
Lipoteichoic acid
Cell wall proteins
Peptidoglycan
Cytoplasmic membrane
Cell wall: detailed structure
Gram-negative bacteria
Pili
Matrix protein
O side chain
Lipid A
Outer membrane
Phospholipid
Periplasm
Peptidoglycan
Inner membrane
Capsular polysaccharide
Proteins


Gram Positive
Gram Negative

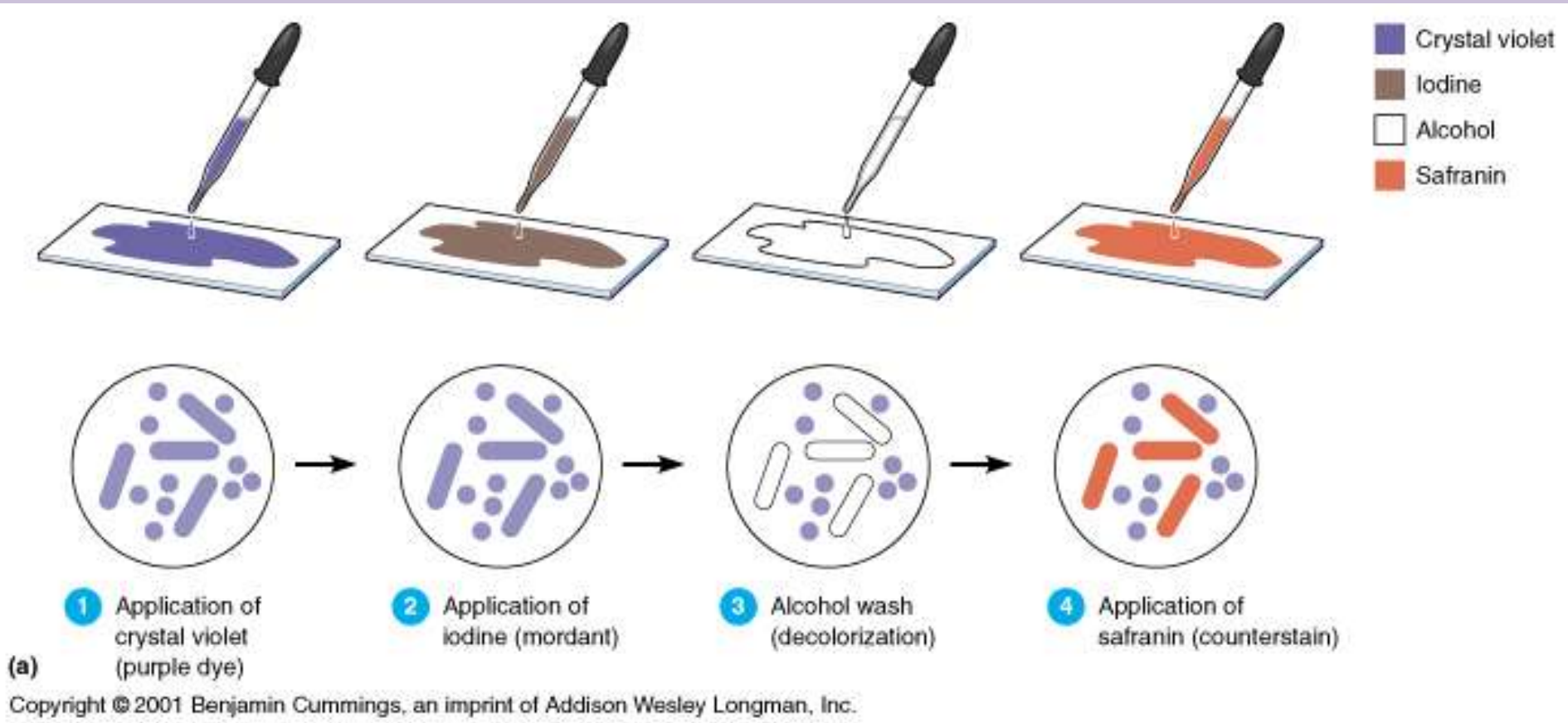
The Chemistry of the Prokaryote Cell Wall:

Christian Gram 1884 - The Gram stain

Differences between Gram positive and Gram negative cell walls:

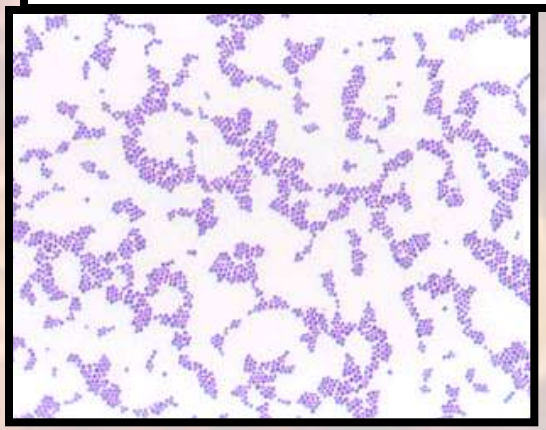


Pengecatan gram



Perbedaan Gram+ dan Gram -

Step	Gram + organisms	Gram - organisms
1. Unstained		
2. Crystal Violet		
3. Iodine		
4. Decolorization		
5. Safranin		



Staphylococcus aureus ***Escherichia coli***

C. IDENTIFIKASI MIKROBIA

adalah proses yang cermat dan sistematis yang menggunakan banyak teknik yang berbeda untuk mempersempit jenis mo. yang hadir dalam kultur mo. yang tidak diketahui.

Teknik identifikasi:

1. Morfologi (menurut morfologi bakteri)
2. Cultural (sesuai dengan tanda pertumbuhan bakteri pada media nutrisi yg berbeda)
3. Biokimia (sesuai kemampuan bakteri untuk memanfaatkan substrat yg berbeda)
4. Serologis (sesuai dengan antigen)
5. Bilologis(sesuai kemampuan bakteri untuk menyebabkan perubahan yang berbeda pada hewan coba setelah di inokulasi oleh mikroba)
6. Flow cytometry
7. Phage typing
8. Analisis Protein
9. Perbandingan sequenses nukleotida .

http://intranet.tdmu.edu.ua/data/kafedra/internal/micbio/classes_stud/en/stomat/ptn/Microbiology,%20virology%20and%20immunology/2/Lesson_03_The%20main%20methods%20and%20principles%20of%20pure%20cultures%20isolation.htm

C. IDENTIFIKASI MIKROBIA (1)

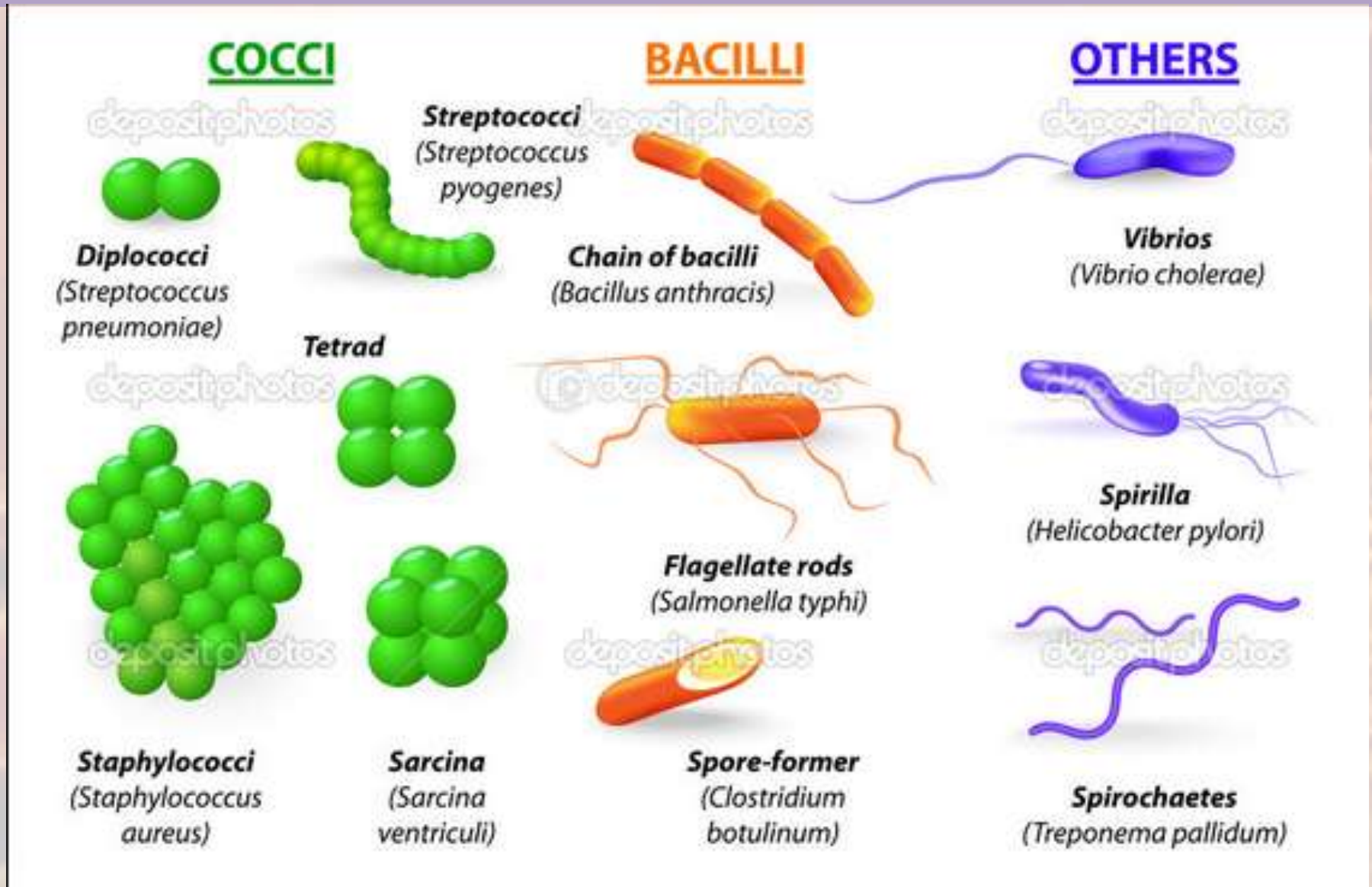
Sifat morfologi :

