

Searching for microbes Part III.

Culture of bacteria & yeasts

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Survey of parts of this slideshow

Multiplication of bacteria, growth curve

Conditions needed for bacterial (or fungal) culture

Bacterial culture – introduction

Liquid media

Solid media: inoculation of a specimen / strain

Solid media: classification and examples

Tale

- It was once a **bacterium, and it was very small**, and so without the microscope nobody has seen it, and in the microscope it was very difficult to see its shape. It was very unhappy, because it emphasized very much its beauty.
- Once, **Mr. Koch came**. He put the bacterium onto a **solid medium** and hide it **into a thermostat**. Bacterium was very happy and quickly started to multiply...

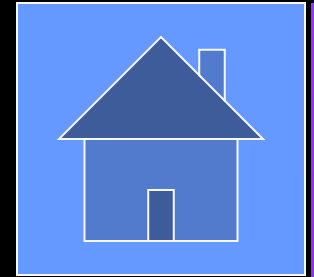
And Day After...

- Pan Koch came, opened the thermostat, took out the Petri dish with medium, and he saw: his liked, tiny bacterium was **visible by a naked eye!** Of course, not as one bacterium, but as a **strain** of totally identical cells, that raised from that bacterium in form of a **colony**
- The bacterium was very happy, and it showed its beauty to mr. Koch. **It showed him its shape, size, profile of its colony,** pigments and many other things.

Multiplication of bacteria, growth curve

Multiplication of bacteria

- Each bacteria has its **generation period**
- During one generation period one makes two, in ten times of the period one makes 1024 bacteria (theoretically) and so on,.
- Ideal multiplication would exist only if we would add all the time nutrients and eventually oxygen and we would remove waste products.



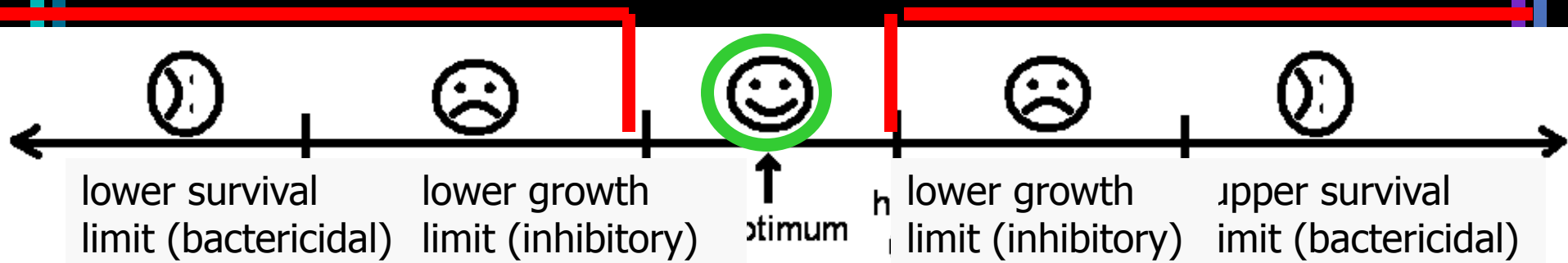
Real growth curve

- **Phase of latence** – we let bacteria grow, but they still do not multiply
- **Exponential phase** – growth accelerates
- **Stational phase** – they grow with the same speed all the time
- **Slowing and stopping of growth** – lack of nutrients, too many waste, or bacteria regulate themselves by „quorum sensing“

Conditions
needed for
bacterial (fun-
gal) culture

Do the conditions for bacterial growth matter?

Of course they do! Majority of bacteria need their temperature, moisture, salts concentration and many other characteristics to be in a quite narrow range.



Values, that enable microbial survival, are not sufficient. They should be able to multiply.

Various microbes need various conditions!

Medically important bacteria

- **Temperature** usually needed around 37 °C
 - but bird pathogens more (42 °C), microbes coming from outer environment less (30 °C)
- **Value of pH** needed around pH 7
 - but gastric helicobacter by far less
- **NaCl concentration** needed around 0,9 % (physiological saline)
 - but staphylococci, that have to be able to multiply on sweated skin, multiplies even at 10 % of salt!

In practice part of parameters (e. g. temperature) is derived from thermostat settings, and remainder (e. g. NaCl concentrations) by composition of the culture medium.

Culture thermostat

Besides box thermostats, like this one, our Institute has a chamber thermostat, too. It is a whole room with 37 °C.

Majority of bacteria is cultured in a thermostat overnight, so about 24 h.



Photo O. Z.

