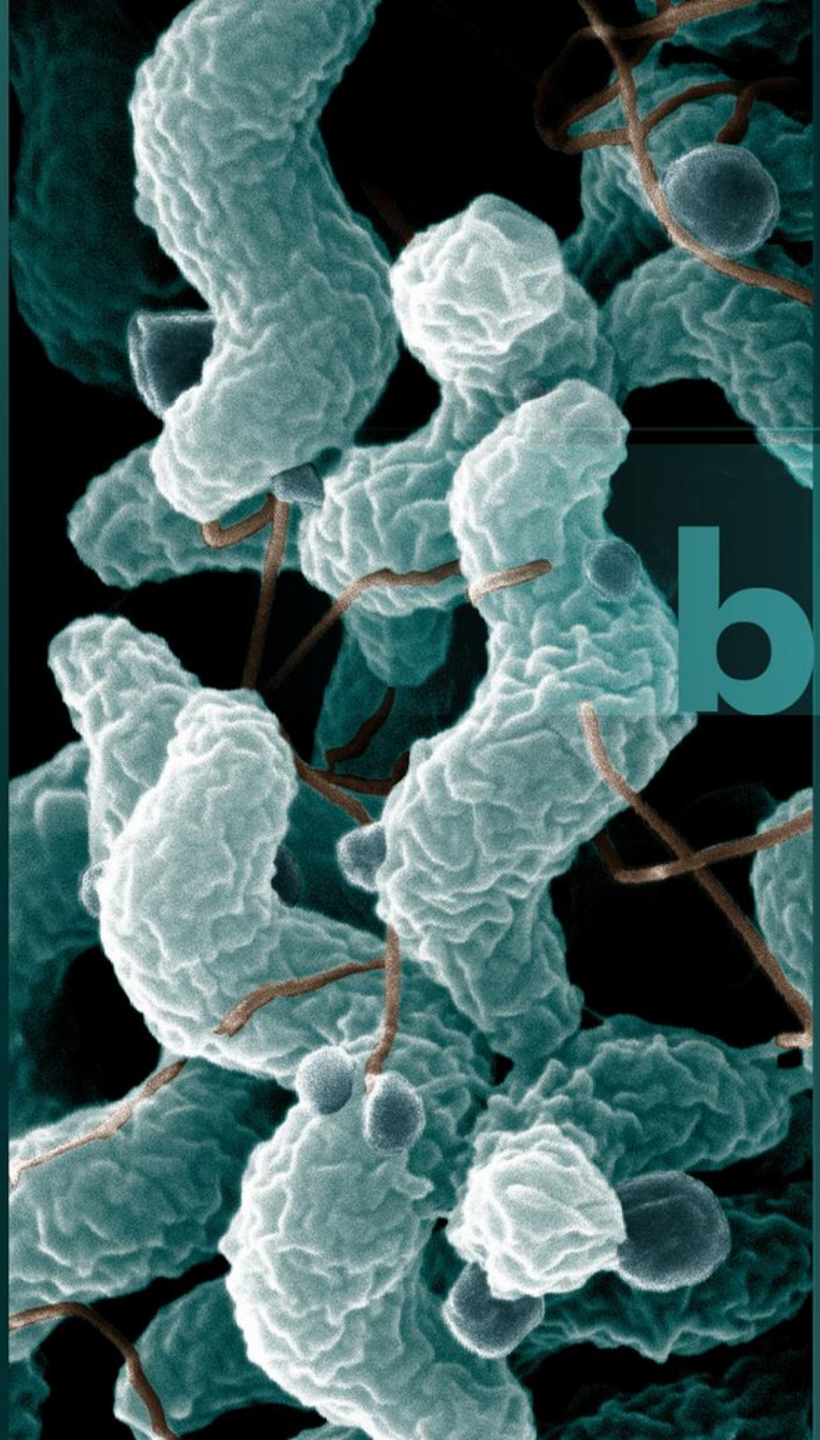
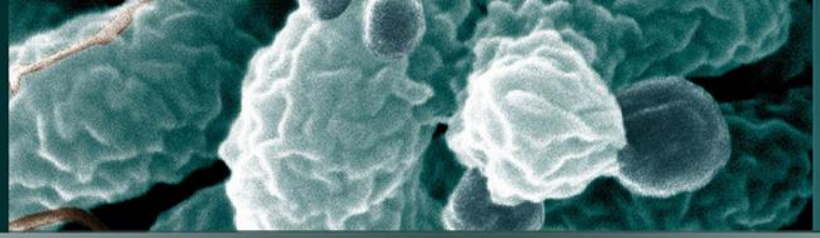


3rd Seminary from microbiology FaF VFU BRNO (theory for lab class no. 2)

bacteria

Diagnostic procedures (I)



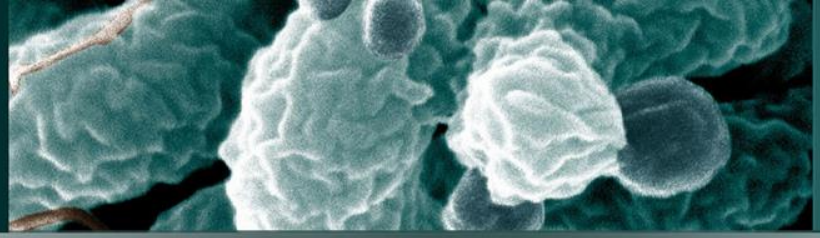
PROCEDURE

Doctor:

- Takes the sample from patient – proper way, dispatch form

Microbiology lab:

- Microscopy + staining (antigens, DNA in sample)
- Preliminary result -> ATB therapy
- Cultivation and isolation of agens
- Determination of sensitivity of bacteria to ATB
- Definitive ATB therapy



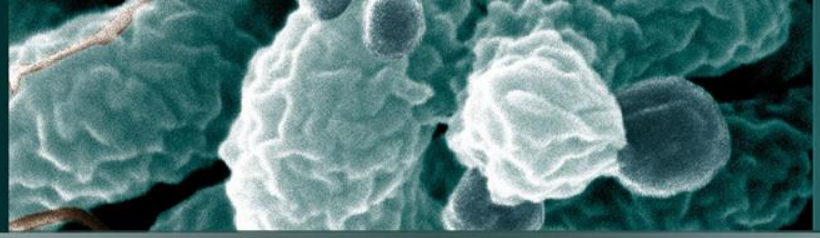
SAMPLING

Throat swab

- In the morning, on an empty stomach, before cleaning teeth
- Helical movement with a sterile cotton swab the surface of both tonsils and palatine arches
- With top of the swab take pus
- Put into transport media

Swab from the nose

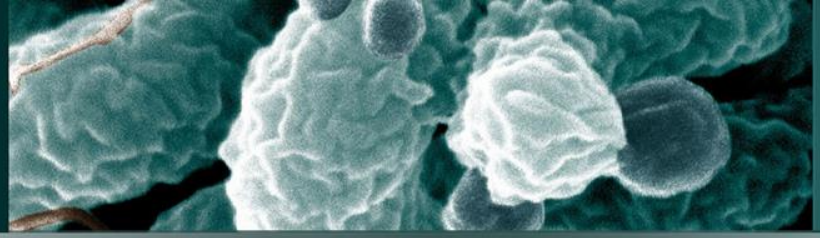
- With a sterile cotton swab both nostrils, so the surface of cotton is covered with secretions
- Put into transport media



SAMPLING

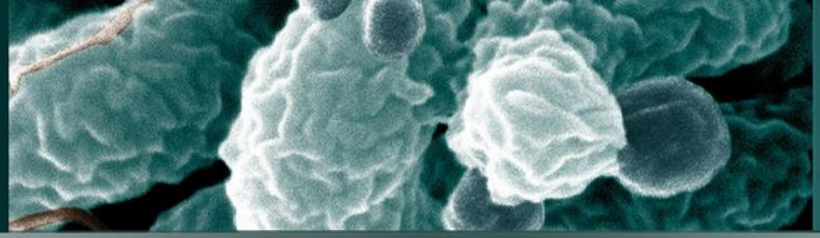
Urine collection

- In the morning
- Properly wash genitals with soap and water, including the external urethral orifice
- Urinate into **sterile** test-tube, collect the midstream urine – e.g. Urinate and after several seconds collect the sample (elimination of skin microbiota contamination)
- Collect 10–20 ml of urine
- Collection must be done out of menstruation
- Deliver to laboratory in 2 hours



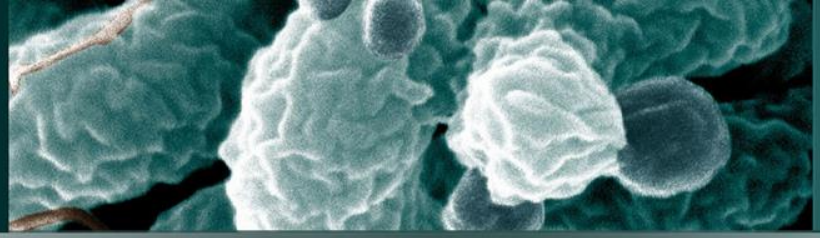
Direct methods

- Microscopy – native preparation
- Simple staining
- Diagnostic staining (Gram method)



Native preparation

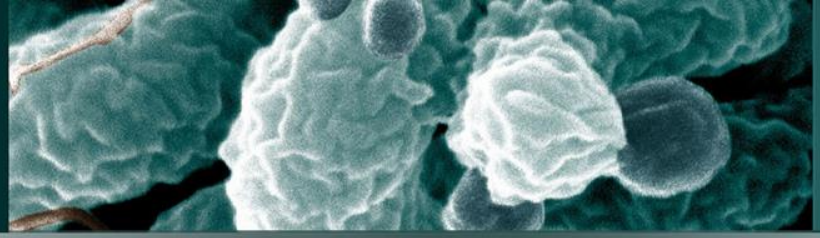
- Microbes in natural unmodified state
- Fysiological solution or liquid medium
- Usage:
  - Less often in bacteriology
    - Rolling movements of Listeria at room temperature
    - Suspect examination of Treponema from chancre
    - Evidence of Leptospira
  - More often in parasitology
    - Stool samples – amoebas and flagellates
    - Trichomonas in soil or vaginal secretion
  - Mycology
    - Preparations from skin, nails and hairs



MICROSCOPY

Fixed preparation

- Sampling to the glass slide
  - Prepared for staining
  - Directly from clinical sample (except urine – very thin - sedimentation)
  - From bacterial cultures
- Fixation
  - Denaturation of bacterial proteins, but friendly
  - Chemical or heat



STAINING

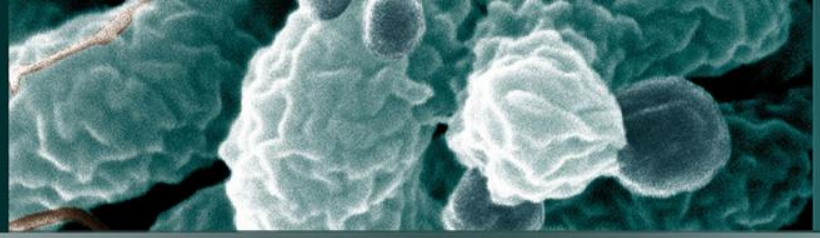
Simple staining

- Determination of size, shape and arrangement of microbes
- Internal organization