- Ukuran, warna, tekstur sel
- Bentuk sel (coccus, batang, spiral, dsb)
- Pengaturan koloni sel (bergandengan, dsb)
- Bentuk konidia, sporangium, rhizoid, dsb
- Cara membelah/ memperbanyak diri
- Sifat permukaan koloni : mucoid, kasar, lembut

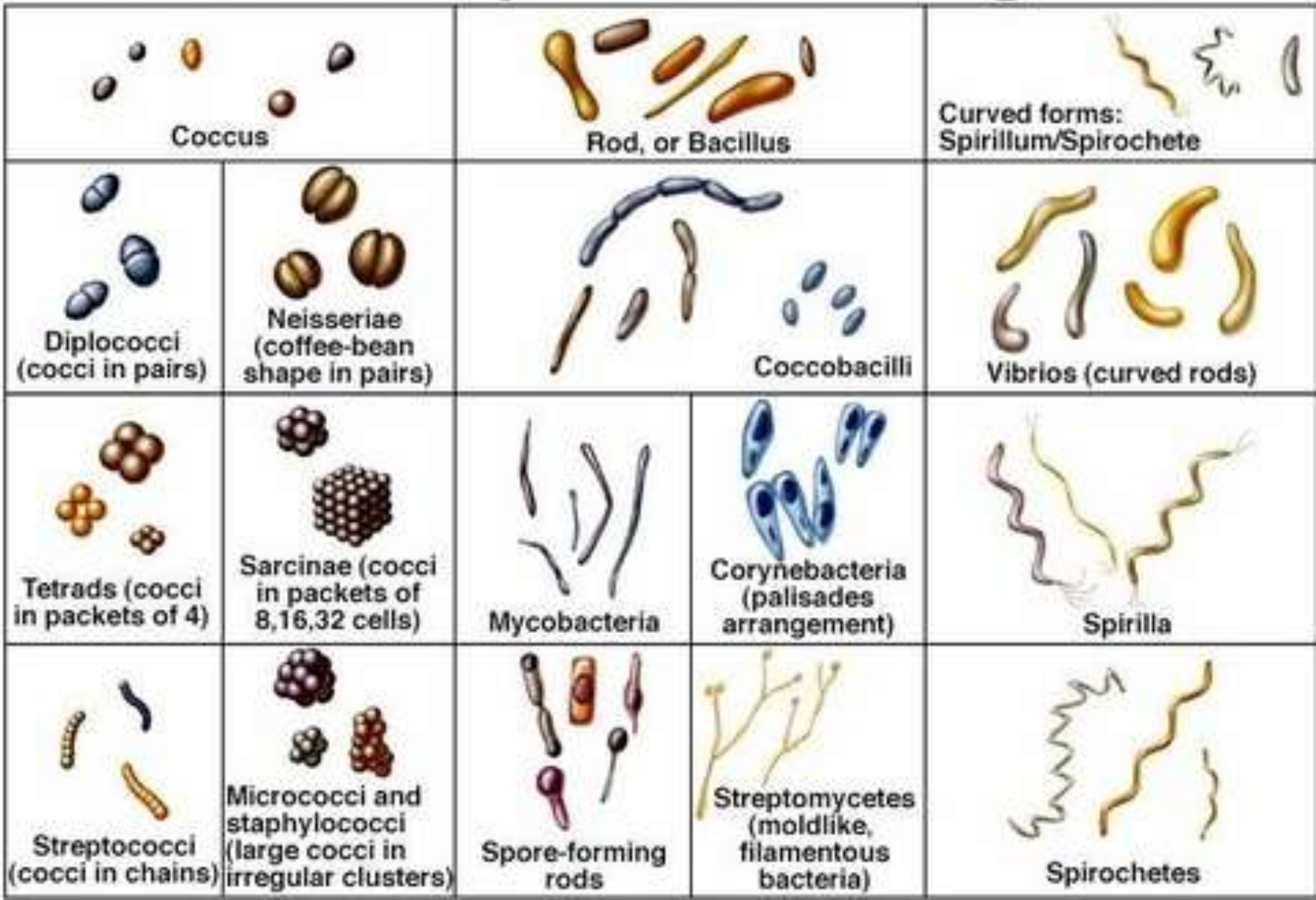
Pedoman :

- Bakteri : Bergey's Manual of Systematic Bacteriology
- Yeast : Identification of Yeast (Kreger van Rij)
- Jamur : John Pitt

Bentuk sel

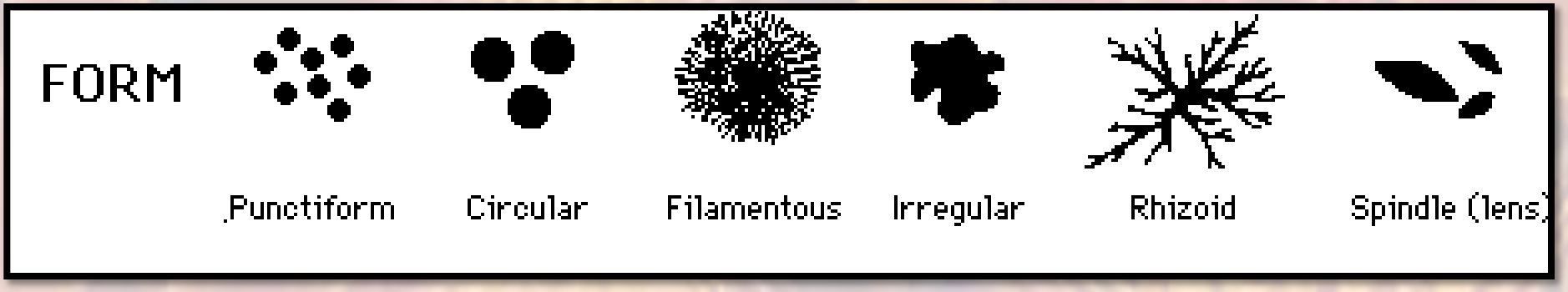

COCCI
BACILLI
OTHERS
Streptococci
(Streptococcus pyogenes)
Diplococci
(Streptococcus pneumoniae)
Chain of bacilli
(Bacillus anthracis)
Vibrios
(Vibrio cholerae)
Tetrad
Spirilla
(Helicobacter pylori)
Flagellate rods
(Salmonella typhi)
Staphylococci
(Staphylococcus aureus)
Sarcina
(Sarcina ventriculi)
Spore-former
(Clostridium botulinum)
Spirochaetes
(Treponema pallidum)

