

Microbiology ST-2

Physiology and metabolism

- Metabolism
 - - catabolism and energy generating
 - - biosynthesis – proteosynthesis
 - - secondary metabolism - ATB production
- growing and multiplication
 - - growth requirements- terminology
 - - grow curve
- Use of nutrition and grow requirements for diagnosis

- For surviving bacteria must have an efficient system for generating energy:
- catabolic - degradative reactions
 - provide subunits for metabolic reaction
 - generate energy - derive energy from oxidation reduction reactions of organic molecules
 - is release in form of high energy phosphate and stored as ATPfor synthesis of all necessary molecules
- anabolic - synthetic processes

- **Unity of biochemistry** - mechanisms for synthesis of energy, the synthesis and functioning of genetic code, identification of metabolic pathway for degrading carbohydrates, proteins, lipids are essentially identical
- Basic nutritional needs - common to all living cells - essential aminoacids
- Specific growth requirement - in procaryotic kingdom - great diversity
- Extra growth factors - bacteria can utilize preformed host components, metabolic adaptation to a single natural host - limiting of host number - great capacity - ability to grow in artificial media

Growth requirements of bacteria

- Source energy, organic carbon, metal ions (Fe), optimal temperature, pH, oxygen acceptance
- - phil, - trophic, - tolerant
- C
 - anorganic, CO₂ - autotrophic (lithotrophs)
 - organický – heterotrophic (organotrophs)
- Temperature
 - thermophil,
 - psychrophil
- pH - usually requiring physiological, neutral pH, some can be
 - acidophil,
 - alcaliphil

- Nitrogen requirements - enzymatic deamination of amino acids to ammonia to form glutamic acid - a key amino acid in protein metabolism
- Phosphorus - important for ATP, nucleic acids and coenzymes
- Iron - part of cytochromes - for cell growth
- Knowledge of nutritional peculiarities of microorganisms can be used for designing culture media - bacteria grow on artificial media

Oxygen requirement

- Not similar to animal cells several bacteria do not strictly require oxygen
- Obligatory anaerob– requiring environment without oxygen, oxygen is toxic for them – type of metabolism is – fermentation, lack of some enzymes for hydrogen peroxide detoxification H_2O_2
- Anaerob aerotolerant – anaerob respiration (fermentation), surviving in the presence of oxygen
- Obligatory aerob – requiring oxygen – metabolism is oxidative phosphorylation (respiration)
- Facultative anaerob – support oxygen and oxygen free environment – fermentation and respiration
- Microaerophil – requiring lower tension of oxygen in atmosphere. Normal oxygen tension is toxic for them

Nutrition factors

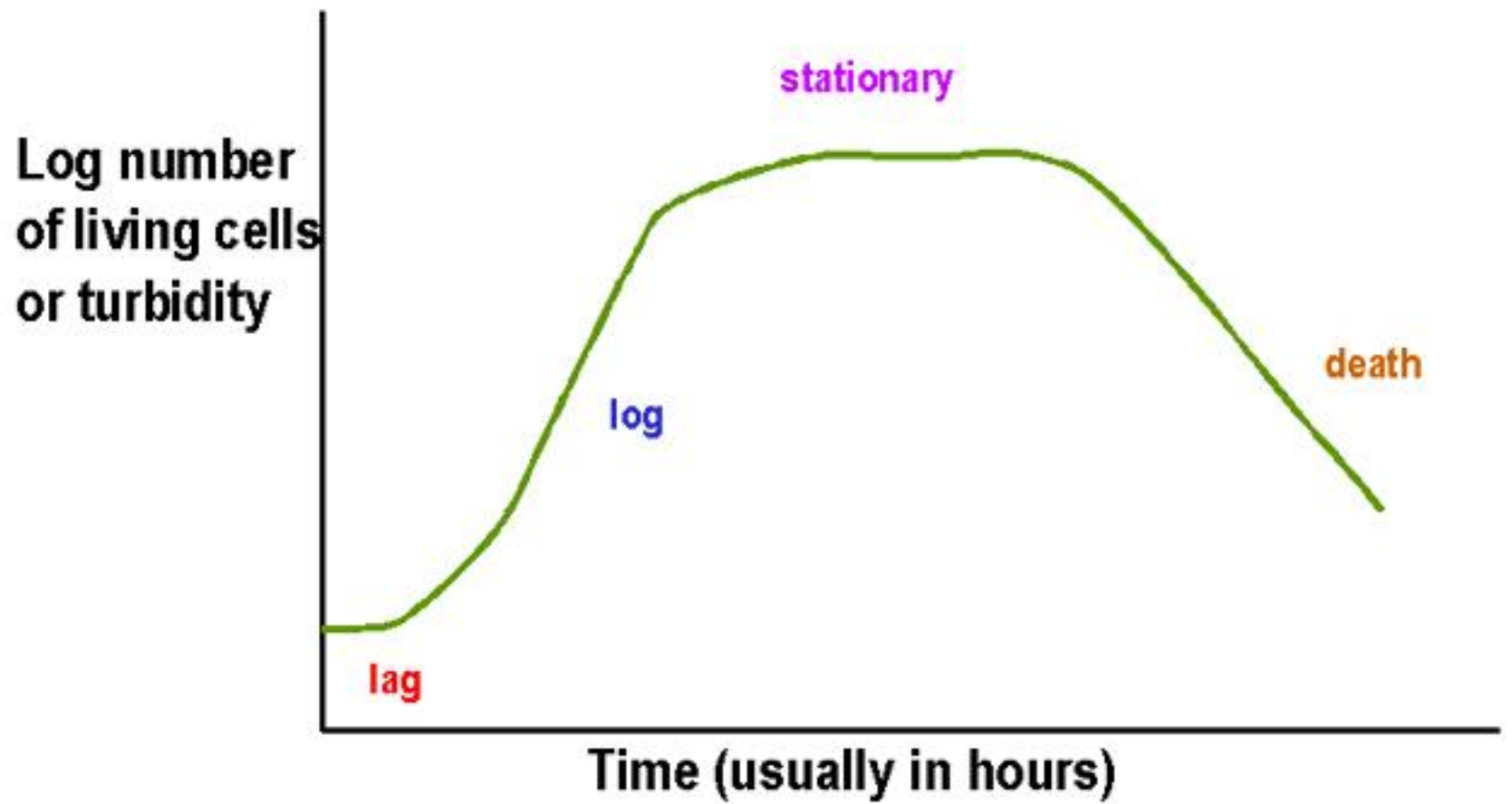
- Environmental sources - usually big molecules incapable to enter the bacterial cell
- First step of metabolism - obtaining nutritional subunits - is performed outside the cell - via bacterial exoenzymes - hydrolysis of macromolecules
- Subunits are imported via plasma membrane and cell wall to cytoplasm - transport - porins, transport proteins
- Catabolic reactions with the aid of endoenzymes start - energy, basal structural molecules - conversion to intermediate - pyruvic acid and carbon - used for energy production or for:
- Anabolic reaction - biosynthesis - peptidoglycan, lipopolysaccharid, nucleic acid, proteosynthesis, replication