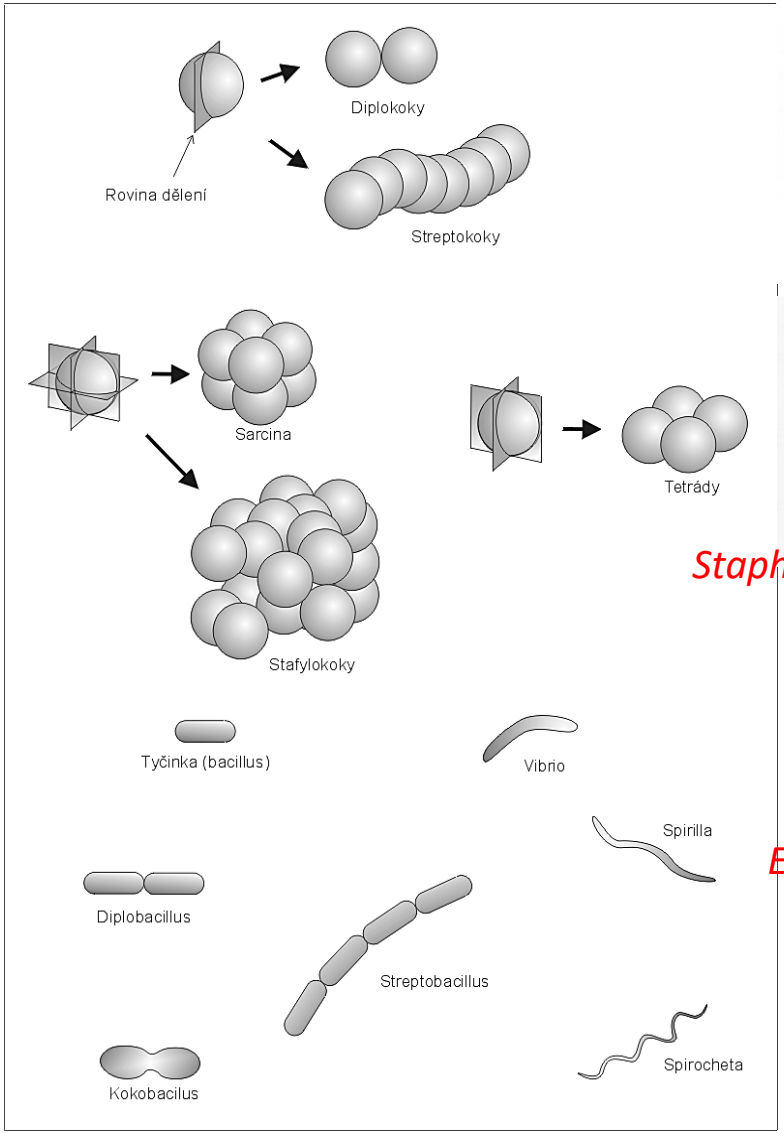
Diagnostic staining

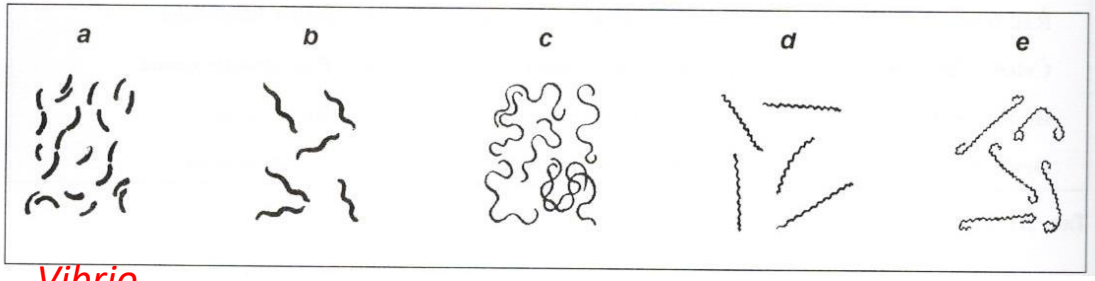
- Diagnosing particular groups of microbes
- Directed to particular structure

  - Gram staining
  - Staining for acidoresistant microorganisms
  - Demonstration of capsules, spores, flagellum
  - Cytological staining – Giemsa

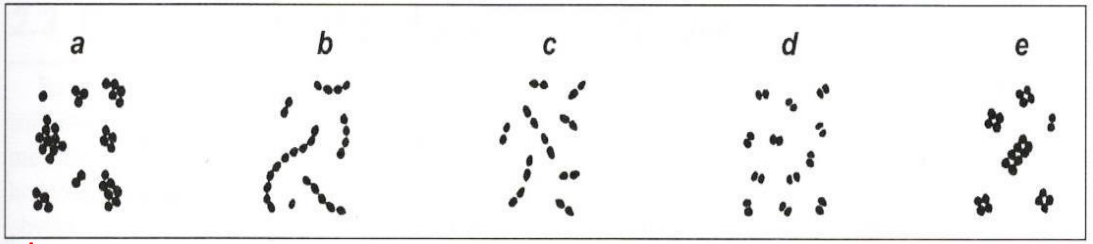


STAINING

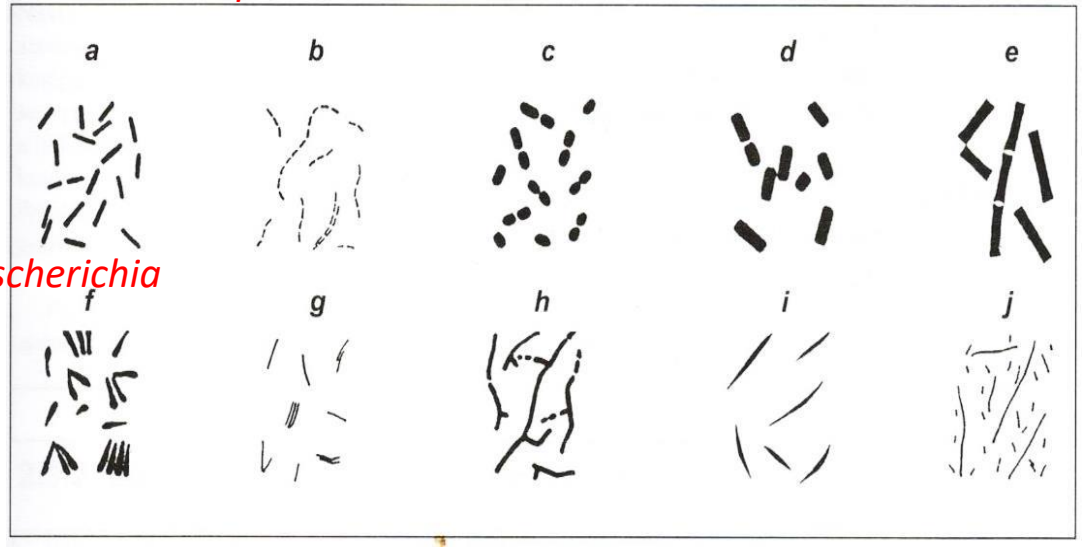




Vibrio

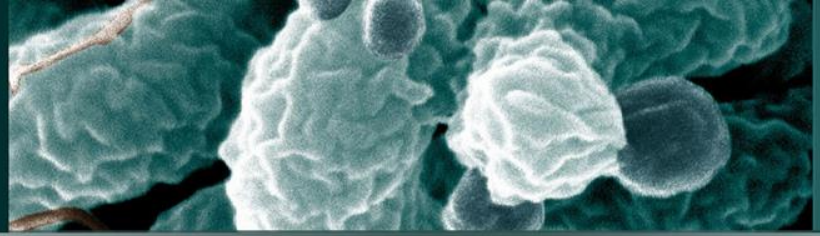


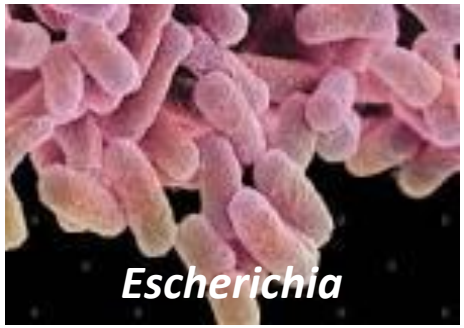
Staphylococcus *Streptococcus* *Micrococcus*