Bacterial shapes and arrangements



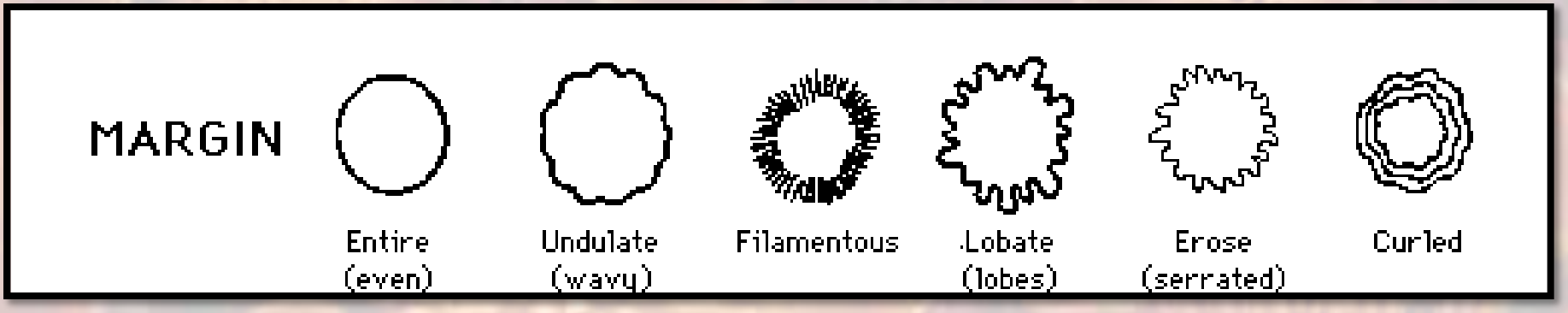
Kenampakan koloni bakteri

BENTUK KOLONI



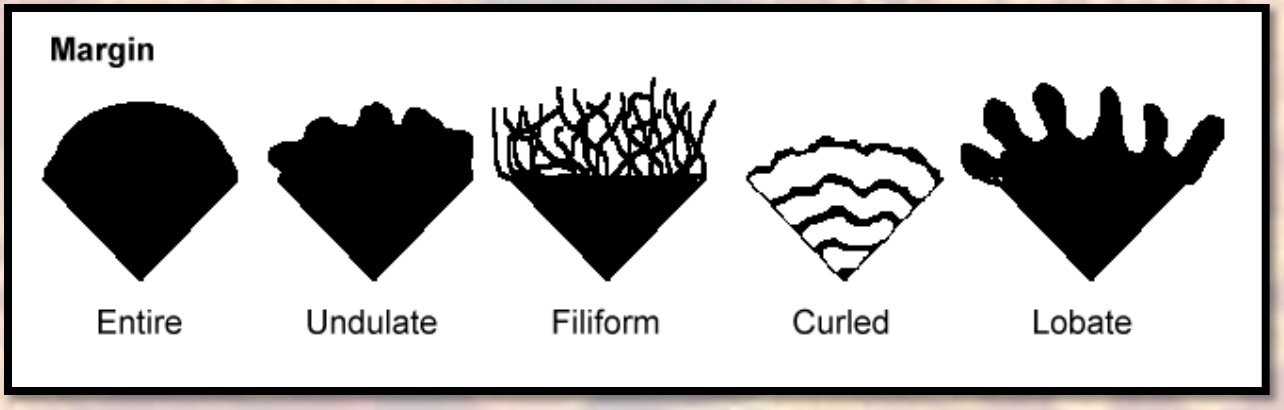
(bulat)

PINGGIR KOLONI

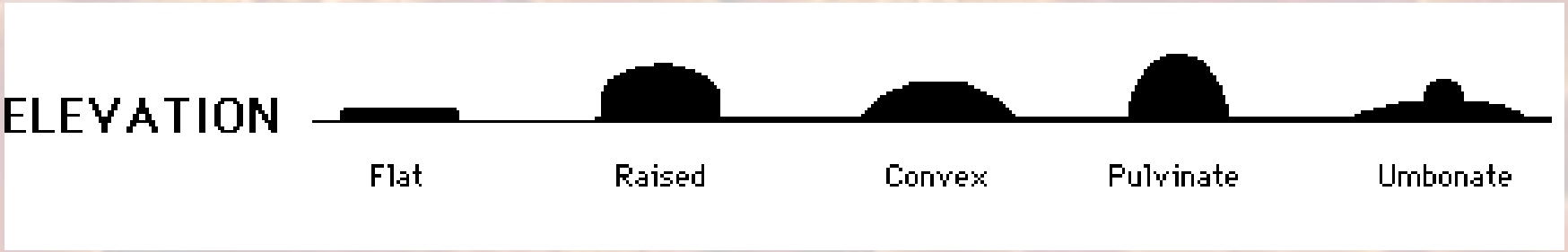


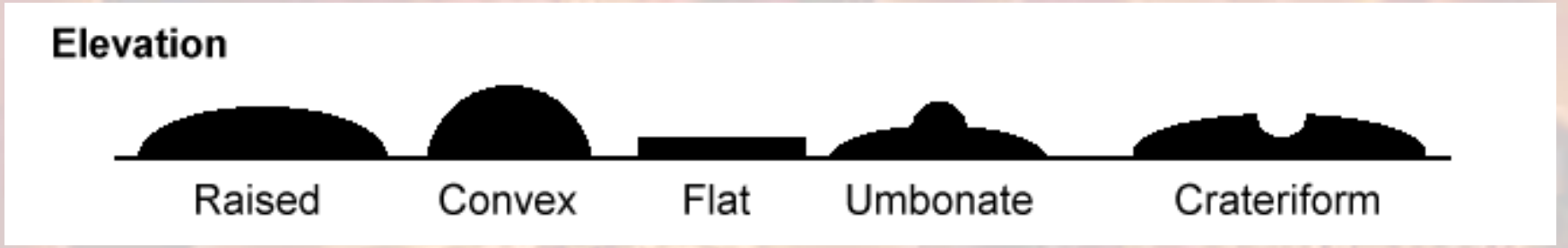
smoot

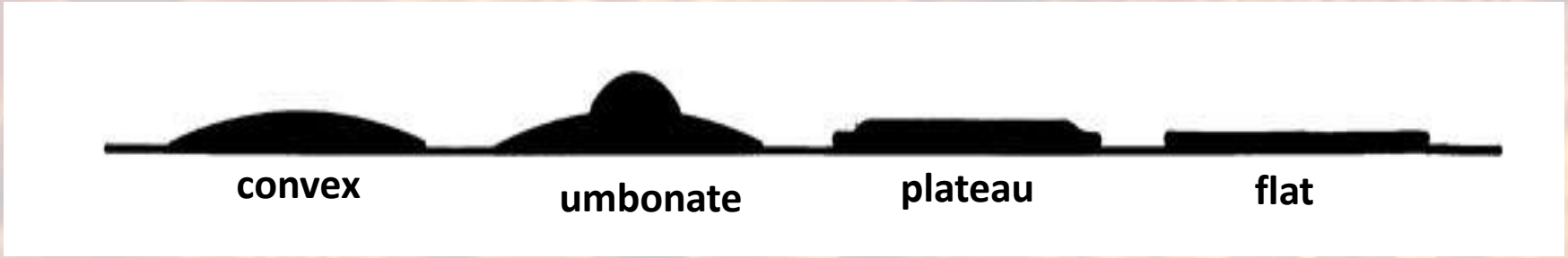
Irregular (erose)