Metabolism of glucose

- Conversion of glucose to pyruvate + energy
- * under aerobic (**oxidative phosphorylation - respiration**)
 - next oxidation to CO₂ + energy in TCA cycle with
acetylCoA
as intermediate and place where meet other metabolic pathways (C derived from lipids...)
- * under anaerobic conditions pyruvate is converted to a variety of end products (**fermentation**) - used for identification
 - no next step, less energy

Growth curve in isolated model

- 1 lag phase - adjustment period, number of viable cells can decline
- 2 acceleration phase - surviving cells starts to multiply
- 3 exponential phase - most rapid multiplication
- 4 stationary phase - environment becomes unfavorable, new generation
- s are just to replace died generations
- 5 decline phase - deaths exceed multiplication
- 6 exponential decline phase - maximal rate of decline, in a period of time half of total number of cell is lost
- 7 autosterilisation



The Growth Curve

Kinetics of growth

- Bacteria divide by binary fission - log function during the period of maximum rate of growth - exponential phase - continuing growth in optimal condition
- Generation time in vitro:
 - is 20 minutes in *Vibrio cholerae* (from 1 cell in 2 days give cell mass 4000 times that of earth)
 - 14 hours in *Mycobacterium tuberculosis*,
 - mammalian cell 8 hoursin vivo generation time of bacteria is longer - forces of host defense and nutritional limitations

Stationary cultivation

- Cultivation in laboratory:
 - Limited amount of nutrient factors (exact force of agar) - stationary phase - visible isolated colonies grown from CFU colony forming unit - a piece of tissue or biological material that will grow in one isolated colony of bacteria. 1 colony consists of several thousands of bacteria

Quantification of bacteria

- It is sometimes important to determine the number of viable bacteria present in clinical specimen:
(in urine - significant bacteriuria is 10^5 viable bacteria in 1 ml of urine)
 - by diluting the specimen and streaking aliquots on the surface of agar plate and counting no of colonies
 - by preparing a dilution and comparing it with standards of known density - McFarland scale
 - measuring the turbidity extinction of liquid sample

Cultivation

- To identify a bacterial pathogen it is necessary to transfer it as a biological sample from site of infection on artificial medium simulating its requirement for growth and isolate grown bacteria in pure culture
- A panel of tests are applied to identify the unknown colony
- This is possible in great majority of bacteria and some yeast - growing on artificial media and being biochemically active - direct detection of pathogen - visualisation
- *Not available for viruses - need vital medium for replication (continuous cell lines, animal model). Indirect detection is more frequently used - via Ab detection.*

Steps in identification of unknown colony

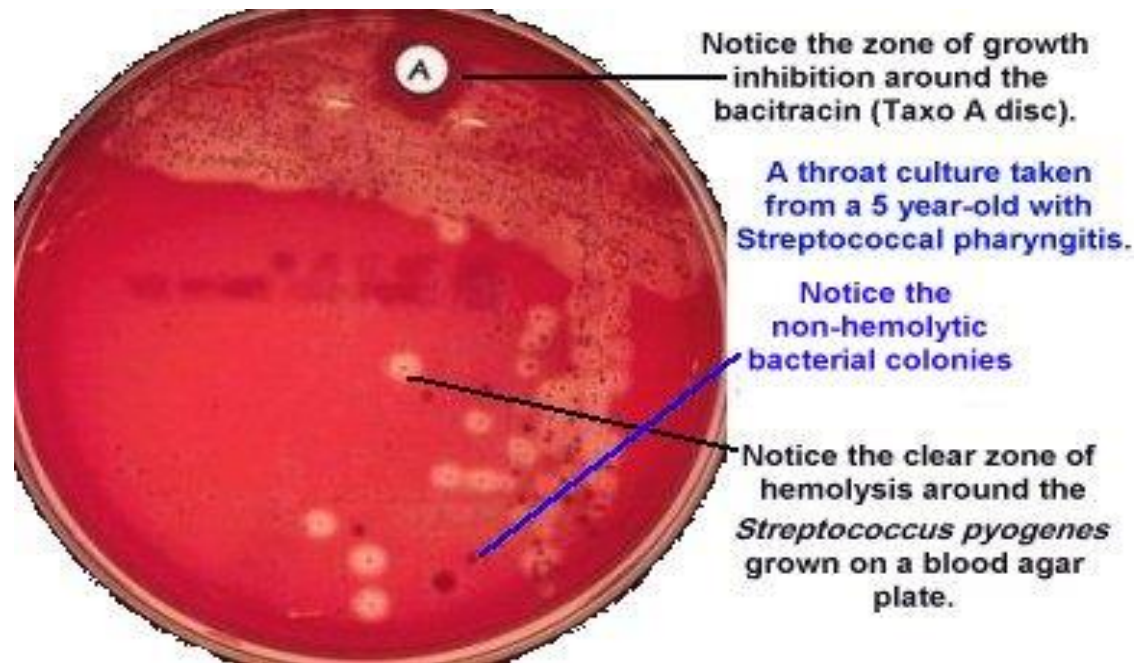
- requirement of oxygen
- Macroscopy of colonies
- Microscopy native (movement) or Gram stain (morphology, cell wall structure) G+,G-,rod, coccus, spiral
 - Cell arrangement diplococcus, regular alignment,
 - Detection of capsule (agglutination, Burri)
- Ability to ferment certain substrate - sugar, aminoacids - (biochemical properties)
- identification of enzymes - (physiology)
 - susceptibility to ATB, and lysis by bacteriophage