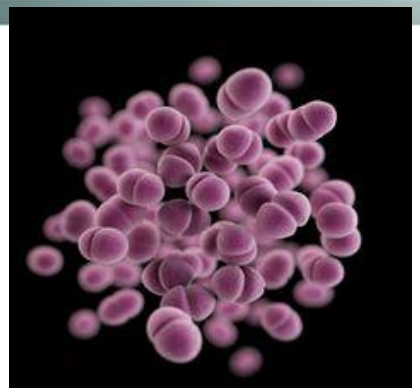


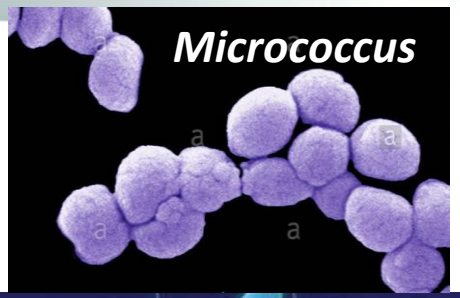
Escherichia

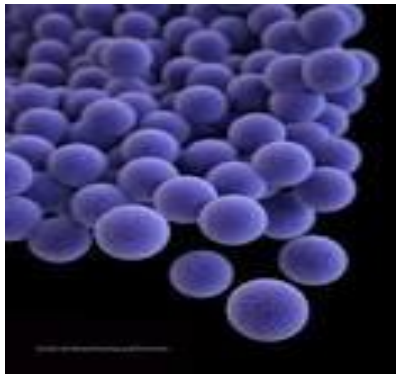
STAINING

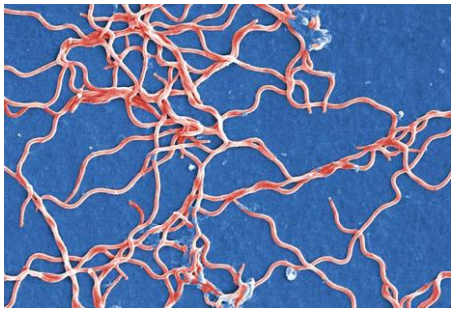


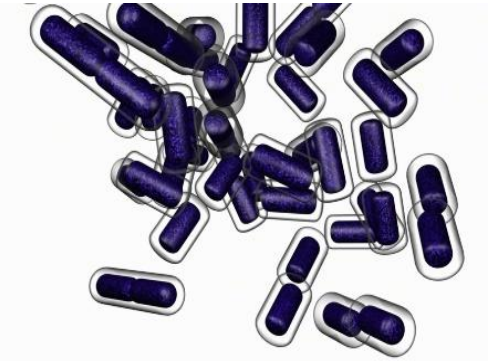


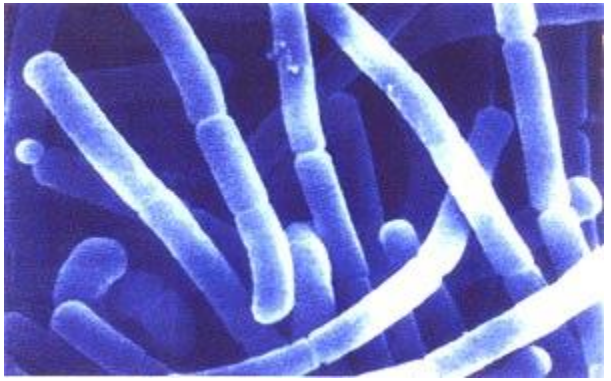


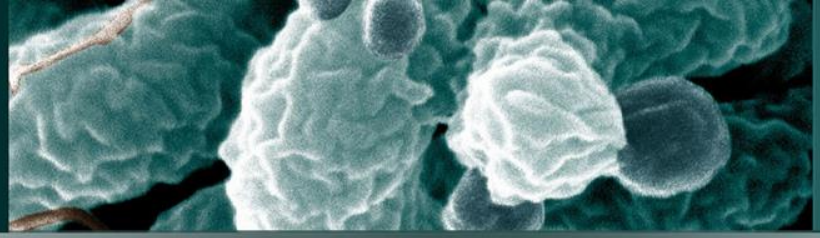




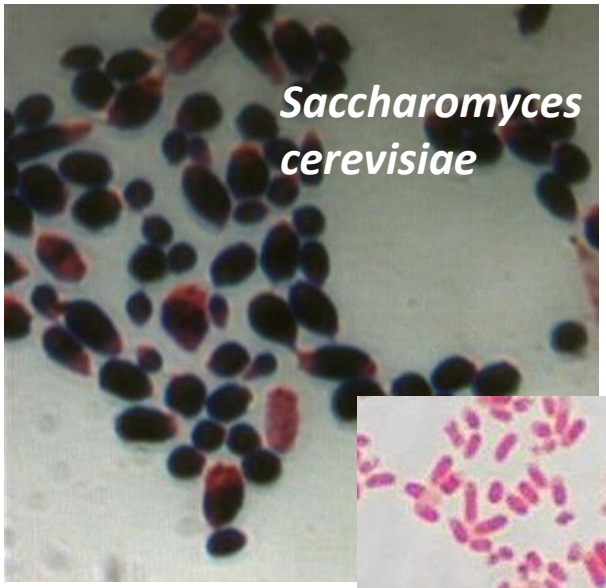


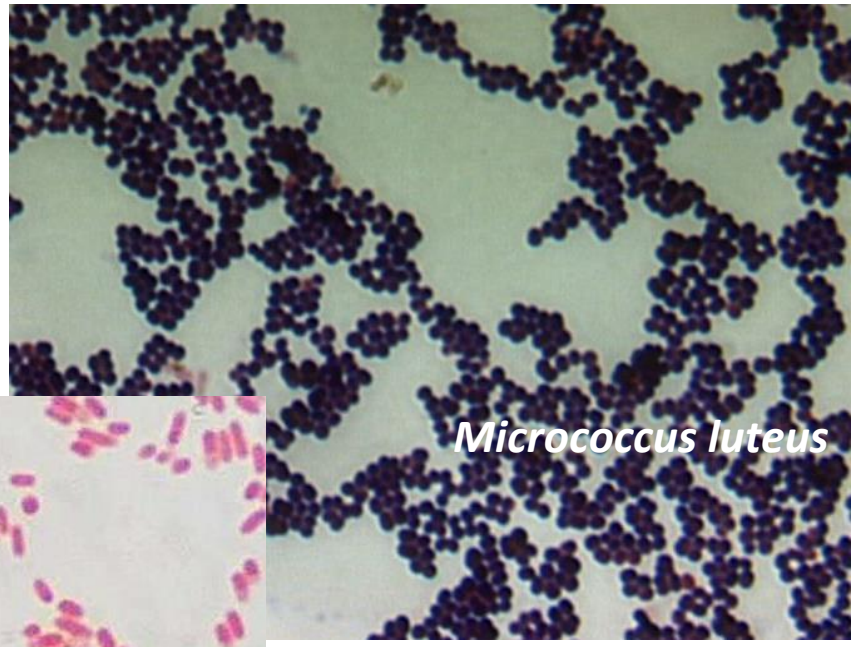


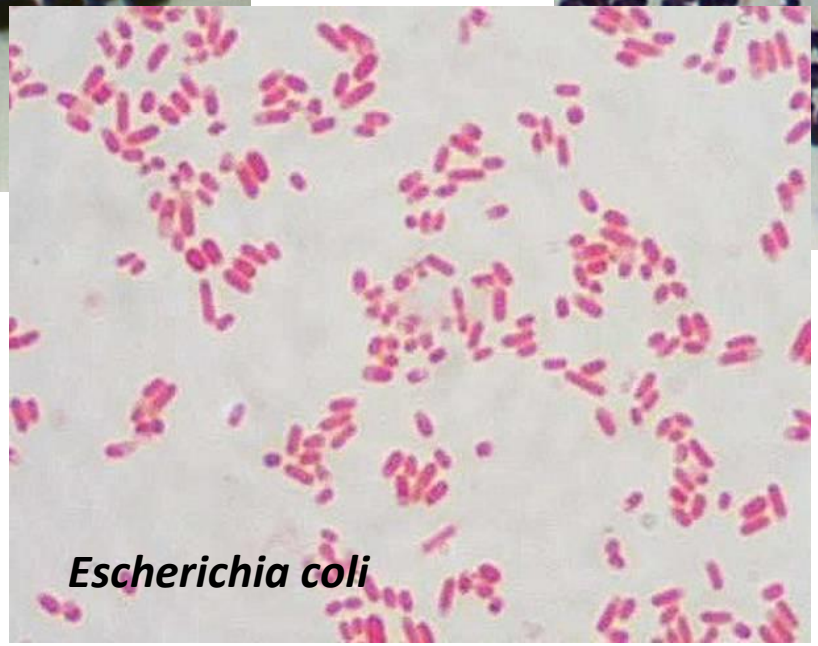




STAINING

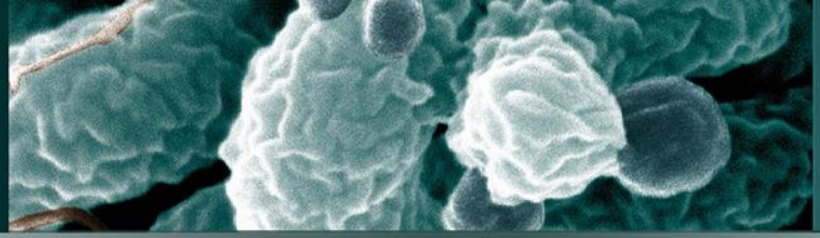






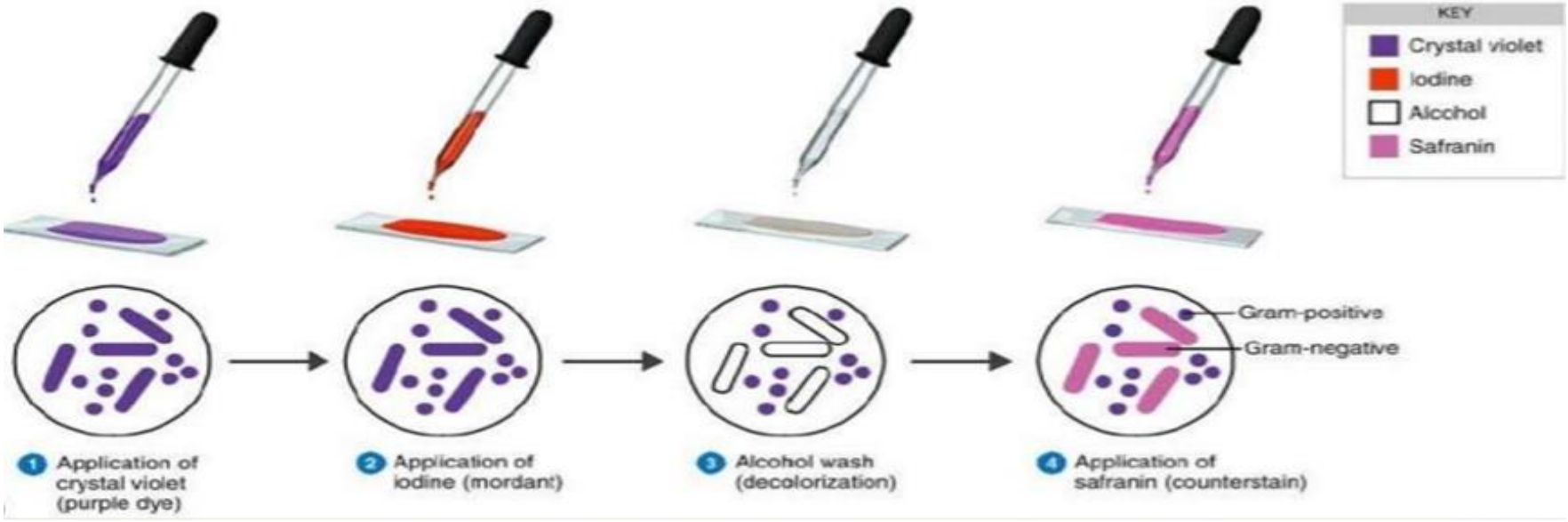
https://www.biolib.cz/

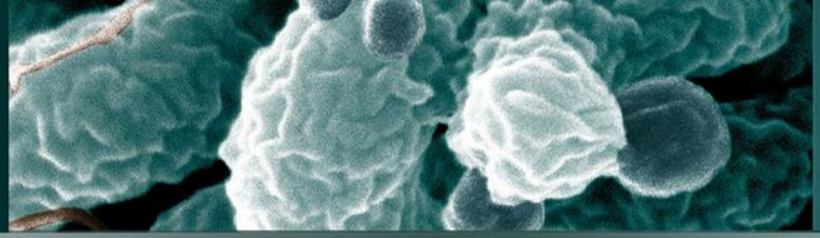
Light microscope



GRAM STAINING

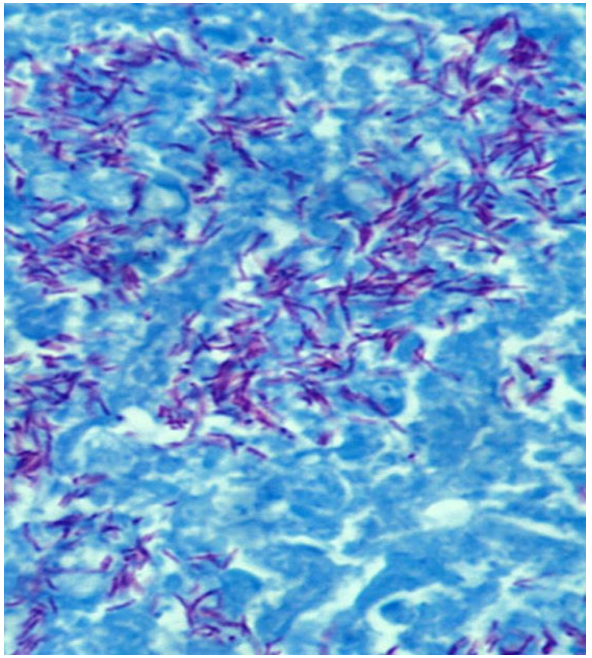
- *Grampositive* (G+) - crystal violet, Lugol solution, safranin
- *Gramnegative* (G-)
- *Gramlabile* (G±)
- not stained (acidoresistant bacilli and bacterial spores)
- some stain poorly (spirochets, e.g. *Treponema pallidum*)



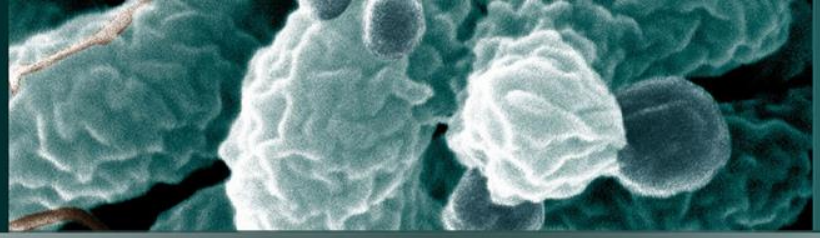


ACIDORESISTANT STAINING

- Microorganisms with structures which do not uptake common dyes
- Bacterial spores
- Spores of yeasts
- Parasite cysts
- Mycobacteria
- Nocardia
- Actinomycetes



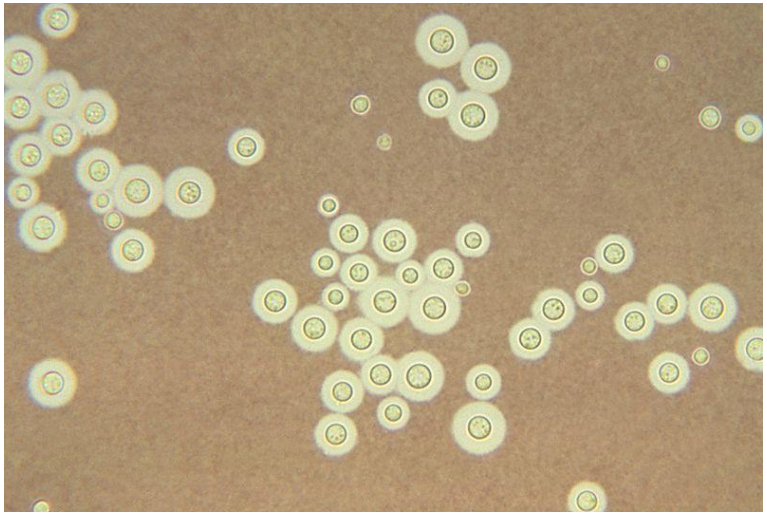
- **Ziehl-Neelsen staining**
  - carbolfuchsin, acidic alcohol, malachite green
- Fluorescence staining for acidoresistant bacilli

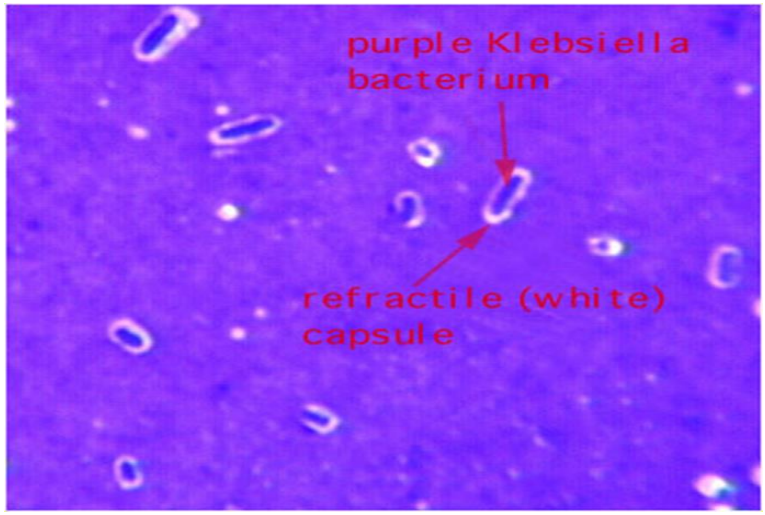


CAPSULE STAINING

- Capsules are hardly stained
- Negative imaging of capsules according to **Burri**
  - negative imaging with ink, carbolfuchsin

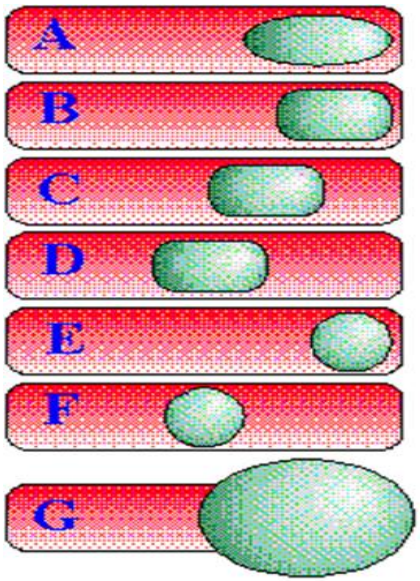
- *Klebsiella pneumoniae*
- *Streptococcus pneumoniae*