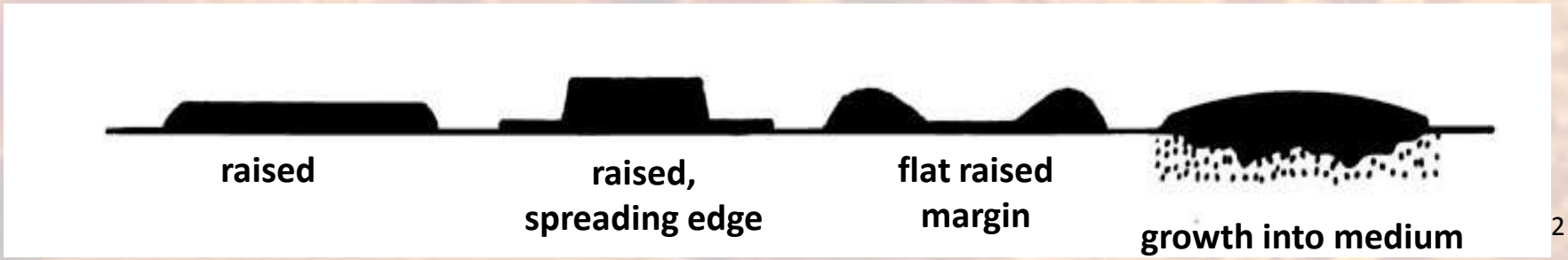


ELEVASI


ELEVATION
Flat
Raised
Convex
Pulvinate
Umbonate

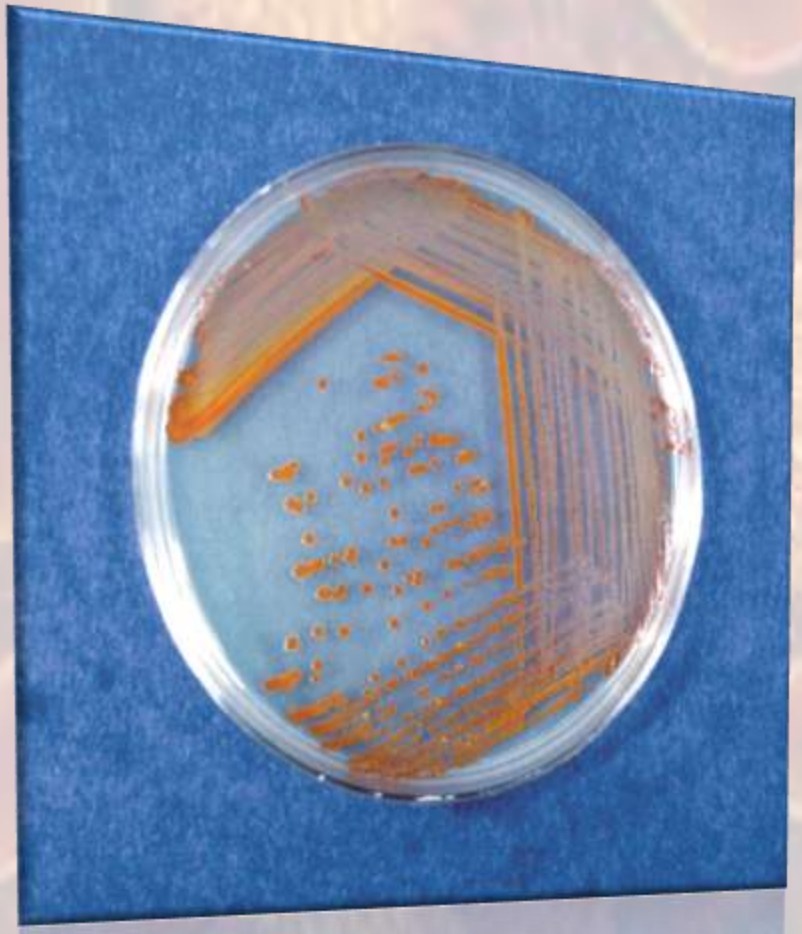

Elevation
Raised
Convex
Flat
Umbonate
Crateriform


convex
umbonate
plateau
flat

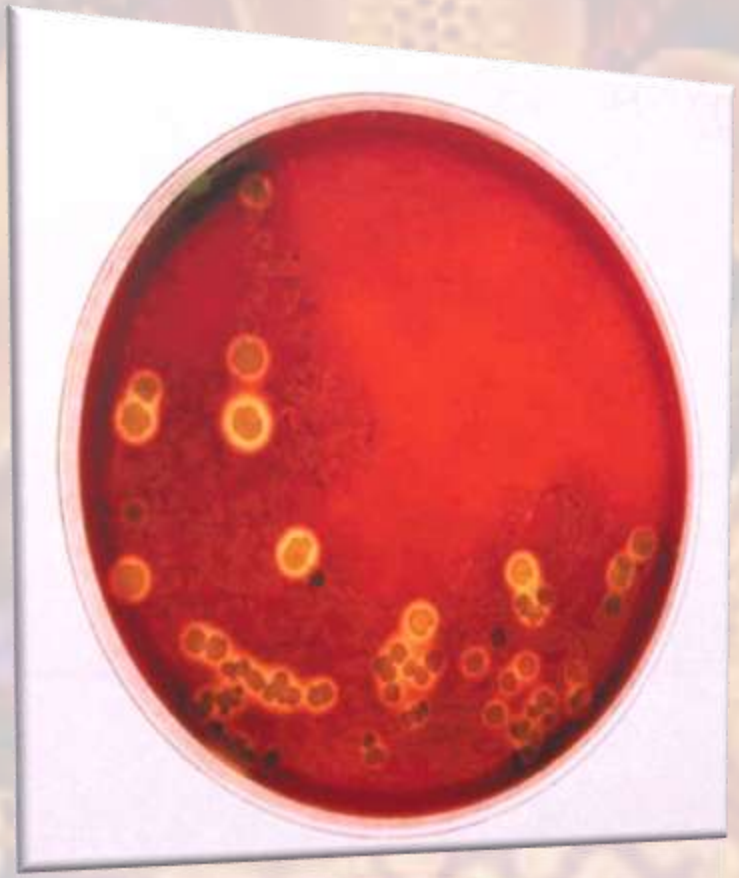

raised
raised,
spreading edge
flat raised
margin
growth into medium

Sifat-sifat koloni

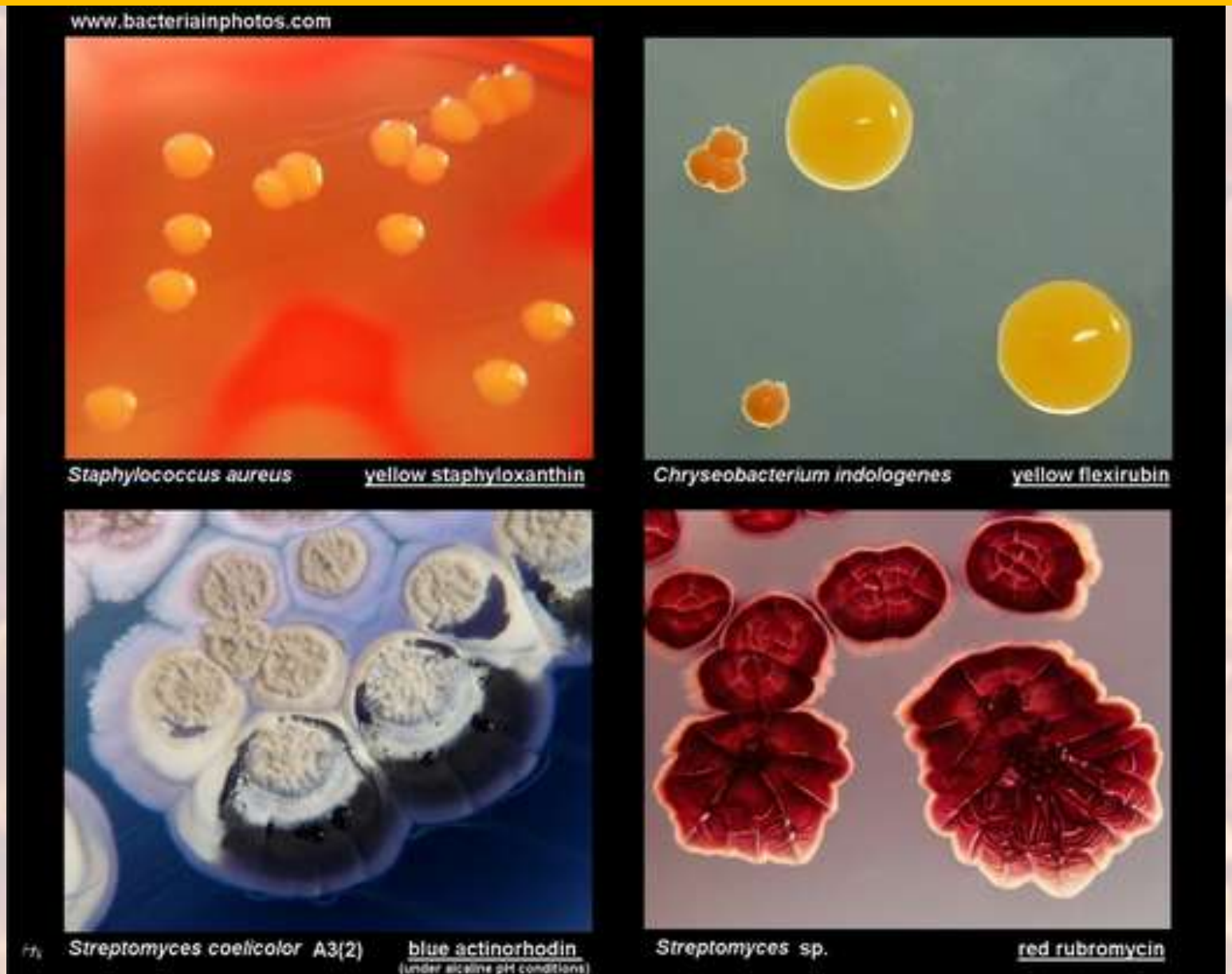
Pembentukan warna



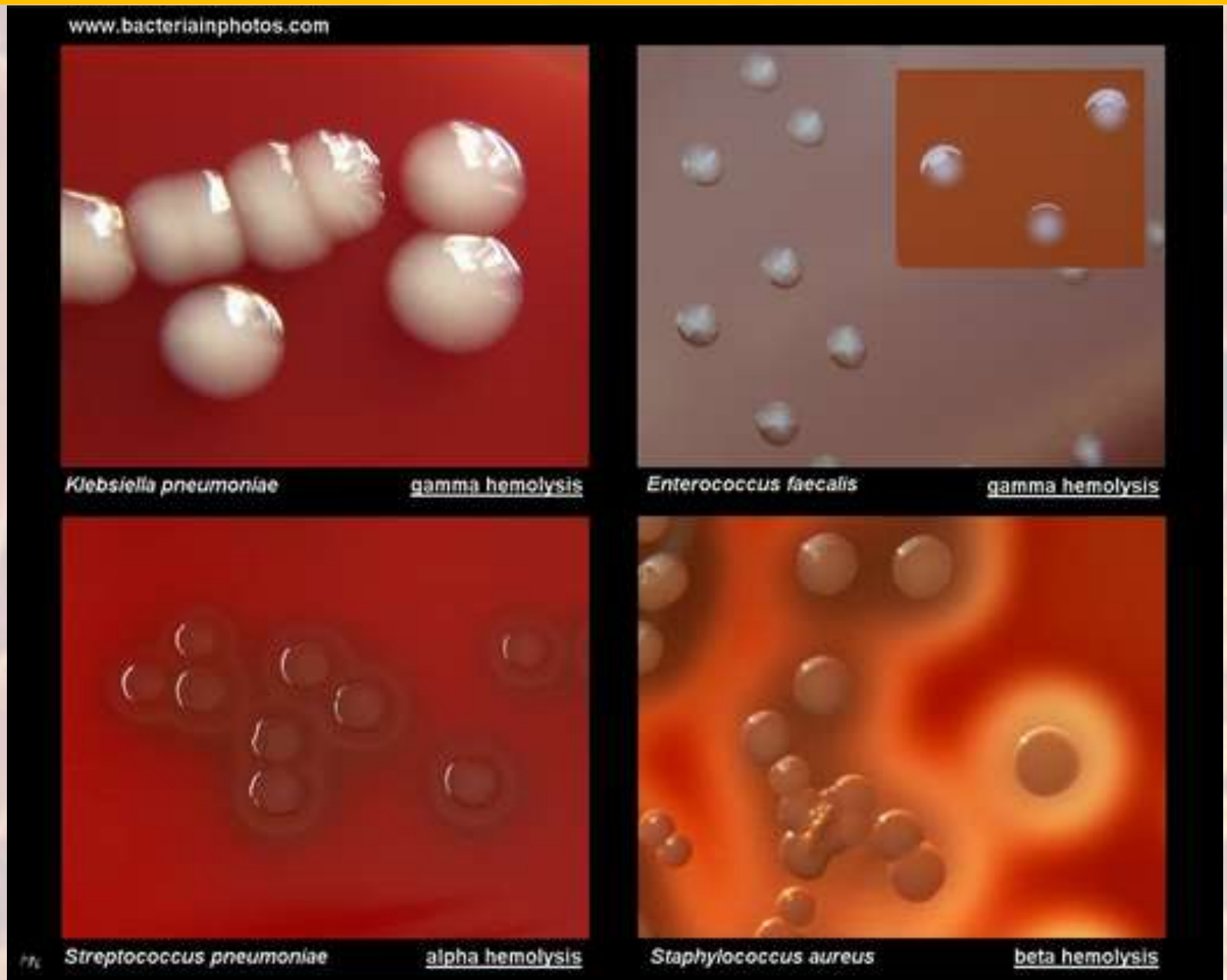
hemolysis



The color of colony



haemolysis


www.bacteriainphotos.com
Klebsiella pneumoniae
gamma hemolysis
Enterococcus faecalis
gamma hemolysis
Streptococcus pneumoniae
alpha hemolysis
Staphylococcus aureus
beta hemolysis

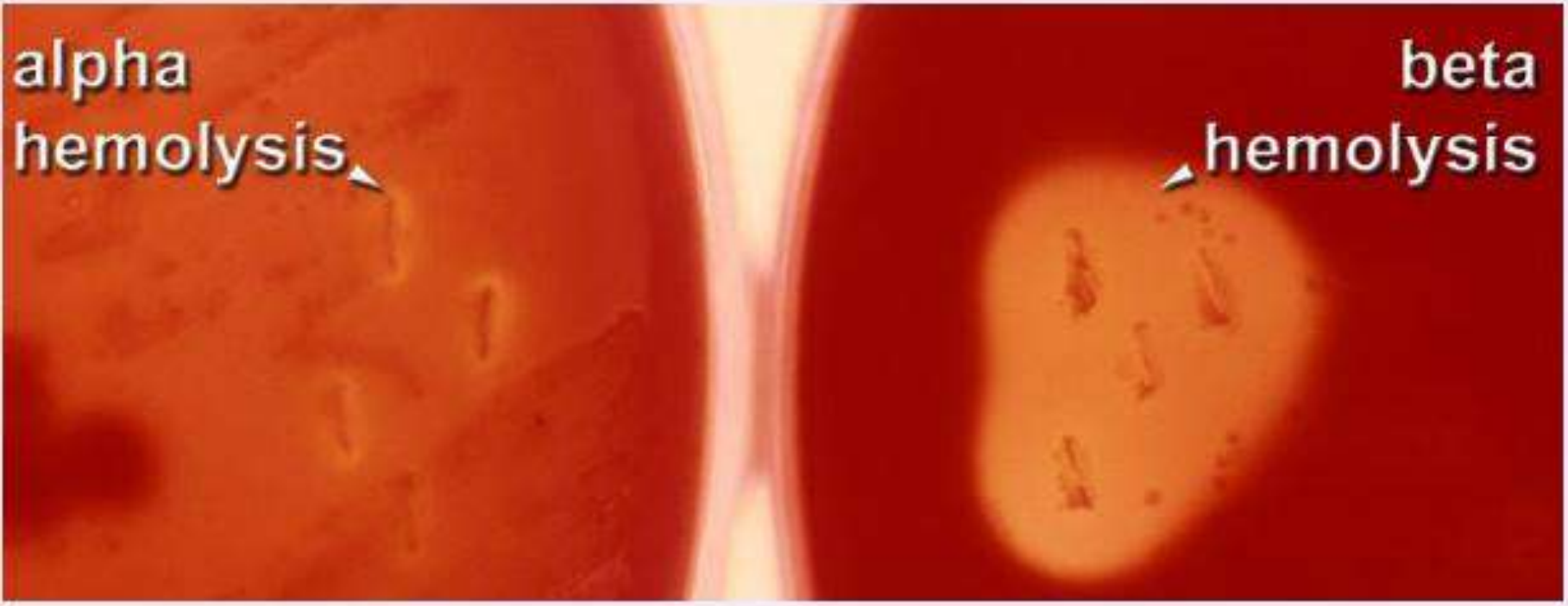
Medical Laboratories

Streptococcus pyogenes: beta, complete lysis of red blood cells, clear area around colony growth.