Sampling

Innoculation of appropriate media a small part of the sampled material is introduced on the surface of medium in Petri dishes and stroken over it

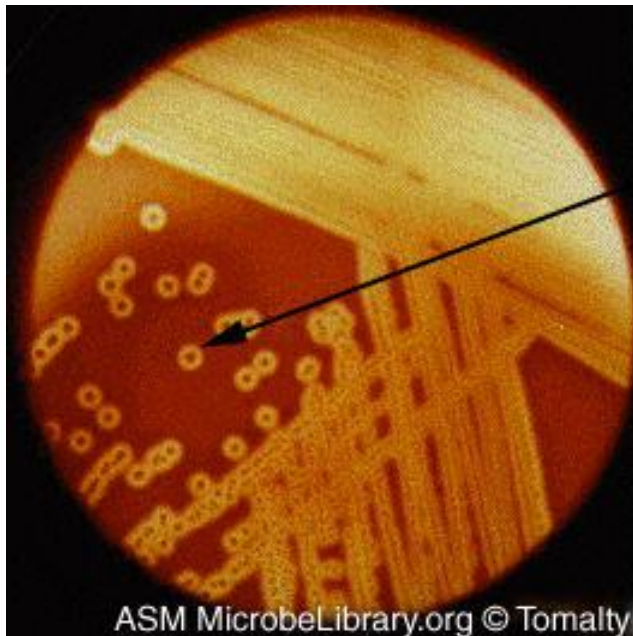
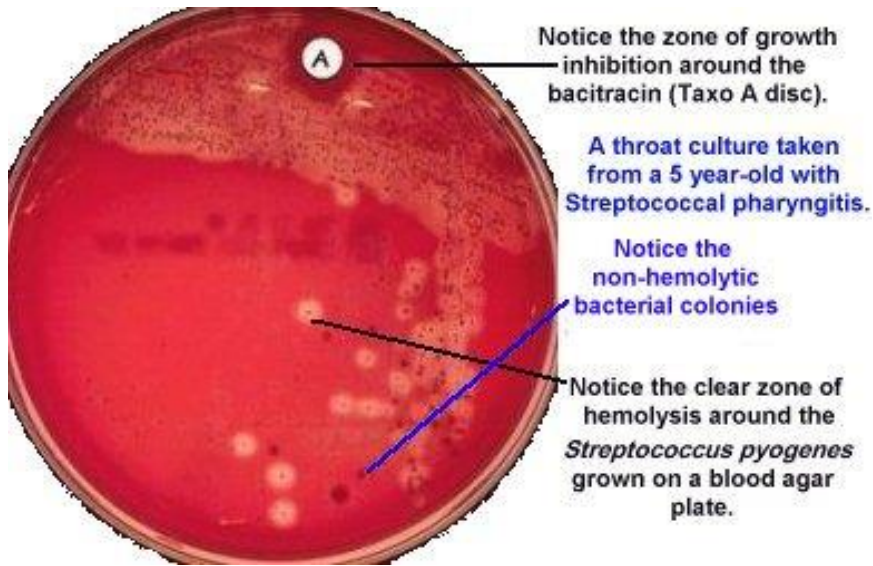
Cultivation- 24 hrs, 37 degC

Correct inoculation with steril loop



Macroscopic:

Morfology of colonies, changes of medium— hemolysis, pigment



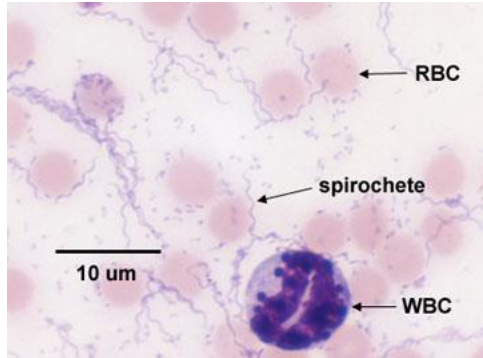
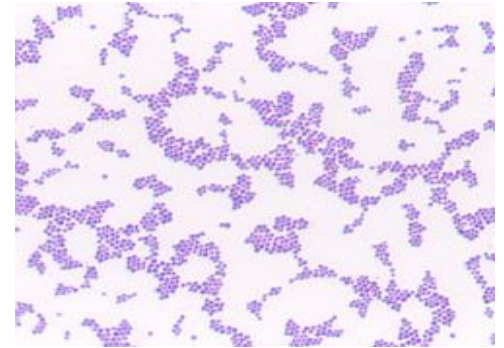
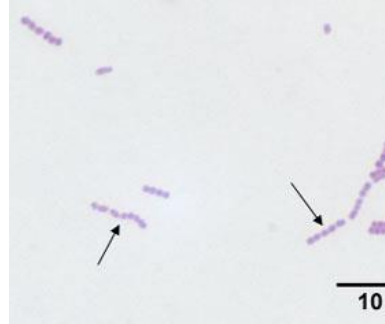
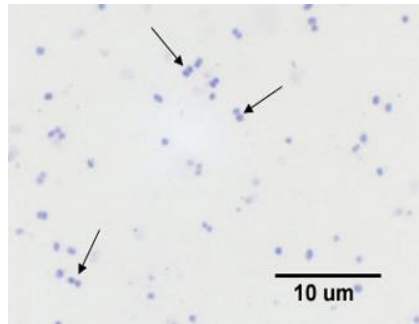
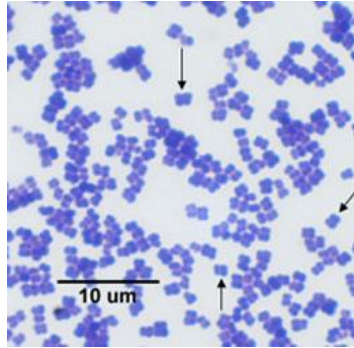
Microscopy