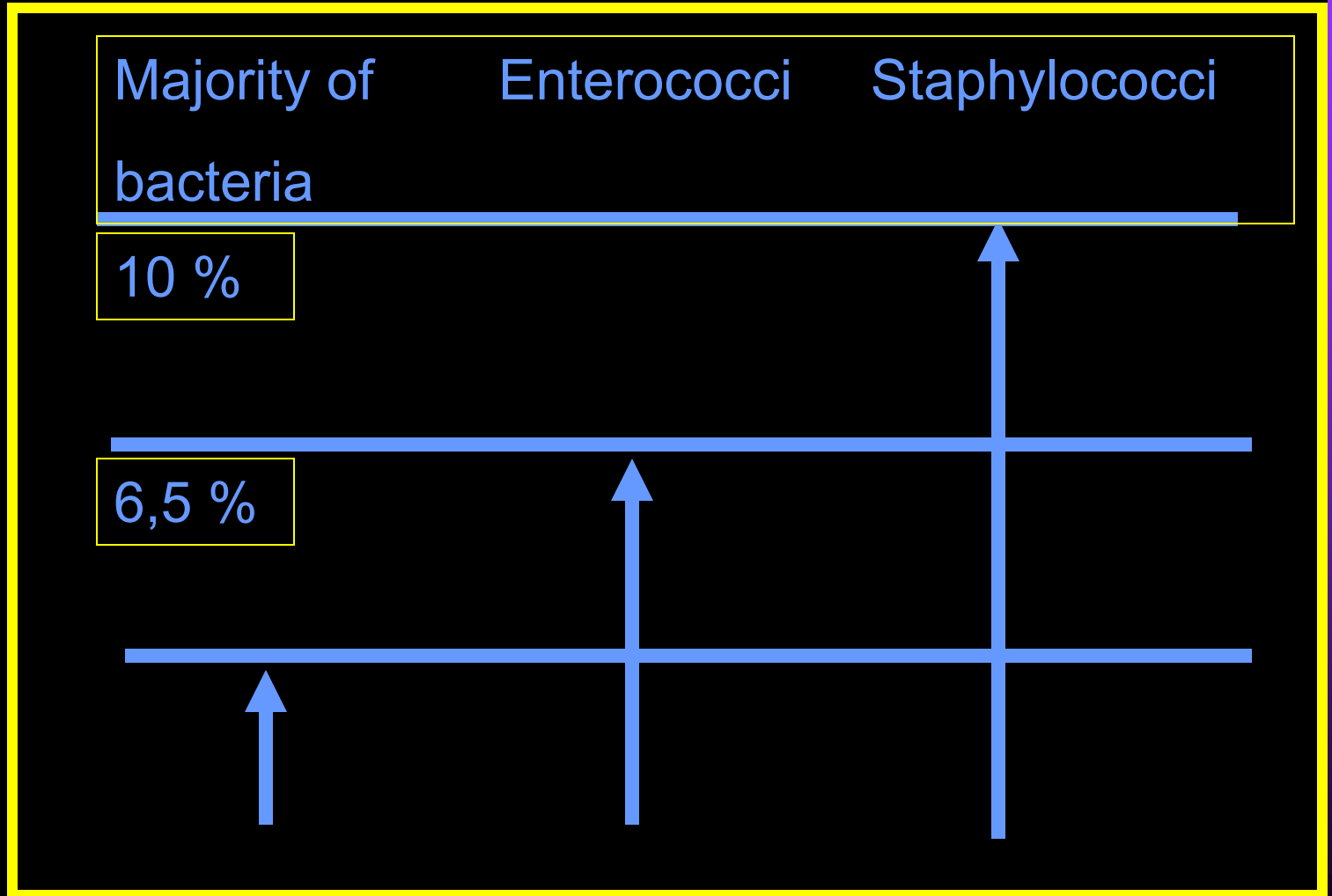
Example of use of relation to temperature in bacterial diagnostics

- *Pseudomonas aeruginosa* grows at 37 °C and 42 °C.
- On the other hand, *Pseudomonas fluorescens* grows at 4 °C and 37 °C.

Besides *Pseudomonas*, *Listeria*, too, and yeasts and molds grow at lower temperature.

Elevated temperature is suitable e. g. for *Campyloacter*

Influence of NaCl concentrations to the growth of some bacterial genera



Besides various NaCl concentrations

- **Adding of sodium azide** enables growth of enterococci, but neither staphylococci, nor streptococci are able to grow
- **Amikacin** enables growth of streptococci and enterococci. Staphylococci, sensitive to amikacin, are eliminated.

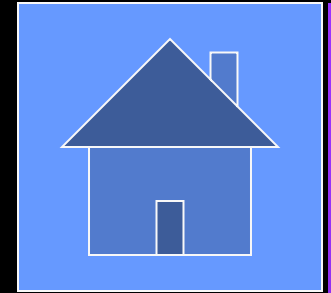
A little about catabolism of bacteria

- We have three types of catabolism:
 - **Fermentation** – nutrients breakdown without need for oxygen. Little energetically effective, but does not need oxygen. Product is e. g. lactate, ethanol etc.
 - **Aerobic respiration** – little nutrients gives a lot of energy, but oxygen is needed. Product is CO_2 and H_2O
 - **Anaerobic respiration** – another electron acceptor
- Type of catabolism is **closely connected with relation to oxygen**. Fermenting bacteria are usually facultatively or **strictly anaerobic**. On the other hand, **aerobically respiring bacteria** use to be **strictly aerobic**.