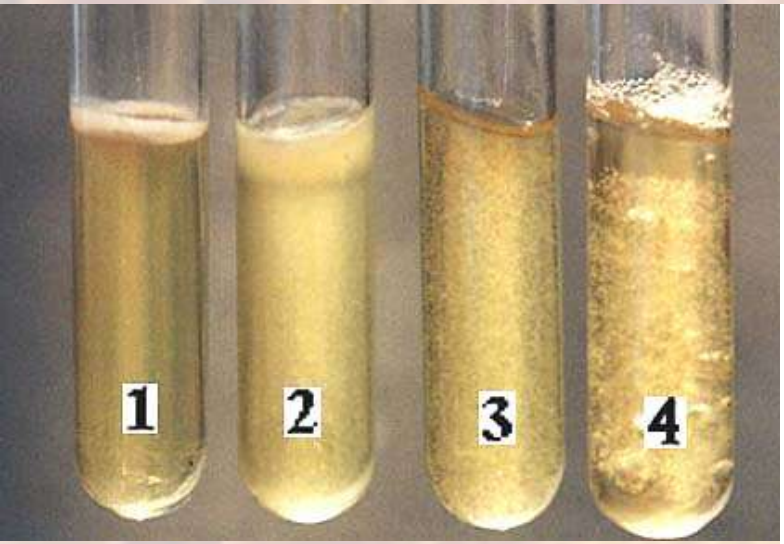
Streptococcus bovis: alpha, incomplete lysis of red blood cells, green area around colony growth.

Enterococcus faecalis: gamma, growth with no blood cell lysis.

Type of hemolysis	Description
α hemolysis:	Colony is surrounded by a zone of intact but discolored erythrocytes that have a green or brownish-green color. This appearance is generally due to the action of peroxide produced by the bacteria.
β hemolysis:	Colony is surrounded by a white or clear zone in which few or no intact erythrocytes are found. This reaction is seen best when the organism is growing under reduced oxygen concentration (peroxide production is thereby decreased). β hemolysis is caused by one or more erythrocyte-lysing enzymes (hemolysins).
γ hemolysis:	Colony is surrounded by no zone of hemolysis. γ is used simply as a synonym for negative in this test.