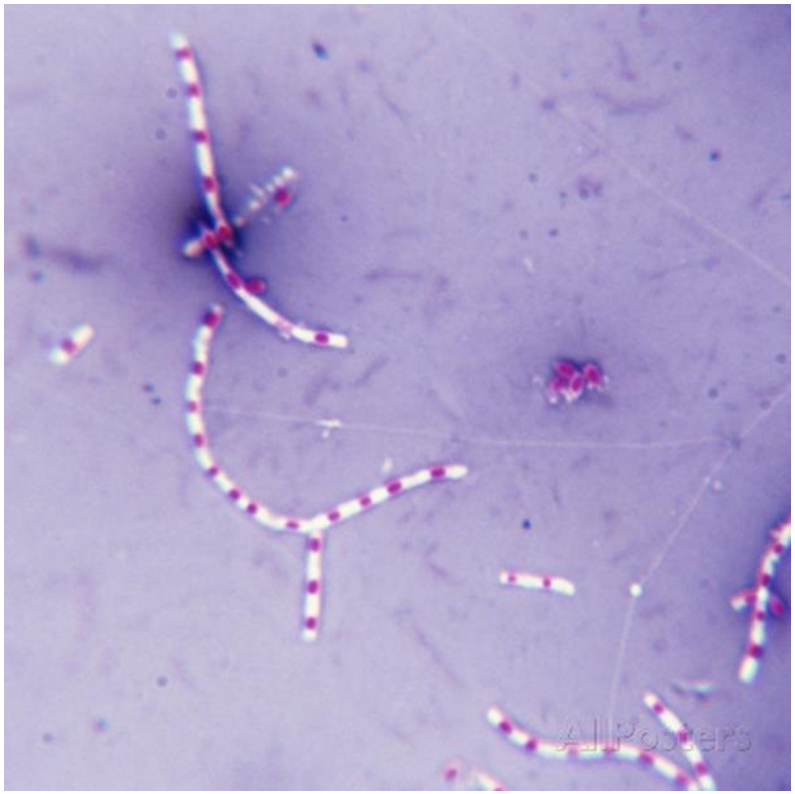


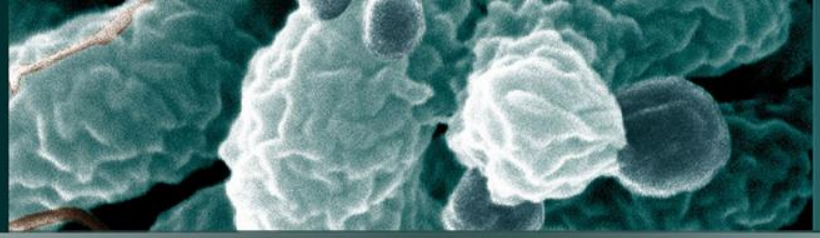


STANING OF SPORES

- According to **Wirtz and Conklin**
  - malachite green, carbolfuchsin or safranine
- *Bacillus*, *Clostridium*

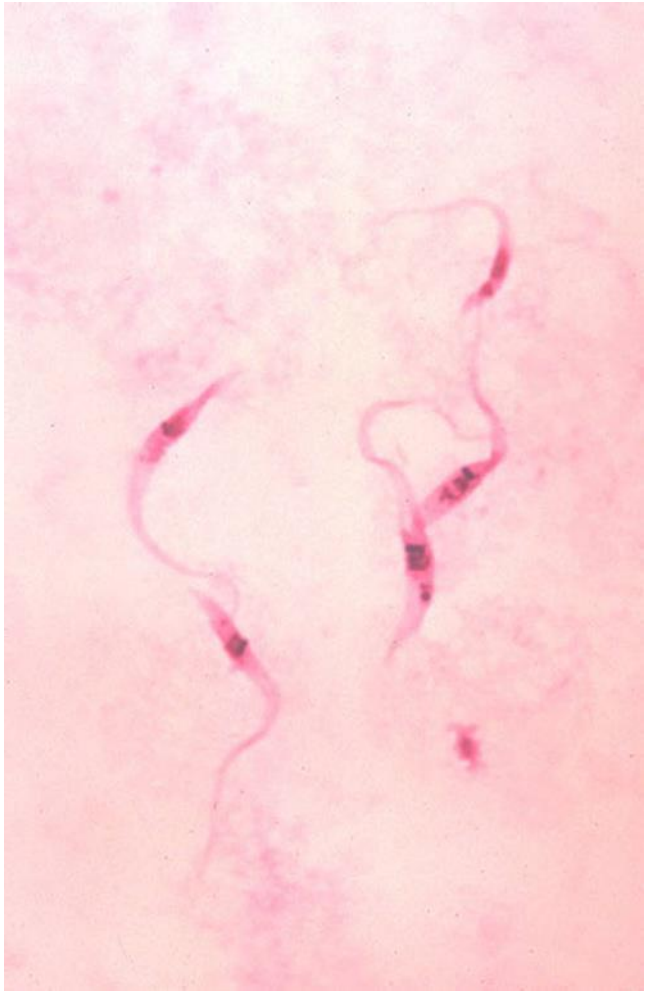




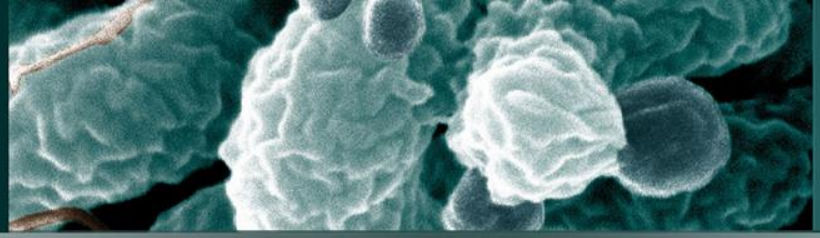


GIEMSA STAINING

- Staining according to **Giemsa-Romanowsky**
  - blood samples
  - protozoa, rickettsia, chlamydia
  - spirochets
  - mycoplasmata
  - malaria, blood parasites

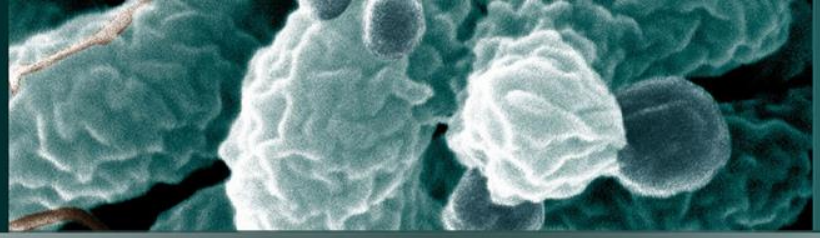


- a) Cationic (alkaline) dye azure B.
  - binds to anionic parts of molecules and stains to blue-grey nucleic acids, nucleoproteins, granules of basophils and secondary granules of neutrophils.
- b) Anionic (acidic) dye eosine Y
  - binds to cationic parts of molecules of proteins and stains red-orange haemoglobine and eosinophil granules.



PROCEDURES

- after microscopic evaluation and staining – isolation and cultivation of bacteria
- evaluation of appearance, colour,..
- growth on different media
- biochemical activity
- presence of antigens
- MALDI-TOF



CULTIVATION OF BACTERIA

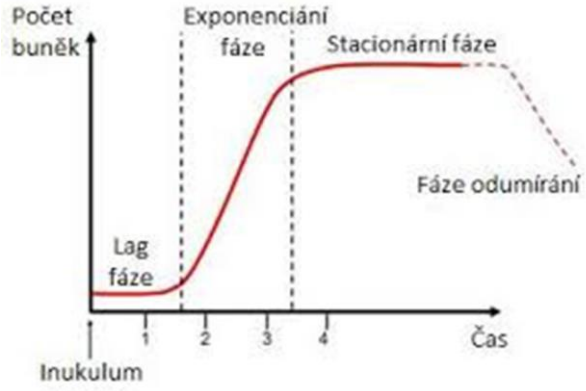
Liquid/growth/ broth media

- liquids → bacteria grow: surface (aerobics), turbidity (from cca 10^6 cells/1ml (microaerophilic) sediment (anaerobic)

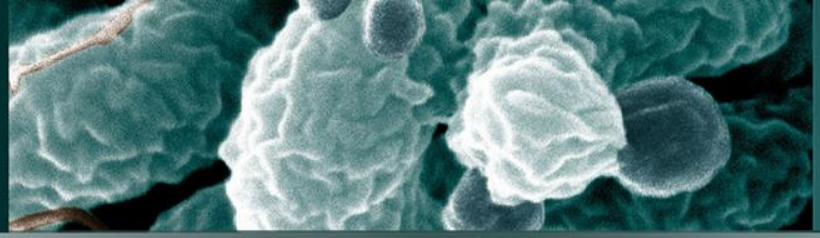
1. Static cultivation

- closed system
- composition and properties of media are changed by bacteria
- factors limiting bacterial growth: exhaustion of nutrients, cumulation of metabolites

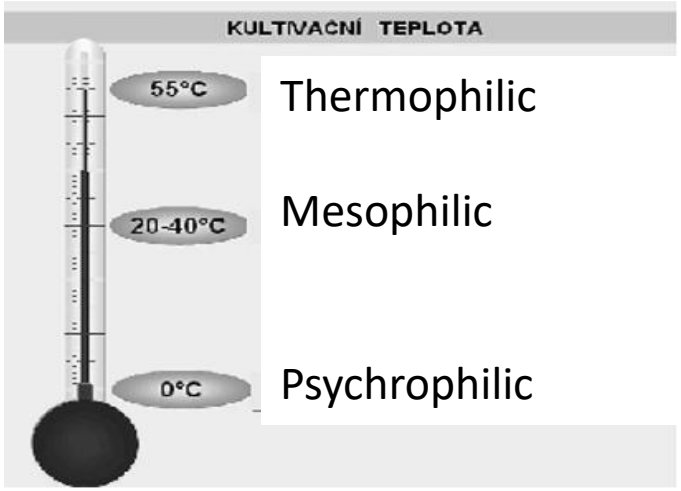
1. Lag phase
2. Phase of accelerated growth
3. Logaritmic, exponential phase
4. Phase of inhibitory growth
5. Stationary phase
6. Declination phase, accelerated dying



2. Continuous cultivation – flow



CULTIVATION OF BACTERIA

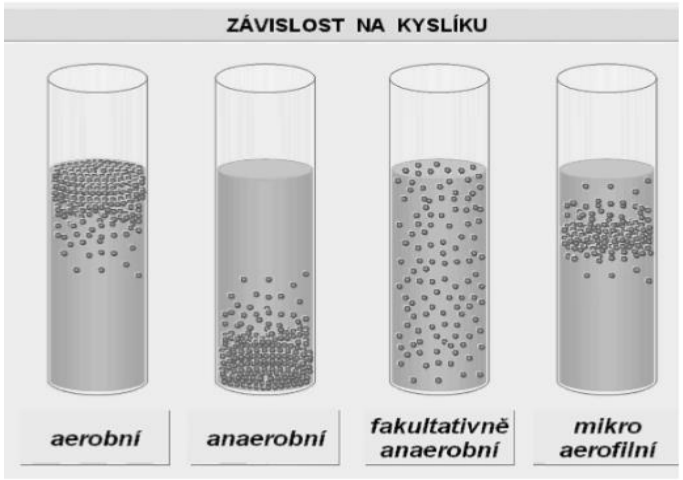


Thermophilic

Mesophilic

Psychrophilic

Aerobic Anaer. Fac. Anaer/microaerofilic



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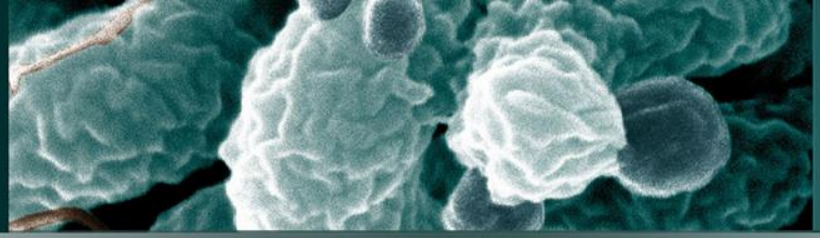
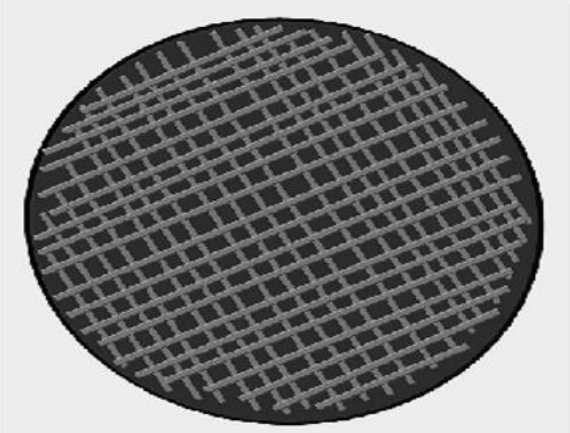
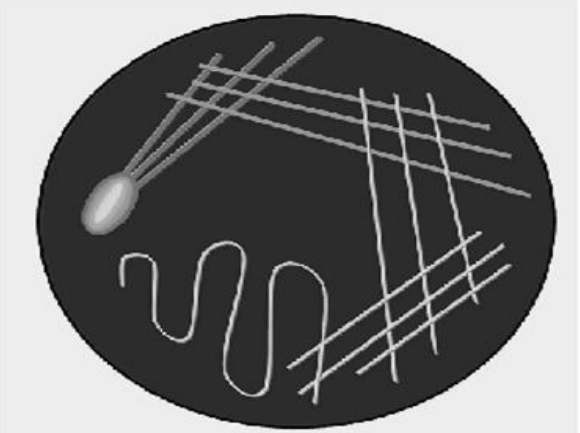