- - magnification,
- - distinguishing

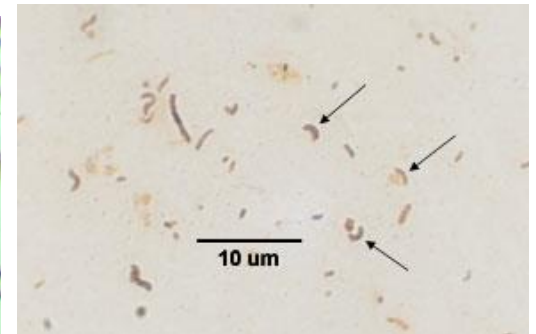
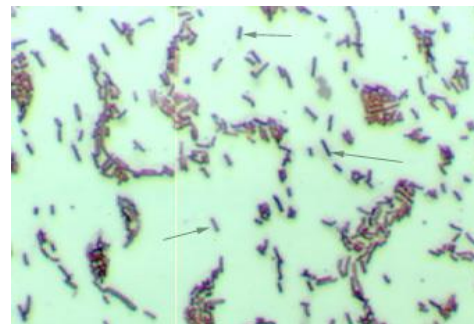
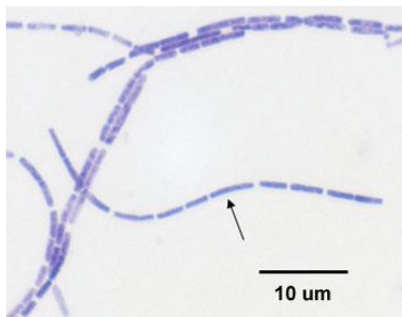
types – according to the method used for visualisation:

- light microscopy,
- fluorescein mikroskopy,
- elektron microscopy

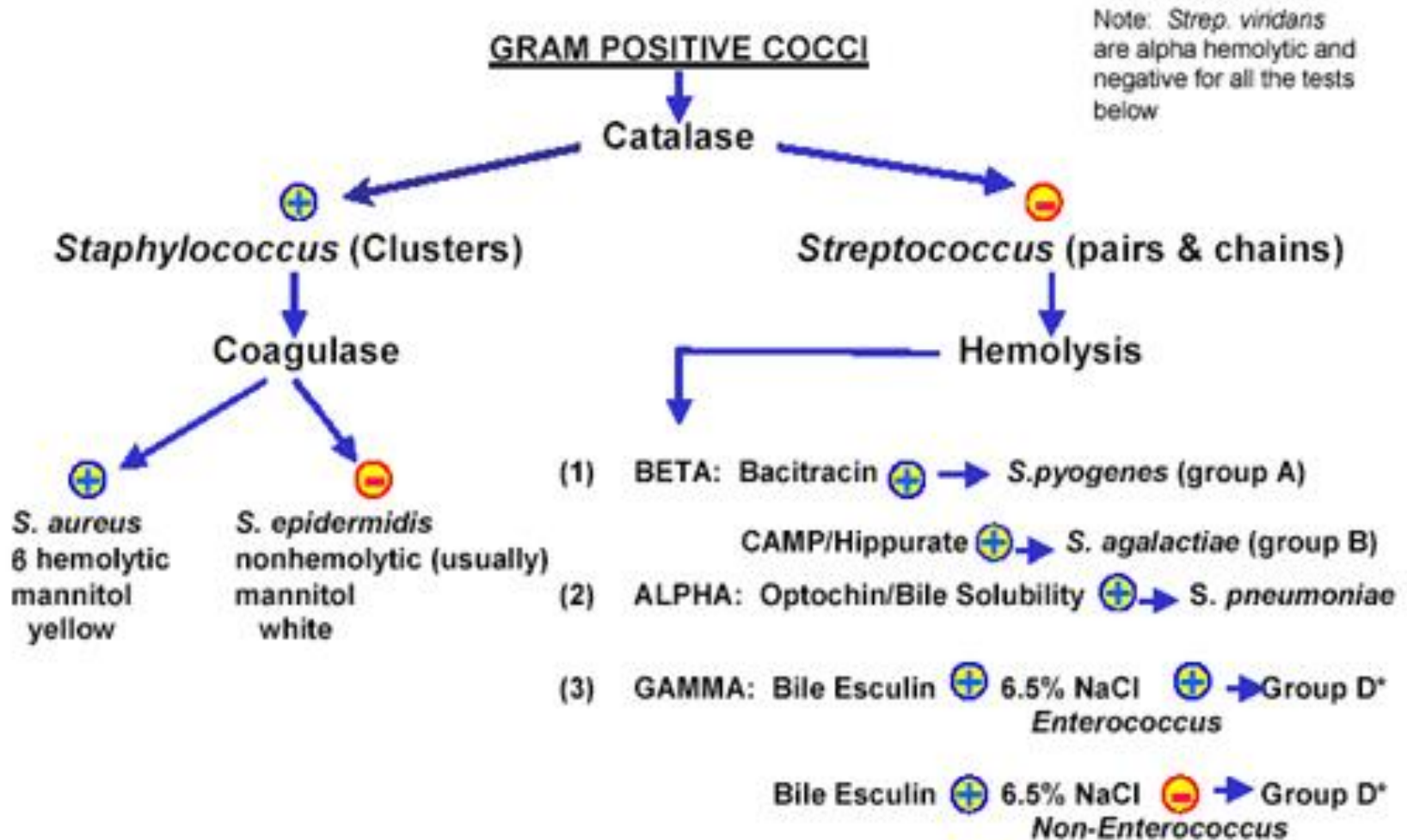
Better visualisation in light microscopy – stained smears



Vibrio, rods with spores, spirals, filamentous, spirochettes, staphylococci, streptobacillus, streptococci, tetrad, diplococci,