Pola pertumbuhan dalam media cair



Corresponding tube no. above	1	2	3	4
Oxygen relationship designation	STRICT (OBLIGATE) AEROBE	FACULTATIVE ANAEROBE	AEROTOLERANT ANAEROBE	STRICT (OBLIGATE) ANAEROBE
Aerobic respiration*	+	+	–	–
Fermentation*	–	+	+	+
Ability to grow aerobically (oxygen tolerance)	+	+	+	–
Ability to grow anaerobically	–	+	+	+
Catalase reaction	+	+	–	–
Reaction in Glucose O/F Medium (for those able to grow well in medium)	O or –	F		
Response to sodium azide in a growth medium	SENSITIVE	SENSITIVE (under aerobic conditions)	RESISTANT	RESISTANT

C. IDENTIFIKASI MIKROBIA (2)

Sifat fisiologi :

- Kebutuhan nutrisi :
  - Kemampuan memfermentasi gula
  - Kemampuan menggunakan gula (asimilasi)
  - dsb
- Kondisi fisik pertumbuhan :
  - Suhu
  - Sinar
  - Oksigen
  - dsb

Sifat fisiologis : kemampuan tumbuh dalam berbagai sumber karbon (API 20 E Test Strips)

Effects of the carbon source on bacterial growth and EPS production by G. hansenii LMG1524

C source	Final pH	Cell dry weight (g/L)	EPS (g/L)
Glucose	3.0 ± 0.2	0.56 ± 0.00	0.10 ± 0.03
Galactose	3.7 ± 0.0	0.24 ± 0.06	0.08 ± 0.03
Sucrose	5.3 ± 0.1	0.28 ± 0.00	0.20 ± 0.03
Glycerol	4.8 ± 0.0	0.19 ± 0.04	0.30 ± 0.17
Sorbitol	5.7 ± 0.1	0.29 ± 0.01	0.21 ± 0.01
Ethanol	3.7 ± 0.1	0.25 ± 0.04	0.18 ± 0.03
Succinic acid	3.2 ± 0.0	0.21 ± 0.10	0.09 ± 0.04

Mean ± standard deviation; n = 2.

Advances in Microbiology
Vol.2 No.4(2012), Article ID:25849,9 pages DOI:10.4236/aim.2012.24062
http://file.scirp.org/Html/11-2270115_25849.htm

C. IDENTIFIKASI MIKROBIA (3)

Sifat metabolik :

- Produksi senyawa kompleks
  - Produksi ekstraseluler polisakararida
  - Produksi ekstraseluler enzim (amilase, protease, lipase, dsb)
  - dsb

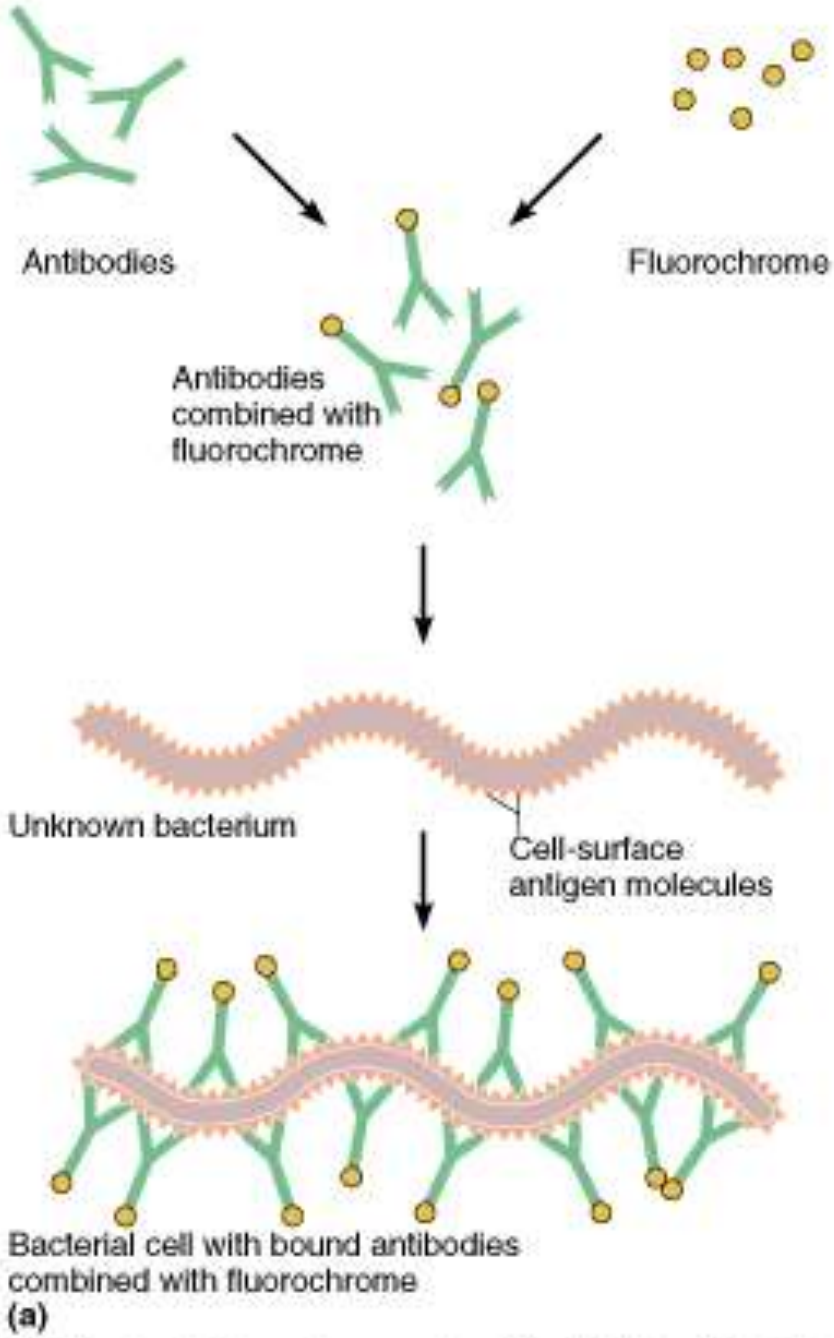
C. IDENTIFIKASI MIKROBIA (4)

Sifat antigen :

- Antigen adalah senyawa yang menstimulasi produksi antibodi jika diinjeksikan ke binatang
- Mikrobia mempunyai struktur fisik permukaan sel tertentu yang dapat berfungsi sebagai antigen
- Keberadaan antigen yang spesifik digunakan untuk identifikasi mikrobia

Uji Serologi / Sifat antigen)

(Pengamatan tidak langsung)



C. IDENTIFIKASI MIKROBIA (5)

Sifat patogen :

- Mikrobia patogen merupakan mikrobia penyebab penyakit.
- Sifat patogenik dapat digunakan untuk identifikasi