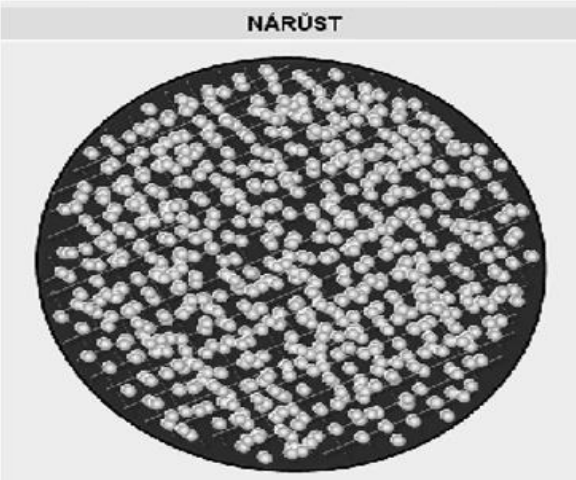
CULTIVATION OF BACTERIA

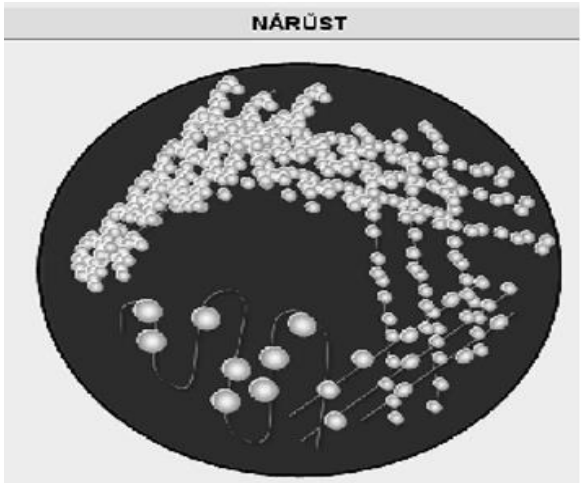
Masive isolation



Cross scattering







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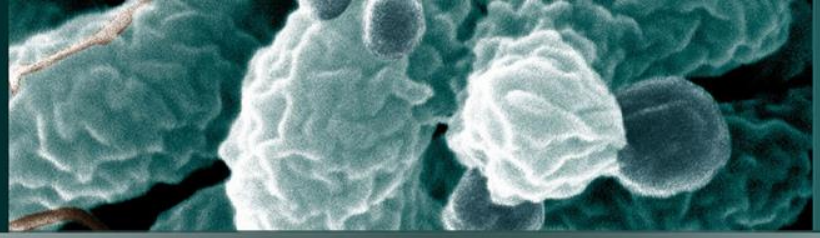
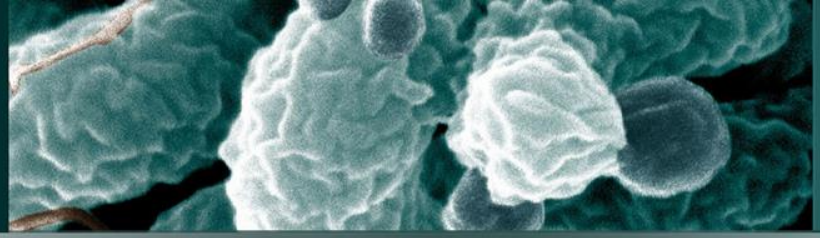


EXPERIMENTS ON ANIMALS

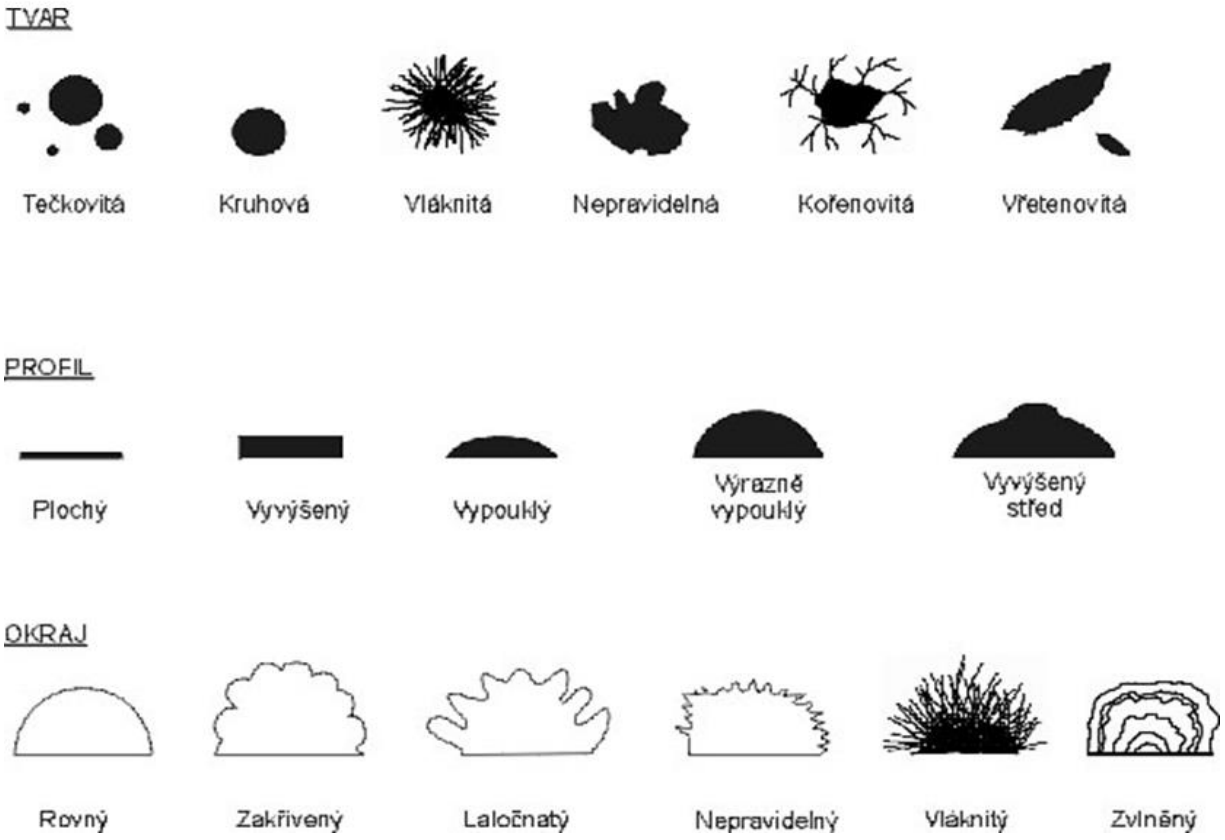
- evidence of infective agens
  - tuberculosis, tularemia, brucelosis, plague, anthrax
- evidence of bacterial toxins
  - botulismus, diphteria, tetanus, anaerobic traumas
- for neutralisation and protective tests
  - vaccinations and imunoglobulins – efficacy is tested
- for preparation of antigens and imunne serums
  - *Treponema pallidum*
- for acquisition of erythrocytes and complement

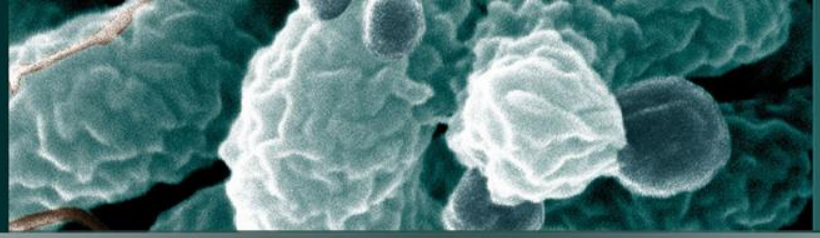


IDENTIFICATION OF ISOLATED STRAIN

Evaluation of colonies

- size
- form
- profile
- edges
- surface
- transparency
- colour
- changes around
- consistency
- smell



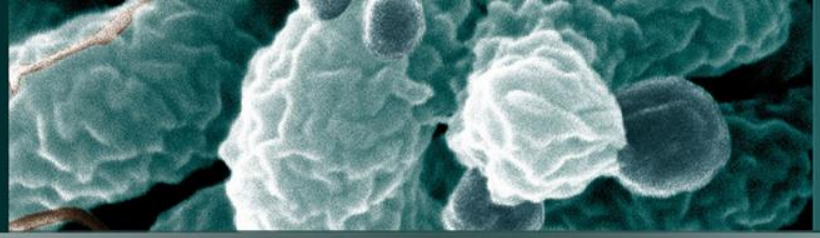


IDENTIFICATION OF ISOLATED STRAIN

Colony v M-phase (mucous):

- colonies have mucous appearance and consistency, they are semi-circular shape bulging, with sharp edges, tendency to join together

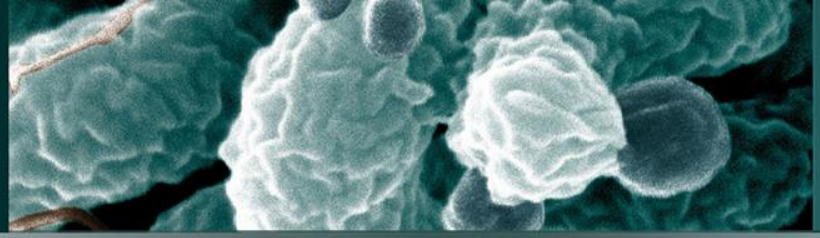
- bacterias are capsulated, bacilli are shorter, sterptococci are mostly in pairs or short chains



IDENTIFICATION OF ISOLATED STRAIN

Colony in S-phase
(smooth)

- more smooth, shiny and flatter then in M-phase
- bacilli are longer, cocci longer chains
- microbes in this phase are most virulent, due to capsules



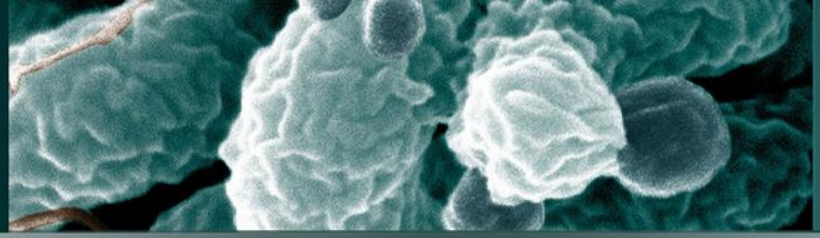
IDENTIFICATION OF ISOLATED STRAIN

Colony in R-phase (rough)