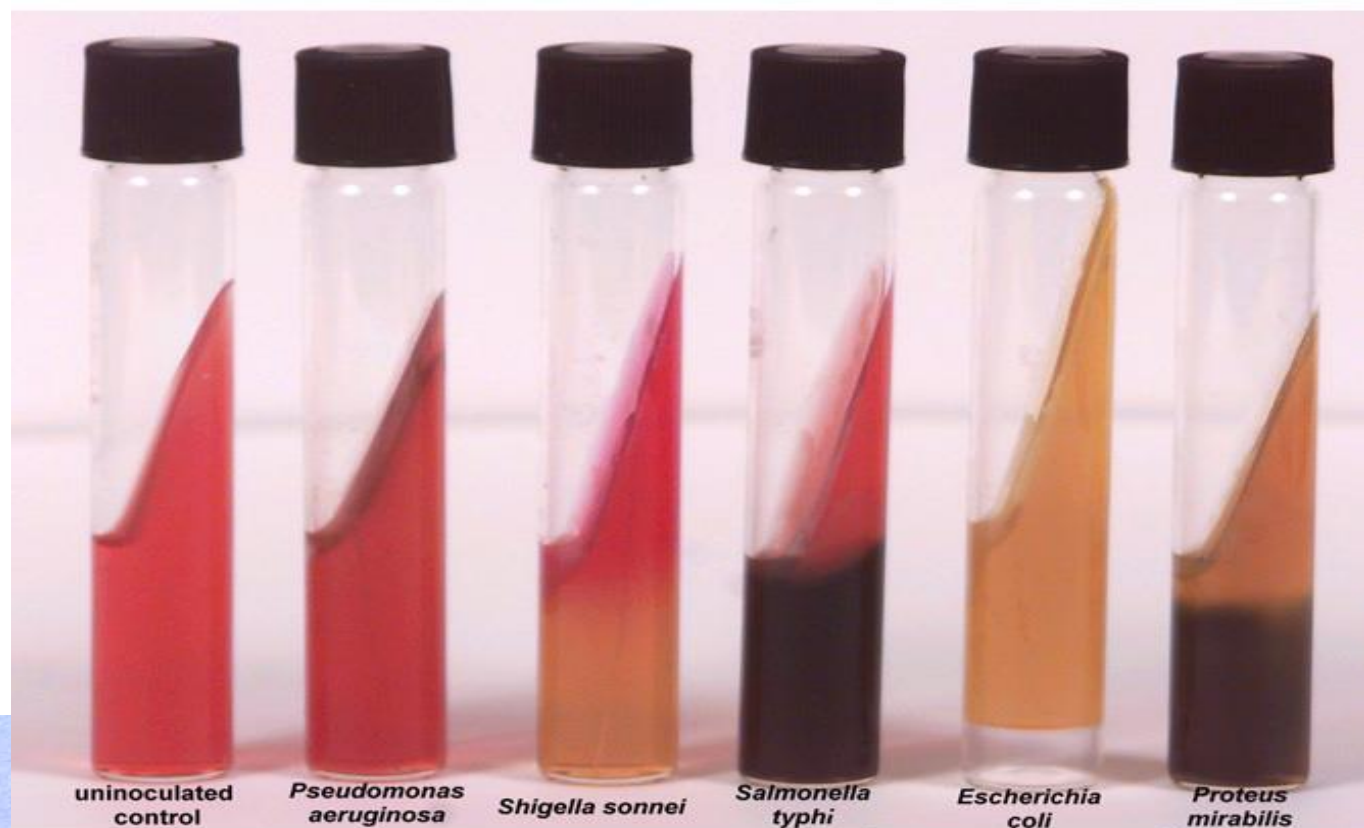


ALGORITHM –TREE OF STEP BY STEP IDENTIFICATION ON THE BASE OF PROPERLY PLANNED BIOCHEMICAL TESTS

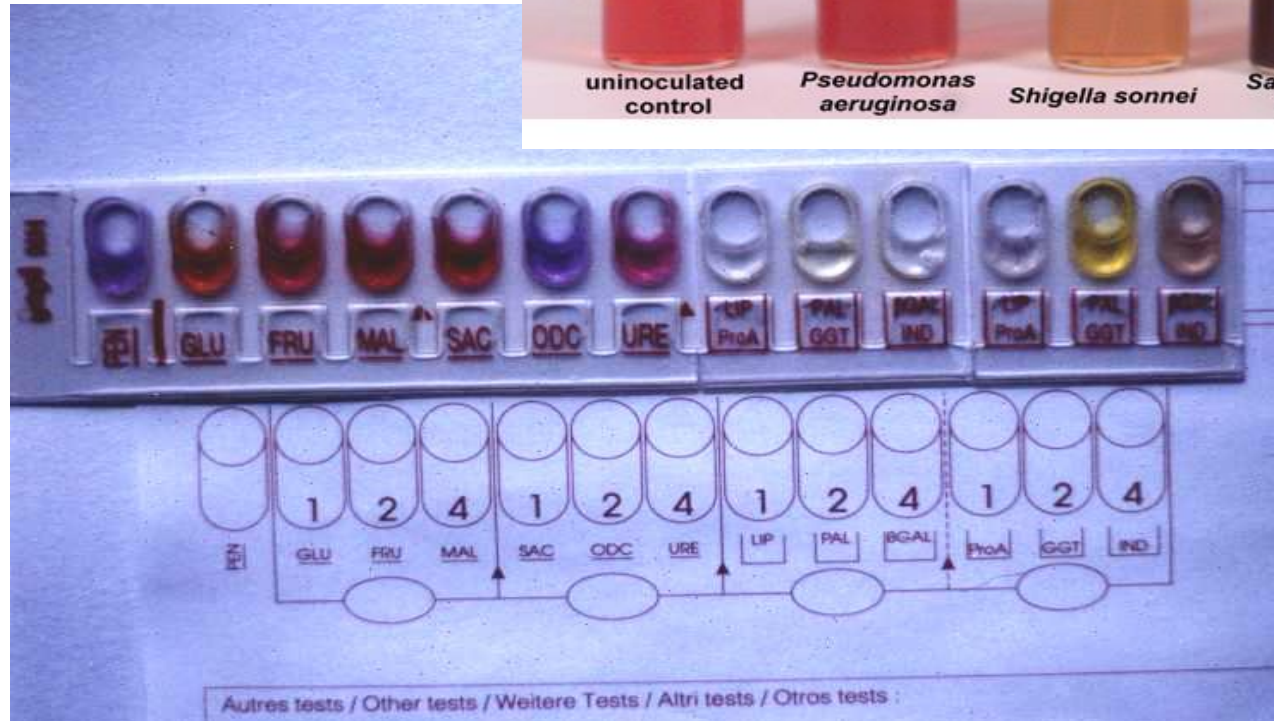


(*can also be Beta or Alpha hemolytic)