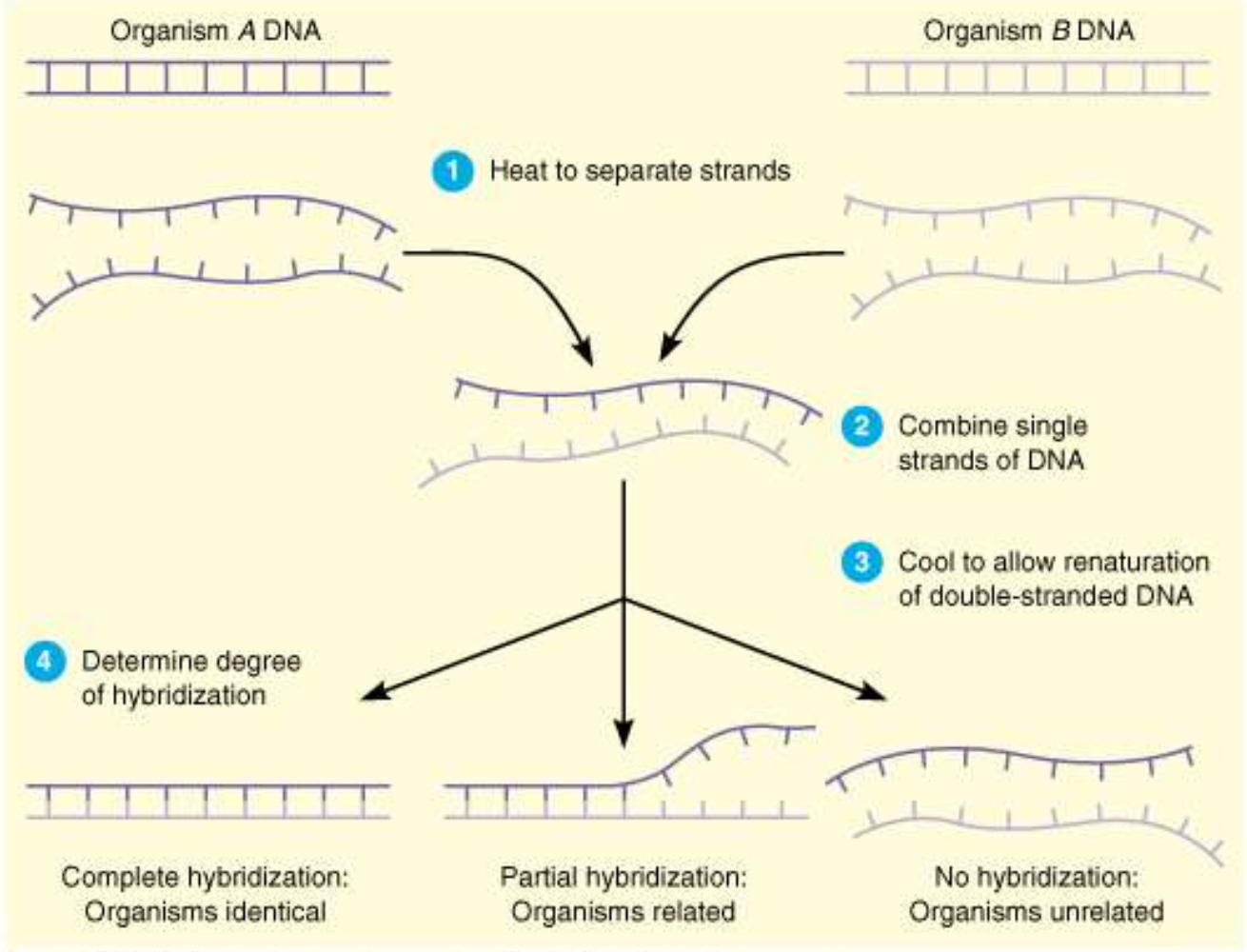
C. IDENTIFIKASI MIKROBIA (6)

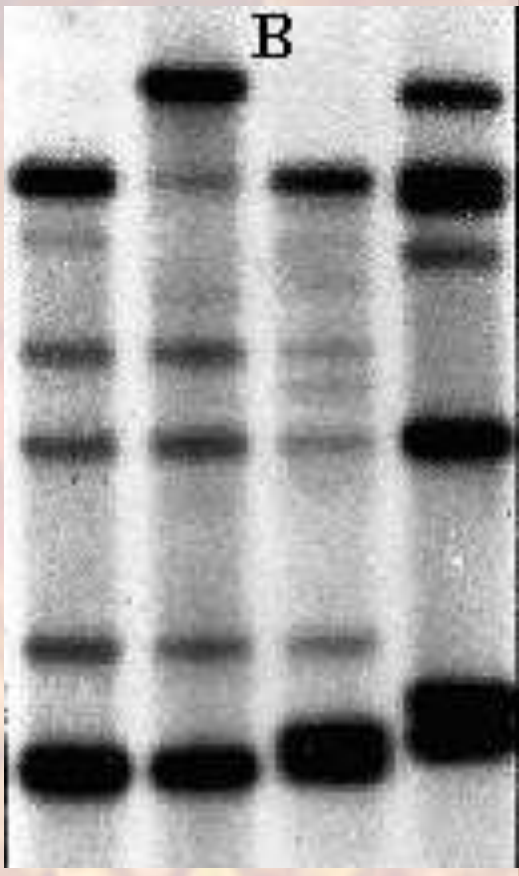
Sifat genetik :

- Analisa genetik sekarang banyak digunakan untuk identifikasi mikrobia

- DNA probe : benang DNA dari spesies yang diketahui dicampur dengan isolat yang akan diidentifikasi.
- Apabila isolatnya dari spesies yang sama, maka DNA akan bergabung membentuk benang ganda.

Southern blot hybridization





B. PENYIMPANAN KULTUR MURNI

1. Penyimpanan sementara :
   - 4 – 10 °C (refrigerasi)

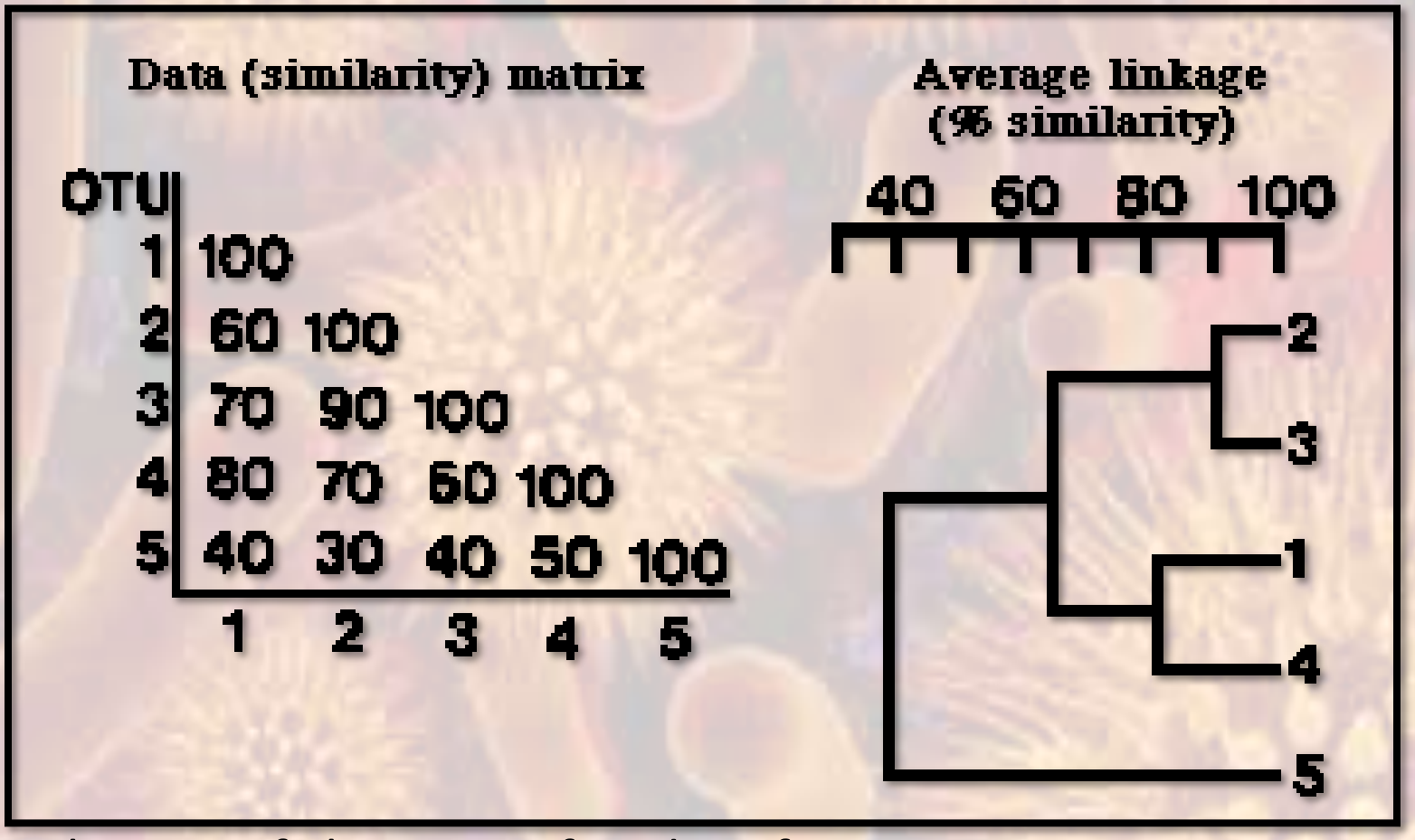
2. Penyimpanan lama :

   tahan sampai tahunan, tanpa mengalami perubahan morfologi dan fisiologi (ditambah cryoprotectant). Macam :
   - Kering beku (liopilisasi)
   - Nitrogen cair : - 196 °C
   - Freezer : - 70 s/d – 120 °C