- rough surface, irregular edges, very often ridged with elevated center

- long bacilli and chains of cocci

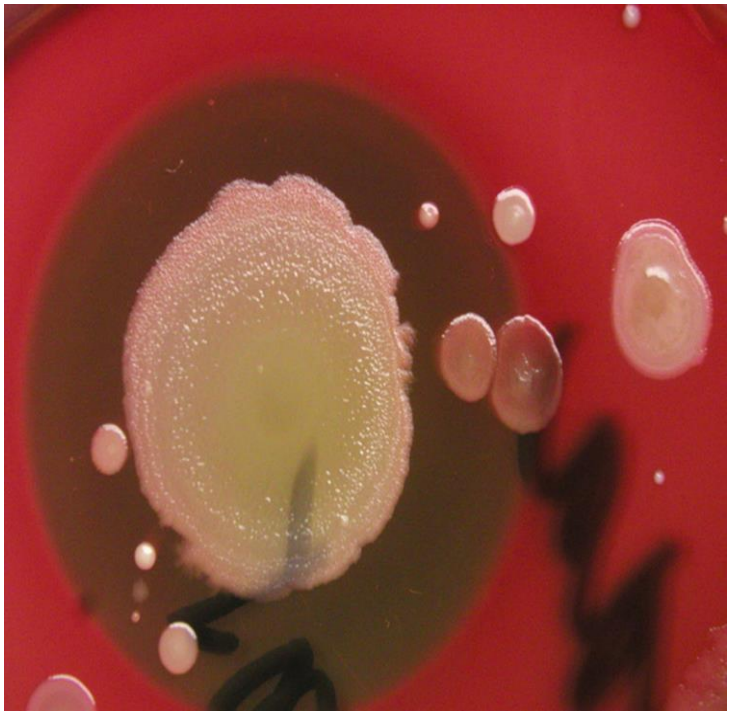
- virulence is reduced or absent



IDENTIFICATION OF ISOLATED STRAIN

Size

- dots
- expressed as diameter in mm



Surface

- smooth, shiny, dim, rough, laminated, grany

Transparency

- transparent, translucent, opaque

Consistency

- stricky, rough, smooth, ingrown into agar

Smell

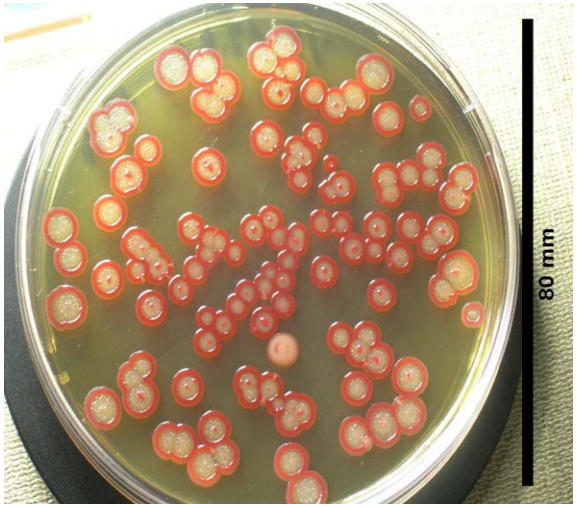
- subjective (fecal, samprogenous, acid, sugary…)

IDENTIFICATION OF ISOLATED STRAIN

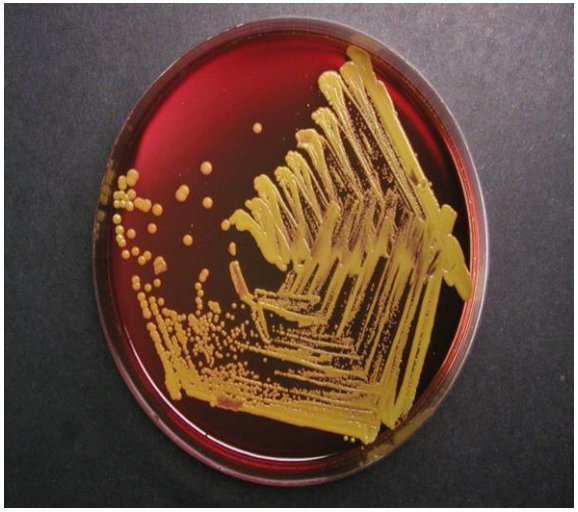
Colour

- Grey-white/ color-less/production of pigment
- change in colour of an indicator, e.g. lactose cleaving or non-cleaving enterobacteriae on selective diagnostic media containing lactose

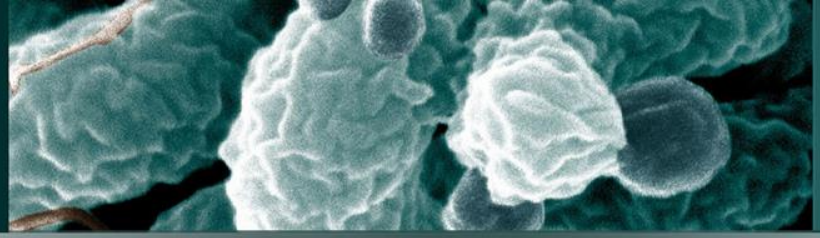
Milk agar – production of pigments



Serratia marcescens



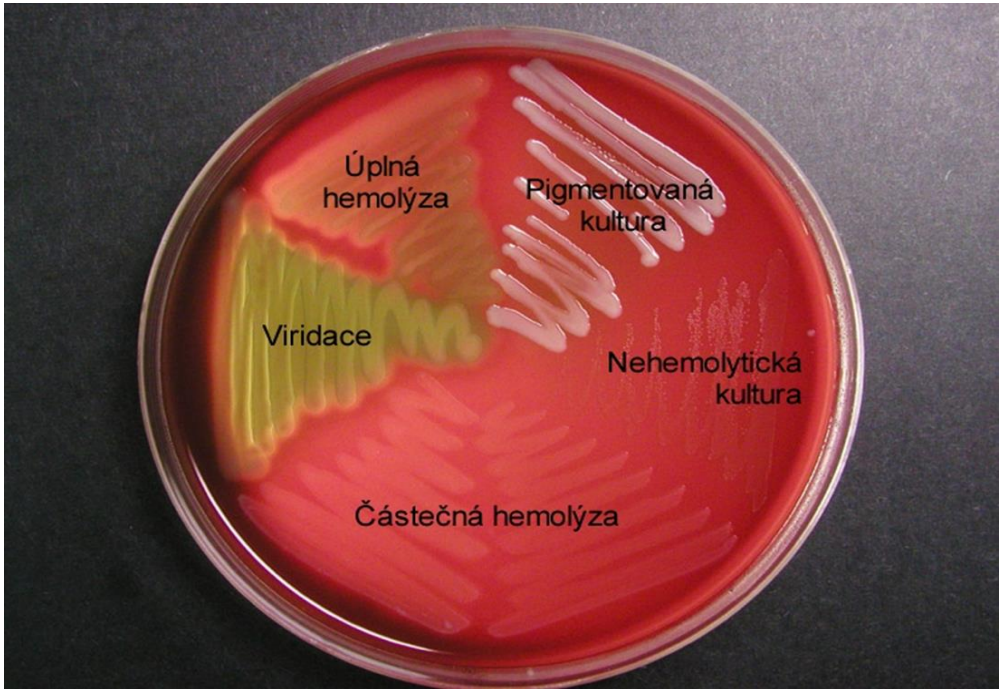
Micrococcus luteus

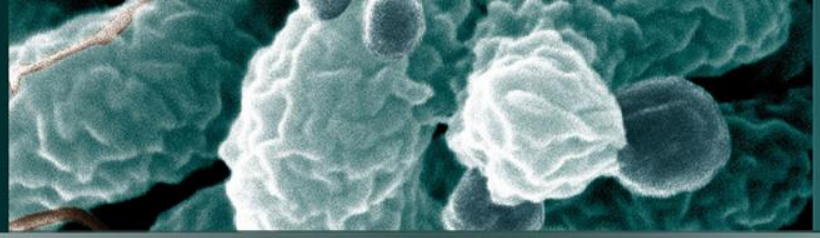


IDENTIFICATION OF ISOLATED STRAIN

Changes in environment

- evaluation of change in colour (production of water-soluble pigments, change in pH – change in indicator colour)
- **hemolysis of erythrocytes** and **greeing** (viridation):

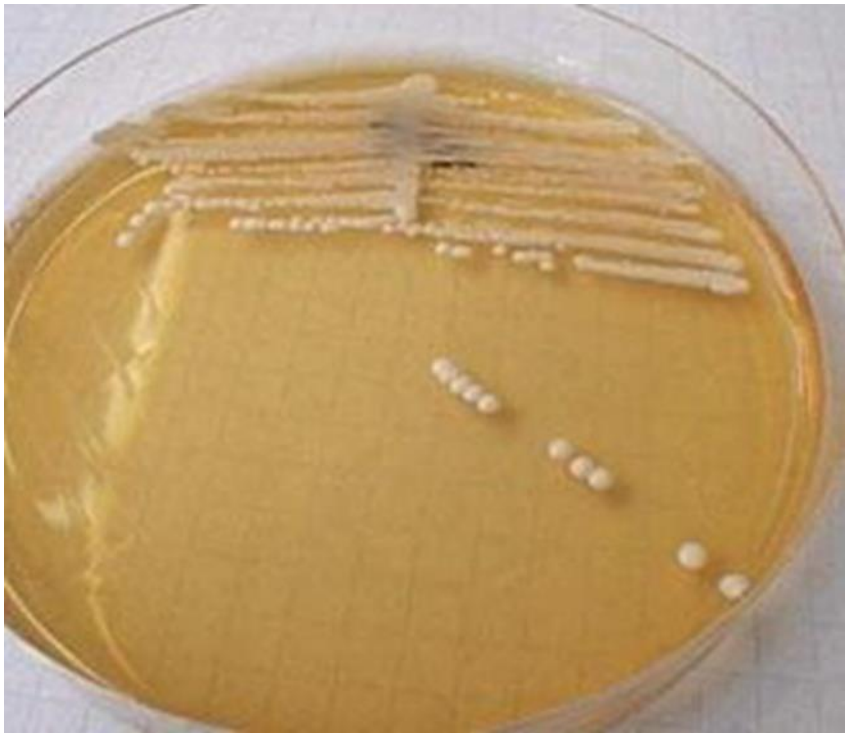


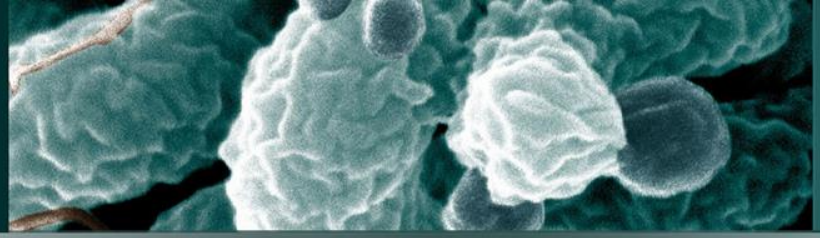


IDENTIFICATION OF ISOLATED STRAIN

Yeasts

- colonies similar to bacterial colonies
- genus *Candida*: mostly white, smooth surface