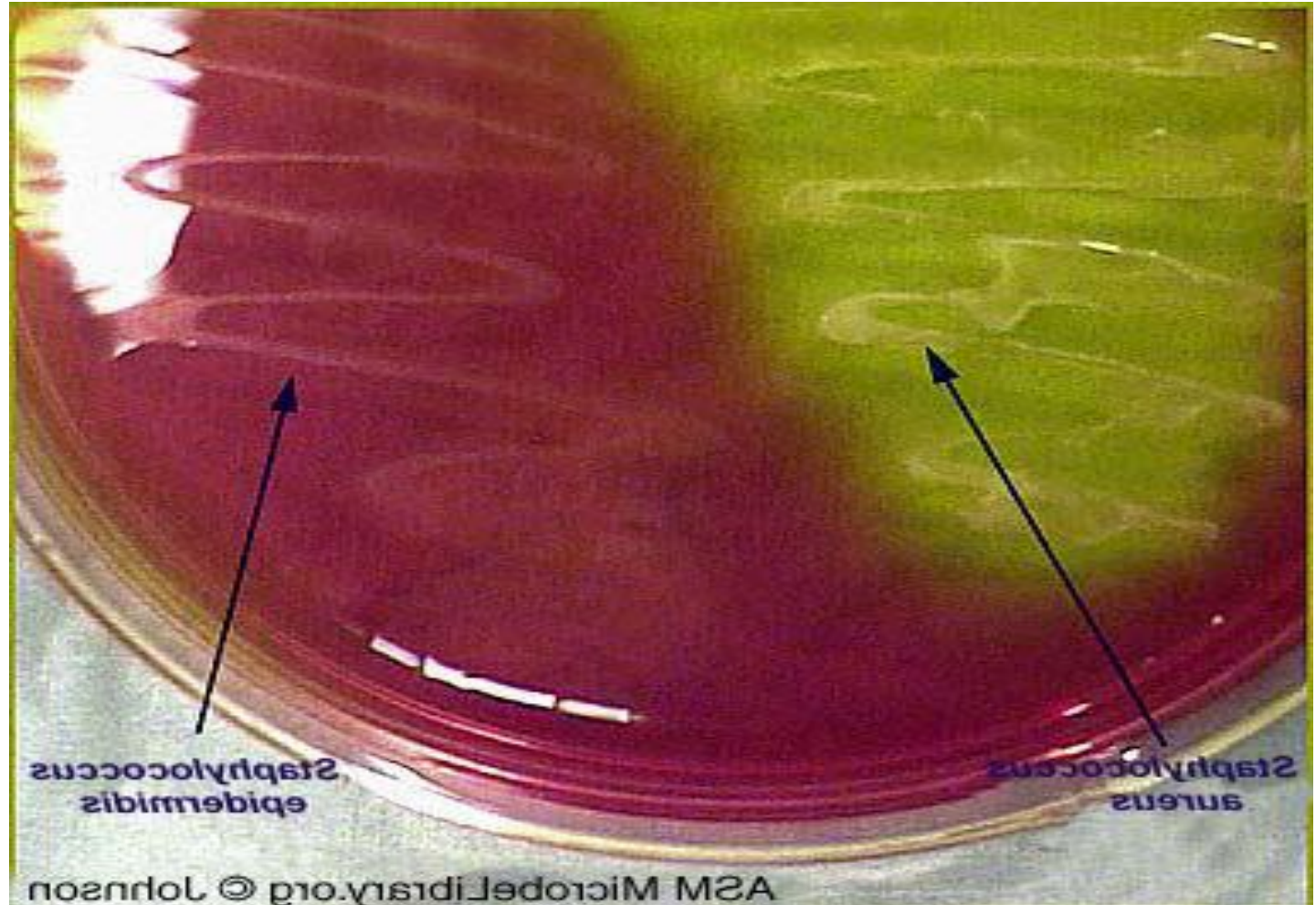
BIOCHEMICAL PROPERTIES



ASM MicrobeLibrary.org©Chamberlain



Change of phenol red (lacmus) in the part where bacteria (staphylococcus aureus) are enzymatically changing mannitol with acidification of medium (yellow). In the left part mannitol is not fermented, resulting in no acidification and no change of red to yellow



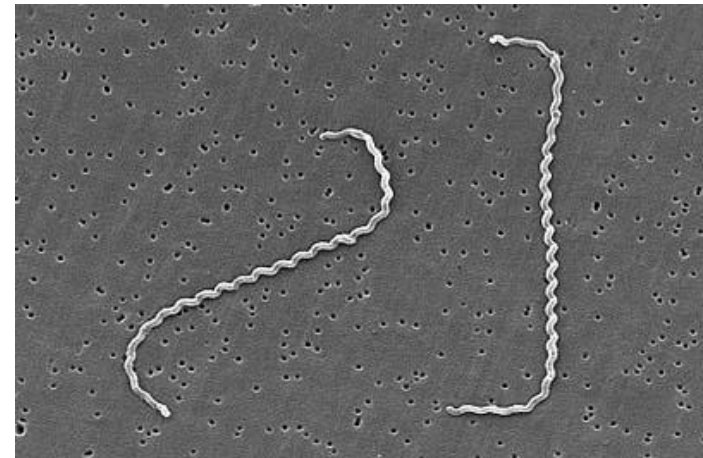
Light microscopy

Background and bacteria are absorbing the light in the same way – bad distinction - native smear – living bacteria (motility, germinating) - stained smear – (higher contrast between bacterium and background) – better distinction

Microscopy in dark field: the sample is enlightened by the beam oriented from periphery (0,1 μ m – 0,2 μ m) – *Treponema*, *Borrelia*, *Leptospira*

Microscopy with phase contrast – the system is visualising phase differentiations of light when passing through objects with different thickness.

3 dimensional picture



Light microscope

Magnification: 2 systems of lenses

- senses of objective
 - 10 x general overview,
 - 40 x parasites, cysts, molds
 - 100 x with immersion oil bacteria
- lenses of ocular 10x

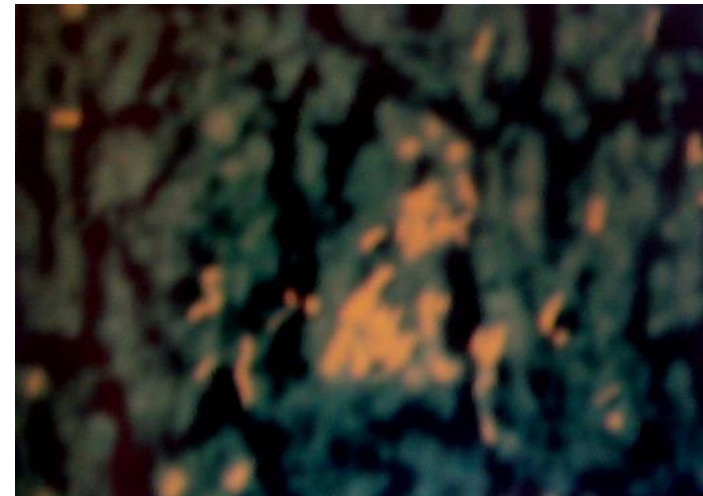
Overall magnification is ocular multiplied by objective magnification.