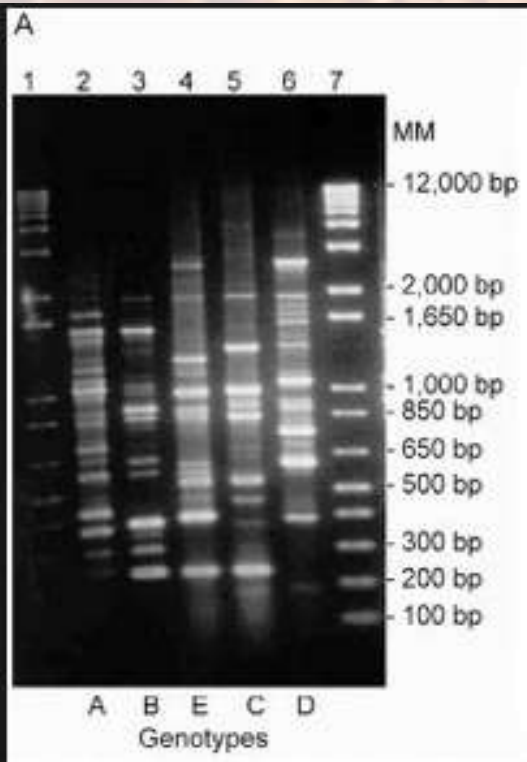
Conventional Classification Major characteristics used in conventional classification:

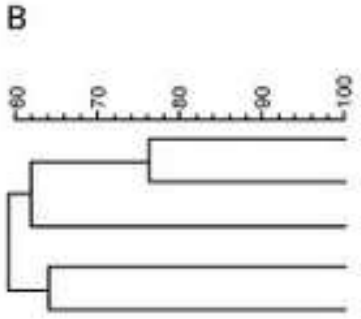
FEATURE:	
Cell shape	Cell wall constituents
Cell size	Energy sources
Colonial morphology	Fermentation products
Ultrastructural characteristics	Growth temperature optimum & range
Staining behaviour	Osmotic tolerance
Mechanism of motility	Oxygen relationships
Cellular inclusions	pH optimum & growth range
Carbon & nitrogen sources	Sensitivity to metabolic inhibitors & antibiotics

Numerical taxonomy, the use of computers. The similarity matrix and conversion to dendrogram (phenogram)



Selection of characters for identification purposes.





Genotype	Species	Specimen	Number
A	*P. putida*	marine sponge	2
B	*P. putida*	rectal swab	3
D	*P. putida*	rectal swab	6
E	*P. fluorescens*	secretion	4
C	*P. putida*	rectal swab	5

Fig. 2: A: RAPD-PCR typing of *Pseudomonas putida* and *Pseudomonas fluorescens* strains. Lanes 1 and 7: molecular size marker 1 kb plus; 2: *P. putida* Mm3 strain from marine sponge; 3: *P. fluorescens* clinical strain; 4-6: *P. putida* clinical strains. B: dendrogram from computer-assisted analysis of profiles shown in panel A.

http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0074-02762009000500002

Summary of bacterial groups (From Bergey's Manual of Systematic Bacteriology)

Section:	Example of Genus:	Habitat:
Spirochaetes	Treponema	Parasites of animals
Microaerophilic Gram-negative curved rods	Spirillum	Aquatic, animal parasites
Aerobic Gram-negative rods	Pseudomonas	Soil, water, animal parasites
Facultatively Anaerobic Gram-negative rods	Salmonella	Intestinal tracts, animal parasites

Summary of bacterial groups (From Bergey's Manual of Systematic Bacteriology):

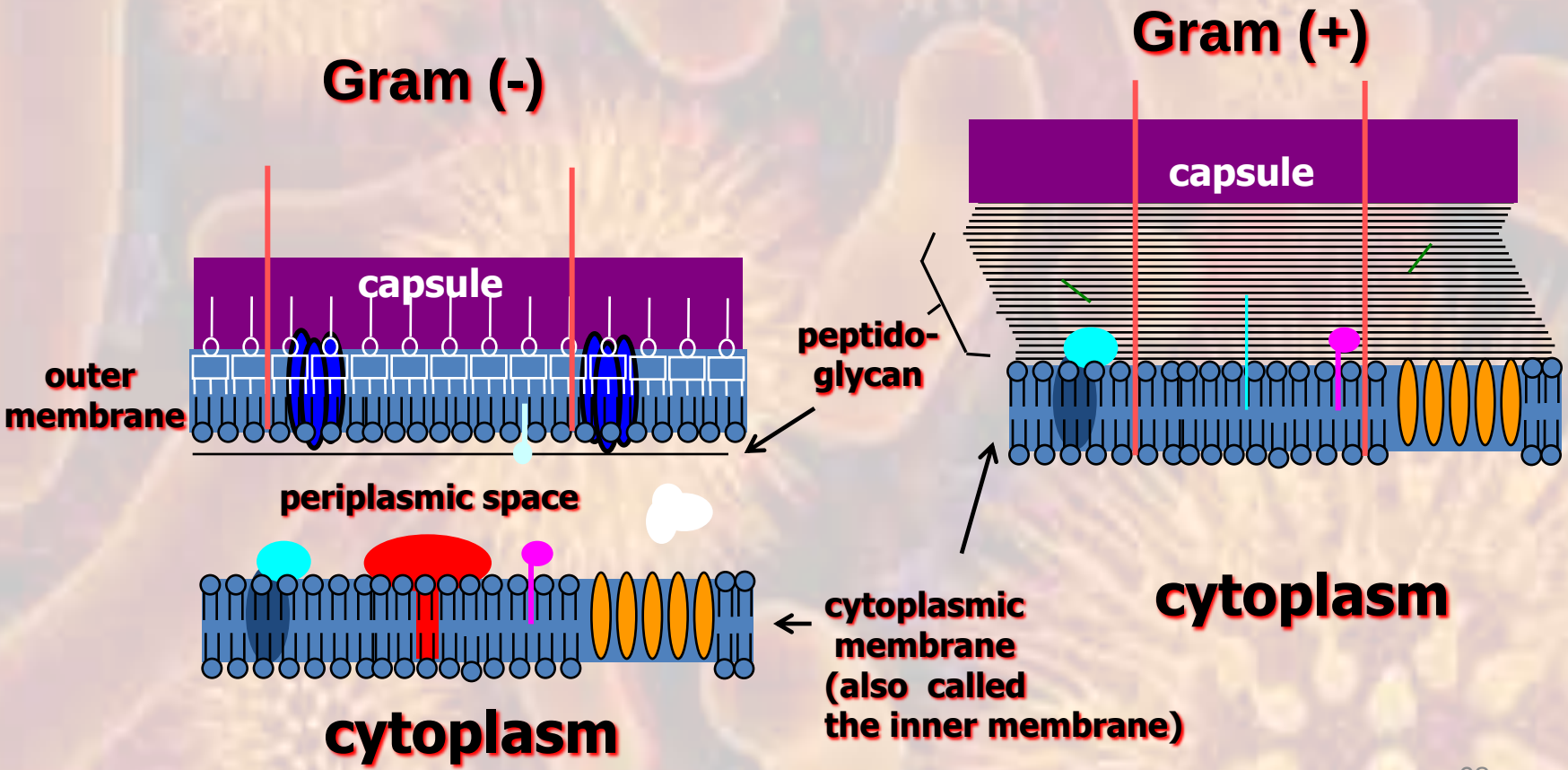
Section:	Example of Genus:	Habitat:
Anaerobic Gram-negative rods	Bacteroides	Animal, insect parasites
Sulphate reducers	Desulfovibrio	Anoxic sediments
Anaerobic Gram-negative cocci	Veillonella	Animal intestines
Rickettsias, Chlamydias	Rickettsia	Arthropods, animal parasites (obligate intracellular)

Section:	Example of Genus:	Habitat:
Mycoplasma	Mycoplasma	Animal, plant parasites, no cell wall
Endosymbionts	Holospora	Symbionts of protozoans, insects, plants
Gram-positive cocci	Staphylococcus	Animal pathogens
Endospore formers	Bacillus	Soil, animal pathogens
Regular Gram-positive rods	Lactobacillus	Dairy products, animal intestines
Irregular Gram-positive rods	Corynebacterium	Soil, animal pathogens

Section:	Example of Genus:	Habitat:
Mycobacteria	Mycobacterium	Soil, plants, animal pathogens
Nocardioforms	Nocardia	Soil
Appendaged bacteria	Caulobacter	Aquatic, soil
Sheathed bacteria	Sphaerotilus	Aquatic

Section:	Example of Genus:	Habitat:
Sliding bacteria	Cytophage	Aquatic
Fruiting bacteria	Myxococcus	Soil
Aerobic lithotrophs	Nitrobacter	Soil, water
Archaeobacteria (archaea)	Halobacterium	Extreme environments
Anoxygenic phototrophs	Chromatium	Waters
Oxygenic phototrophs	Anabaena	Soil, water
Actinomycetes	Streptomyces	Soil


Thanks so much


Gram (-)
capsule
outer membrane
periplasmic space
cytoplasm
Gram (+)
capsule
peptido-glycan
cytoplasmic membrane (also called the inner membrane)
cytoplasm

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