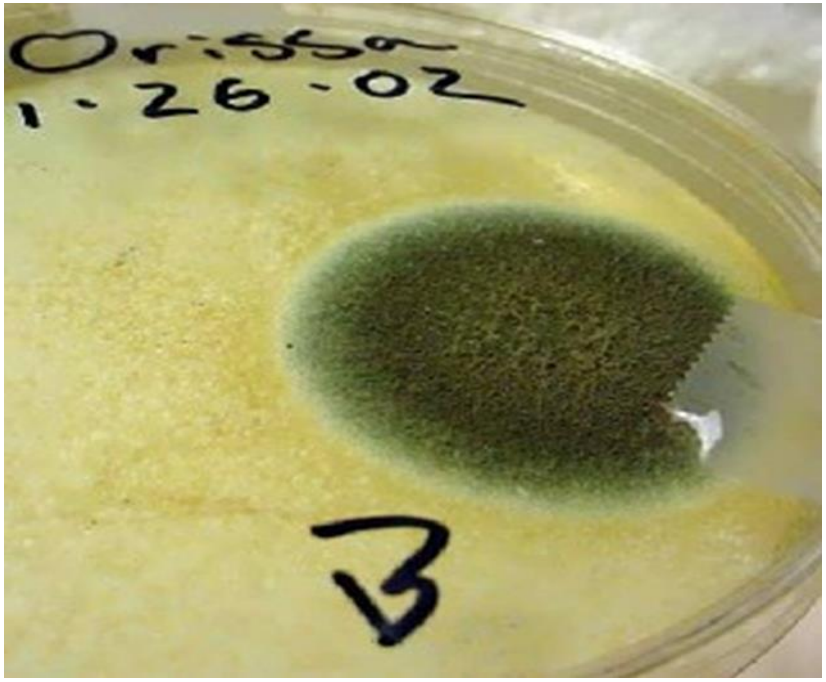




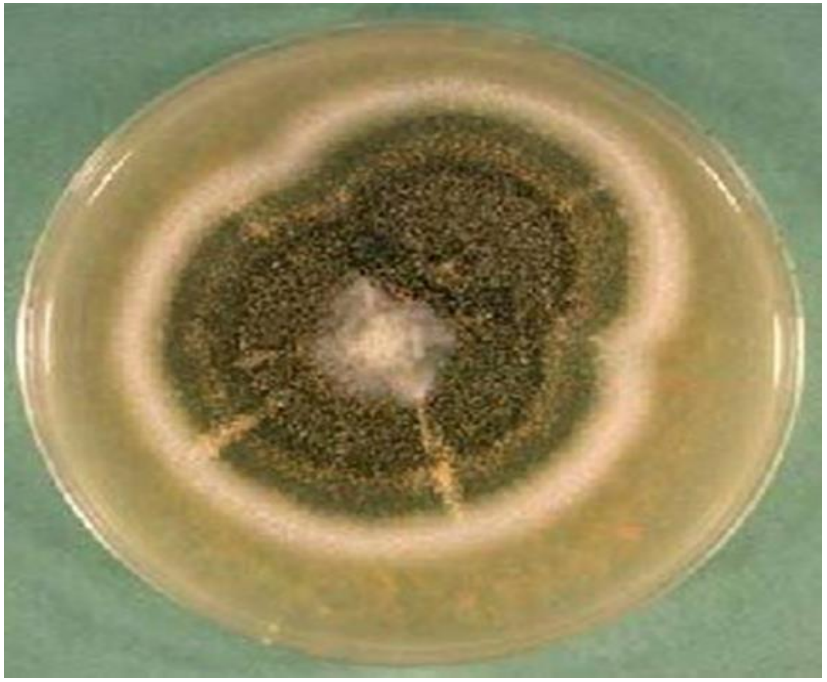
CULTIVATION OF MOULDS

Moulds

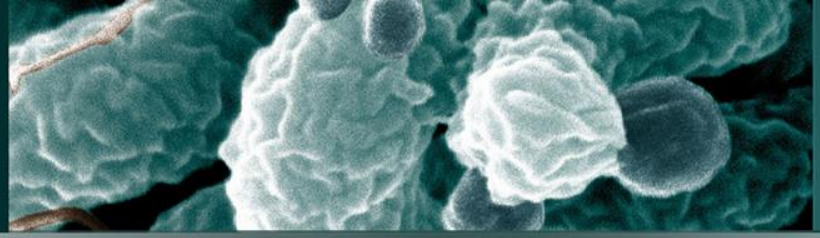
- white-grey-greenish colonies, irregular edges
- changes in colour from edges to center



Trichoderma harzianum



Aspergillus nidulaus

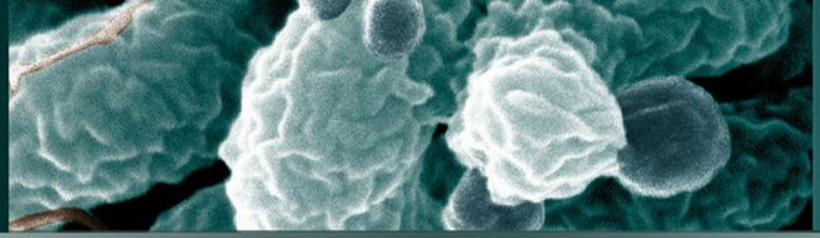


PROCEDURES

Direct methods (microb – part – product):

- Microscopy – evidence in sample and identification
- Cultivation – evidence in sample and identification
- Biochemical identification – only identification!
- Evidence of antigens – evidence in sample and identification
- Nucleic acids – mainly evidence in sample
- Experiment on animals – mainly evidence in sample

Indirect metods (antibodies)



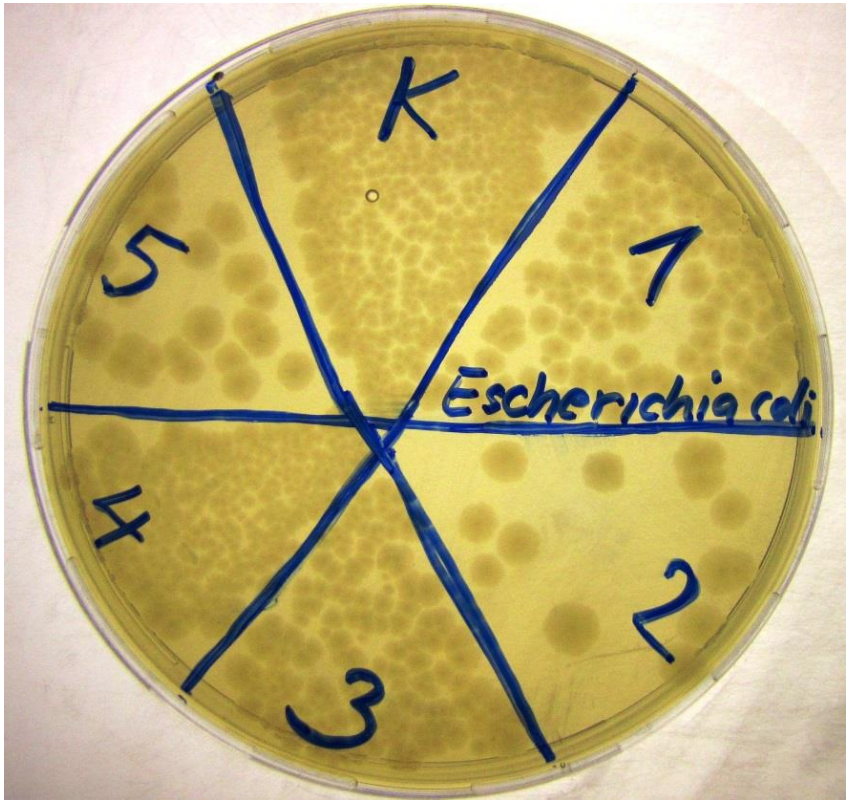
Lab class no. 2

Evaluation of efficacy of disinfectants and antispetics, evaluation of colonies, Gram staining, isolation inoculation

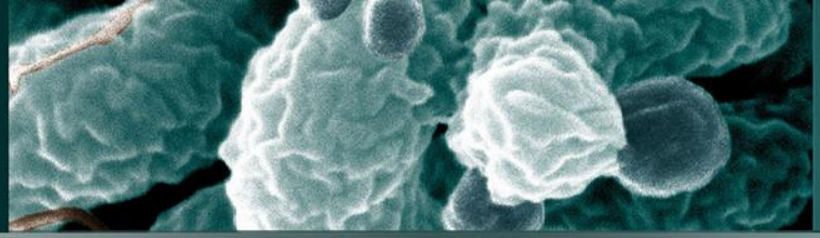
Aims:

- ***Evaluation of efficacy of disinfectants and antiseptics.***
- ***Evaluation of colonies***
- ***Gram staining***
- ***Isolation inoculation***
- ***Microscopy – stable preparates***

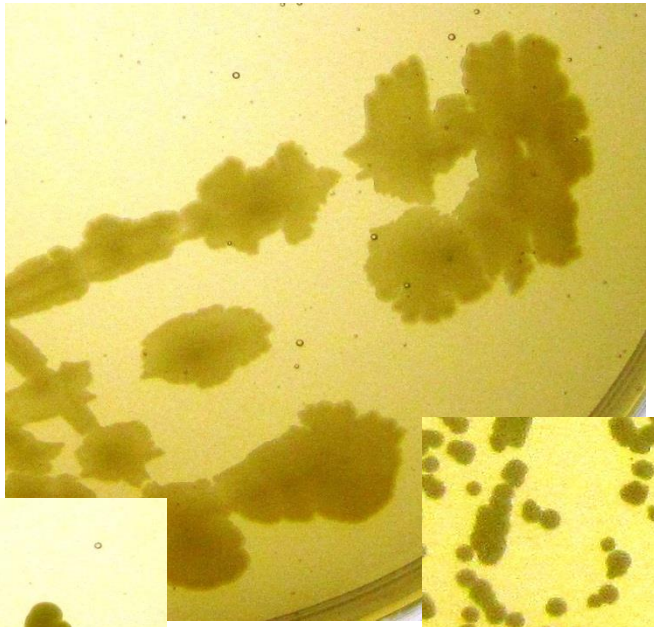
- ***Evaluation of efficacy of disinfectants and antiseptics.***

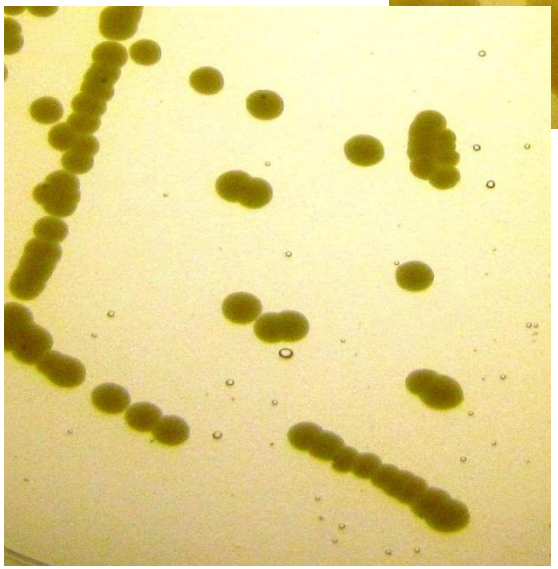


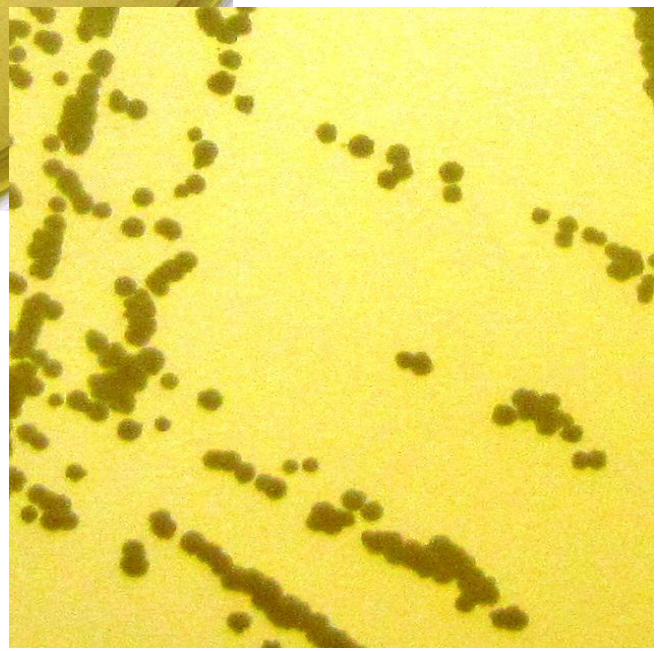
Results in % of efficacy of antiseptics as graphs calculated from number of colonies, which grow on disinfected surface (sterile water as control)