Distinction capacity: wave length of the light beam and the angle in which the light beam enter the lense of objective – numeric aperture

Light microscope: 1 - 2 μm – the smallest distinguishable

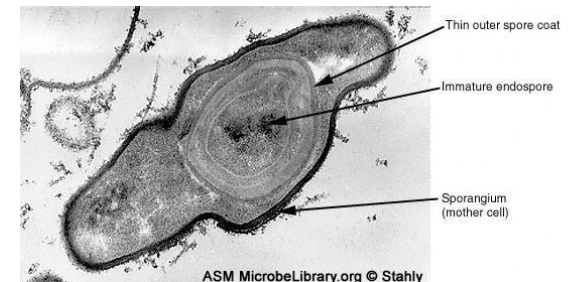
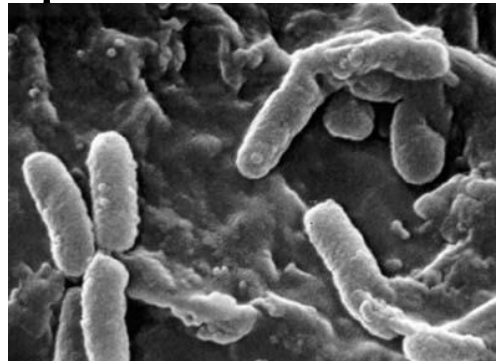
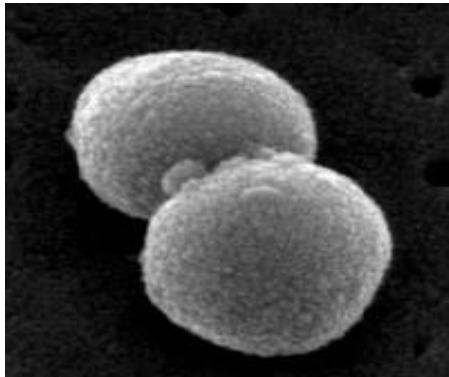
Fluorescein microscopy

- Using Hg vacuum lamp which is emitting light of shorter wave length than in the light microscope
- They use fluorochromes – compounds that are able to absorb short waved ultraviolet or ultrablue light and to emite the energy of highere wave lenght. Fluorocromes are used for preparing the smear – fluorescein staining – after lightening it with shortwave light
 - fluorescence: Fluorochrome is targeted to the structure by antibodies against the said structure

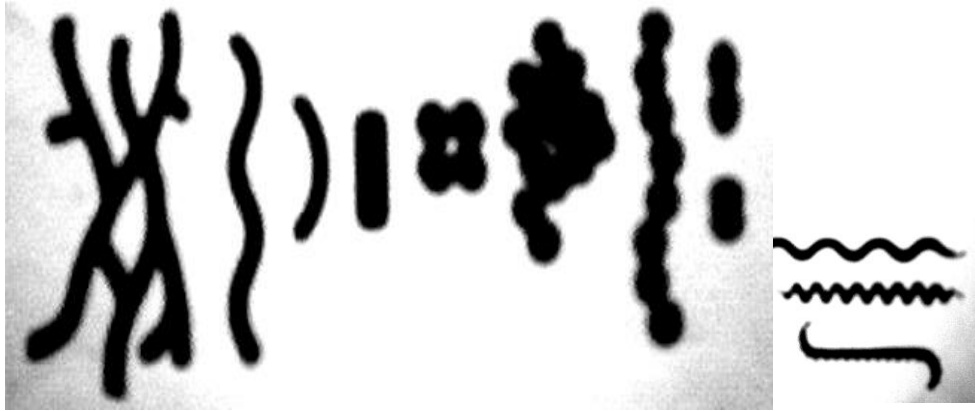


Electron microscopy

- Using magnet – not lenses – for targeting the beam of electrons through the sample to the screen. This process is using much more shorter wave lengths of the light, the magnification is higher and distinguishing many times better.
- Visualisation of viruses and subcellular structures
- 2 types – transmission – particles go directly through the sample
 - scan – particles go through the sample in the angle – 3 dimensional picture



Types of formes in light microscope

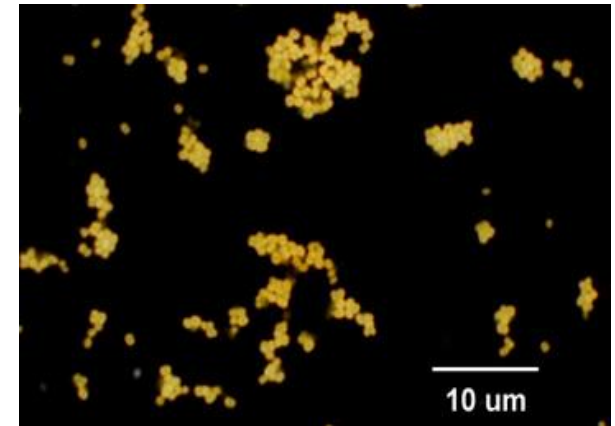
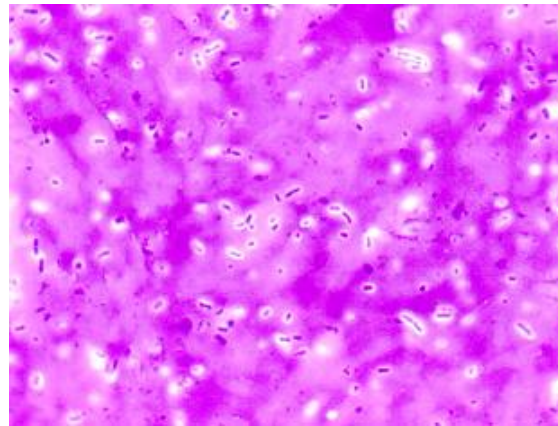
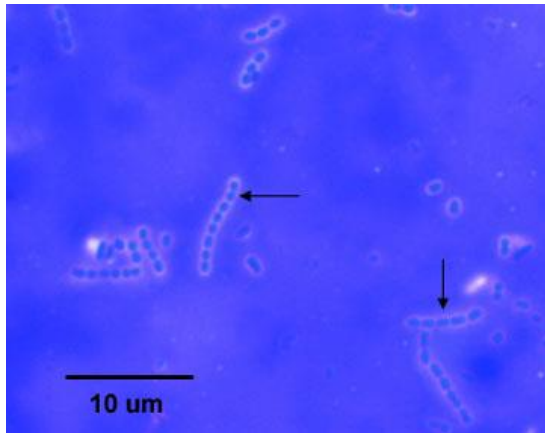
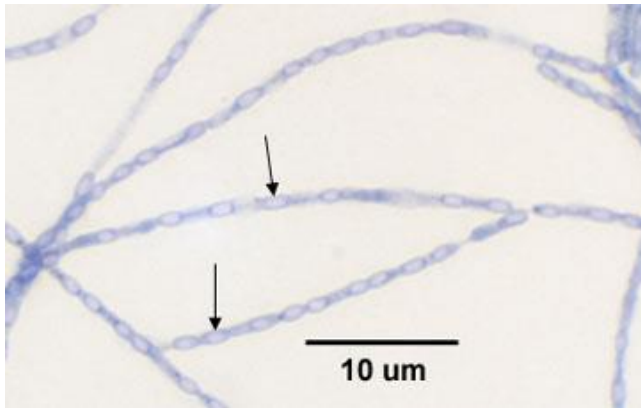


Branched filamentous formes, spirals, vibrio, thick rod, tetrad, cluster of cocci, chain of cocci, diplococci – coffee beans, candle and flame.- Different thickness of spirals in spirochetes *Leptospira interrogans* ?

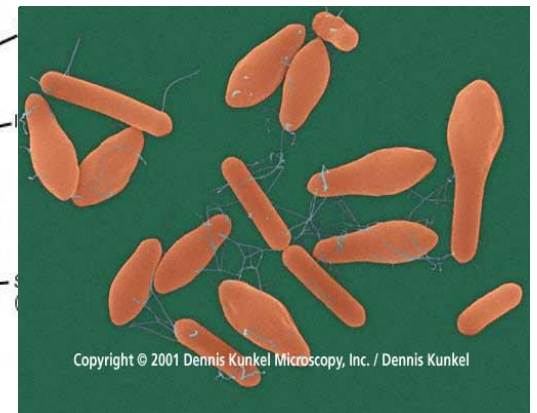
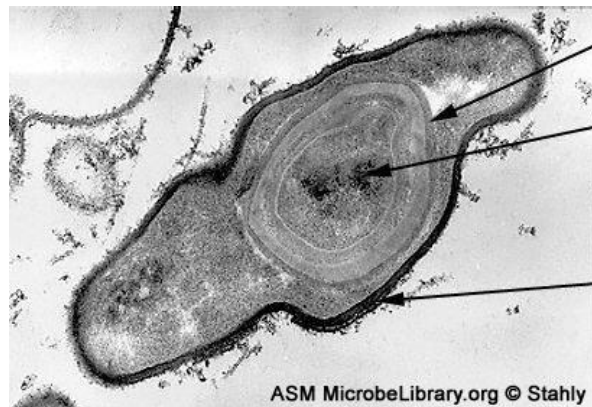
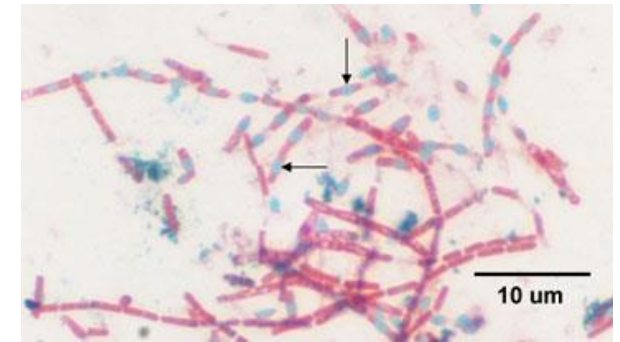
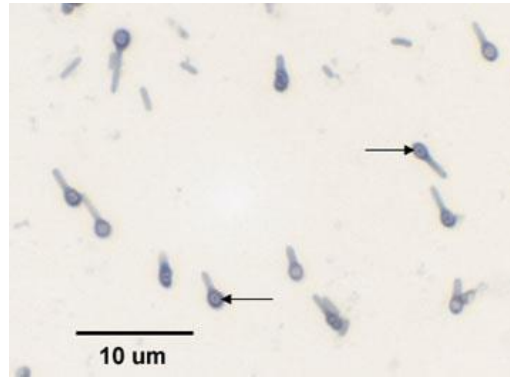
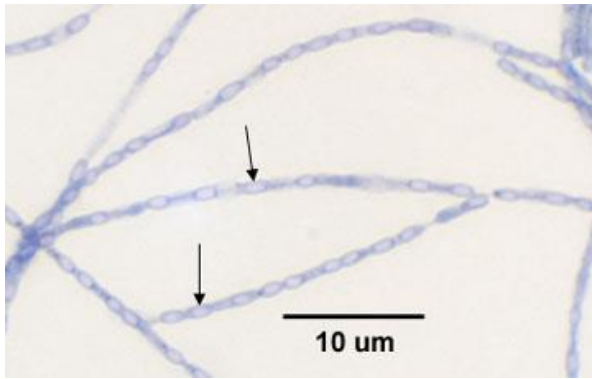
Negative staining

augmentation of the contrast against dark background

- Burri method for capsule visualisation,
- Gram staining used for not stained structures (spores)



Visualisation of bacterial spores



Identification – panel of appropriate steps - algorithm:

Macroscopy:

Morphology of colonies and change of medium – hemolysis, pigment,

Microscopy:

Native smear (motility), orientation staining (one color, arrangement of bacteria), differentiation staining: Gram staining, Burri smear (visualisation of capsule), metachromatic granules, spores

Biochemical properties

fermentation of sugar, utilisation of amino-acids, identification of enzymes, resistance to outside conditions

Typisation

identification of antigen with appropriate antibodies - serotypisation,

Others:

patogenity to animals, nucleic acid identification PCR