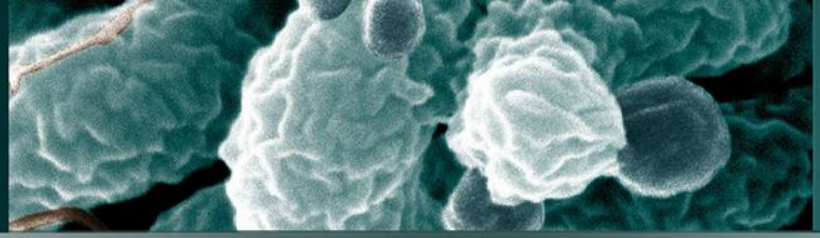


- ***Evaluation of colonies***

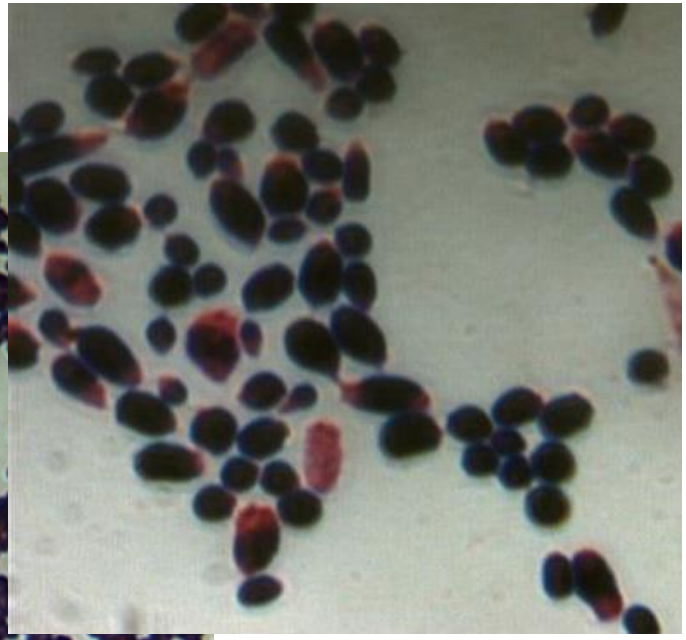


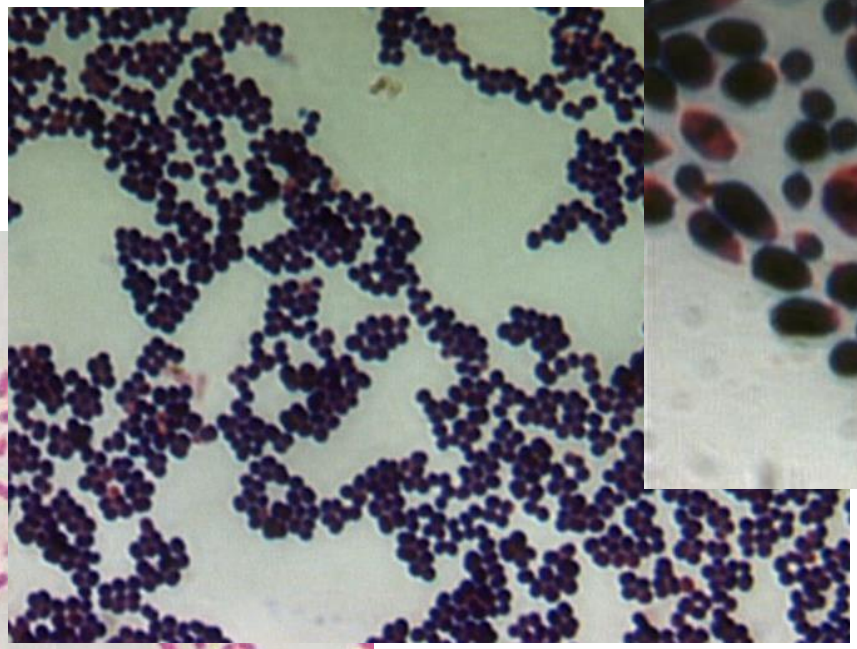


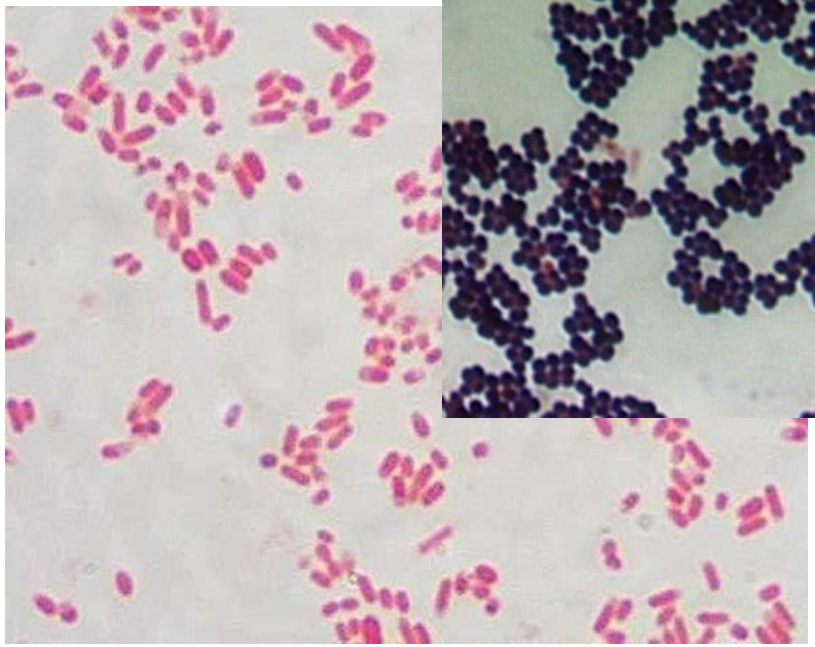




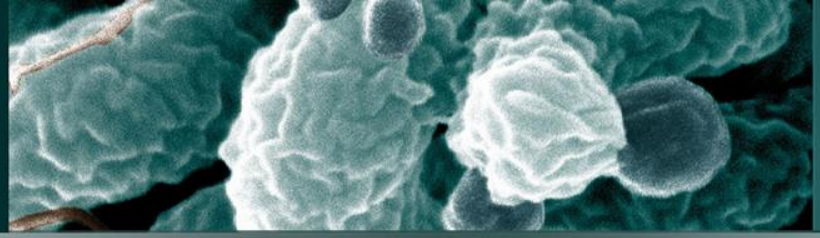
- ***Gram staining***





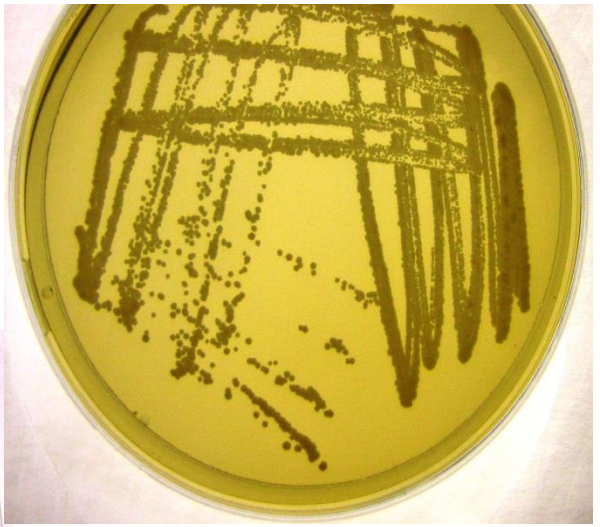


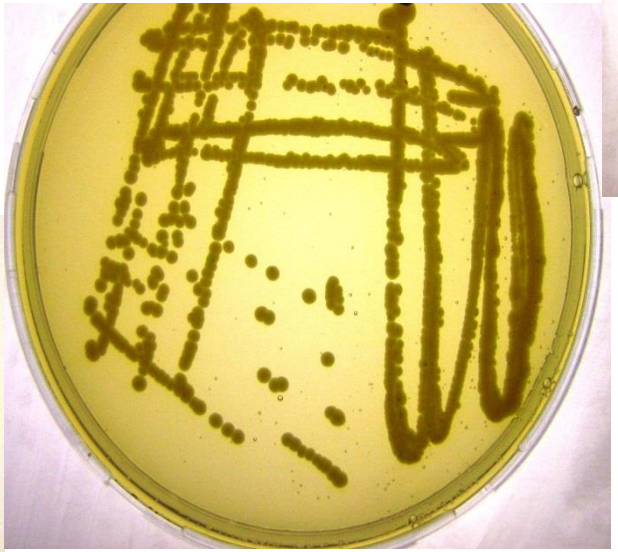
procedure, principle, picture or photo of preparates from lab class

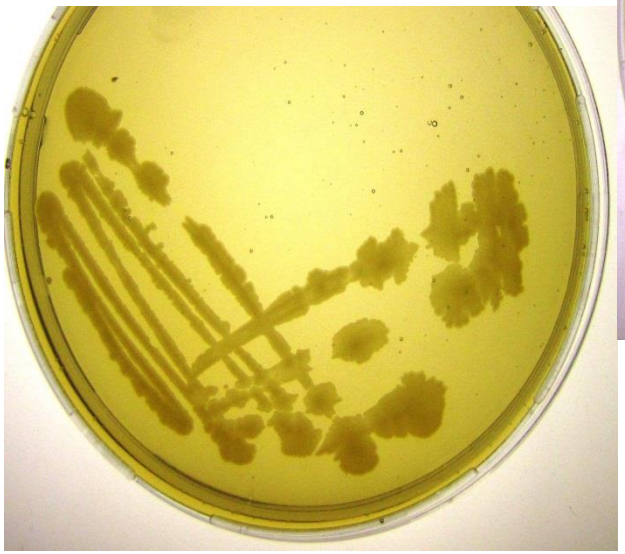


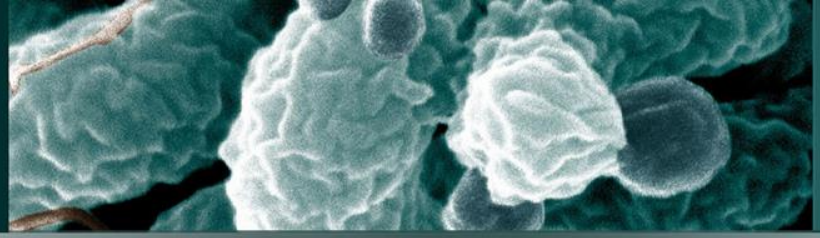
- ***Isolation inoculation***

Describe principle, what is the aim, picture



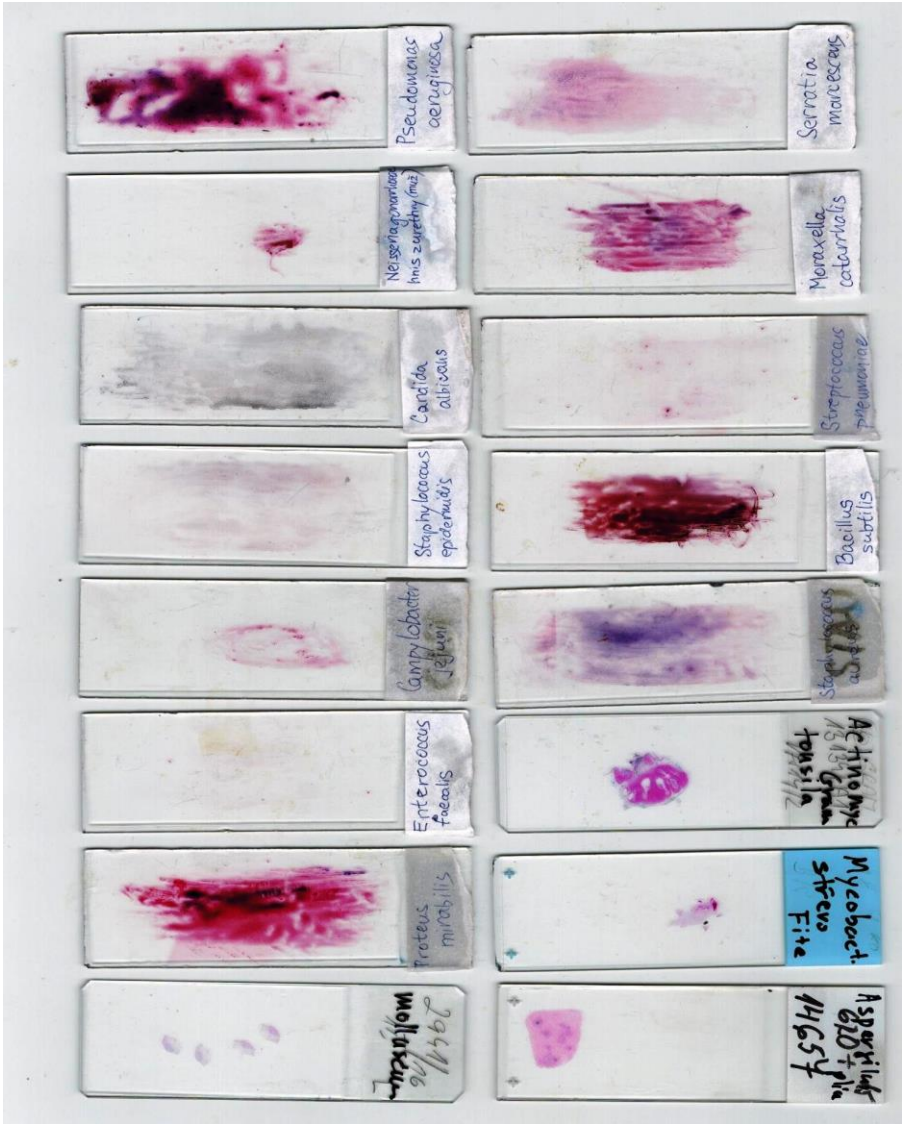


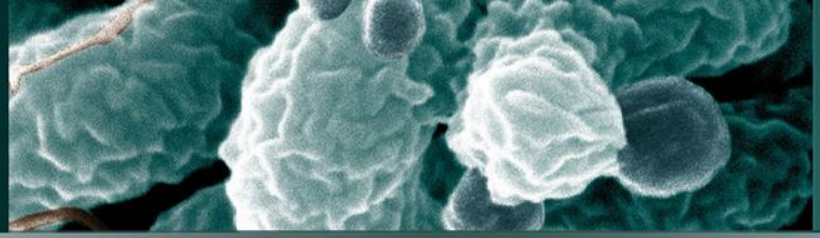




- ***Microscopy – stable preparates***

Pictures or photo. Describe the microbes: cocci, bacilli, G+, G-, aerobic or anaerobic – part of normal microbiota? Where can we find them (skin,..)? Do they cause any disease?





Questions for lab class test:

- Describe Gram staining and what is its usage
- Why we use cross scattering
- What is hemolysis and for which genus of bacteria is it typical
- Explain what does it mean acidoresistant and name one example of such microbe
- Describe growth curve of a microbe during static cultivation
- What parameters do we describe in microbial colonies
- What is viridation and for which genus of bacteria is it typical
- What is typical size of bacteria and of yeasts
- Name 2 examples of G+ cocci and 2 examples of G+ bacilli
- Name 2 examples of G- cocci and 2 examples of G- bacilli