Relation to oxygen

- **Strict aerobes** grow only in presence of oxygen
- **Strict anaerobes** they grow only in environment without oxygen
- **Facultative anaerobes** and aerotolerant bacteria (it is not possible to differentiate them) grow at all conditions
- **Microaerophile bacteria** grow only in conditions with traces of oxygen
- **Capnophile bacteria** need more CO₂

Growing anaerobic bacteria



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Bacterial culture – introduction

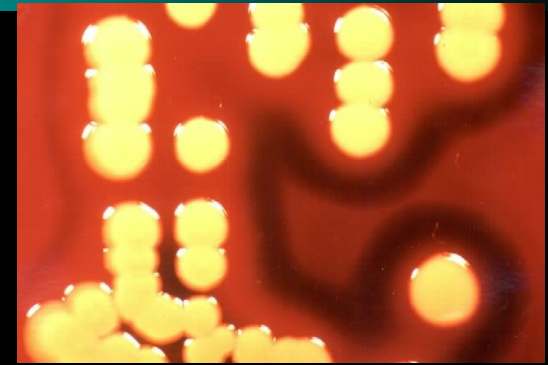
Why we culture bacteria

- Why bacteria are cultured in the laboratory?
 - To **keep them living and to multiply them**. This is gained by cultivation in both liquid and solid media (jelly-consistence media, based on agar algae)
 - To obtain **strain** – solid media only; invented by Robert Koch 
 - To **differentiate and divide** them mutually – diagnostic and selective media are used, for identification

Specimen and strain

- **Specimen** is taken from a patient. Specimen contains cells macroorganism, various number of microbial species (zero to maybe twenty) and more items
- **A strain – an isolate** – is a population of one bacteria, isolated from a specimen on a solid medium
- To gain a strain, **we have to grow a bacterium on a solid medium and inoculate carefully**

Term „colony“



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- A colony is **a formation on a surface of a solid media**. It is developed from one cell or a small group (couple, chain, cluster)
- In some cases number of colonies on an agar shows us **number of microbes** in the specimen – or more precisely, number of „colony forming units“ (CFU)
- Description of colonies has an important place in bacterial diagnostics

Solid media

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Is it good, or bad, that various bacteria need different conditions?

- **It is bad**, because it complicates definition of conditions, that would be suitable for majority of bacteria
- **It is good**, because this enables to use it in diagnostics (e. g. growth ability on medium with 10 % NaCl differentiates well staphylococci)

Media globally versus media in medical microbiology

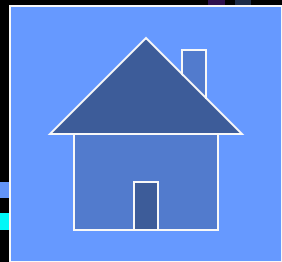
- **In industrial microbiology** or in some other applications we use mostly chemically defined media. We know their composition, and it is possible to observe, how much of something increased or decreased.
- **In clinical microbiology** we have no need to know a detailed composition. Often some parts of media are not definable (red blood cells, yeast extract).

Liquid media and solid media

- **Liquid media** are based on je meat-peptonic broth (extract of cooked beef meat + protein hydrolysate). They are used mostly to multiplication. It is difficult to evaluate the result, in fact, only „non turbid broth – turbid broth“ (growth – no growth)
- **Majority of solid media** are based on the same broth, but supplied by an agar alge extract. Bacteria grow slower on solid media, but the result is very variable, and it is possible to get a strain.

Various specimens – various cultivation

- How the specimen type influences culture?
 - Specimens, where **microbes use to be rare** are inoculated into liquid media only. Microbes multiply quickly. **Example: conjunctival swab**
 - Specimens, where **the amount of microbes may vary, but even small amounts are important** are cultured on both liquid and solid media. **Example: wound swab**
 - Specimens, where **usually we have many microbes, eventually even common microflora**, are cultured on solid media only. **Example: throat swab**

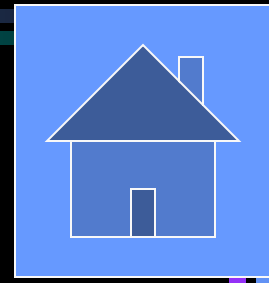


Liquid media

Liquid media

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Classification of liquid media

- Liquid media have two categories only:
- **multiplying media** are common and universal. Example: **broth** for aerobic culture and **VL-broth** for anaerobic culture (VL = viande-levure, from french – contains meat-yeas extract)
- **selectively multiplying media** were developed to multiply some bacteria and to supress multiplication of other. Example: **selenite broth** for salmonella

**Solid media:
inoculation of
specimen and
strain**

Solid media

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Solid (agar) media

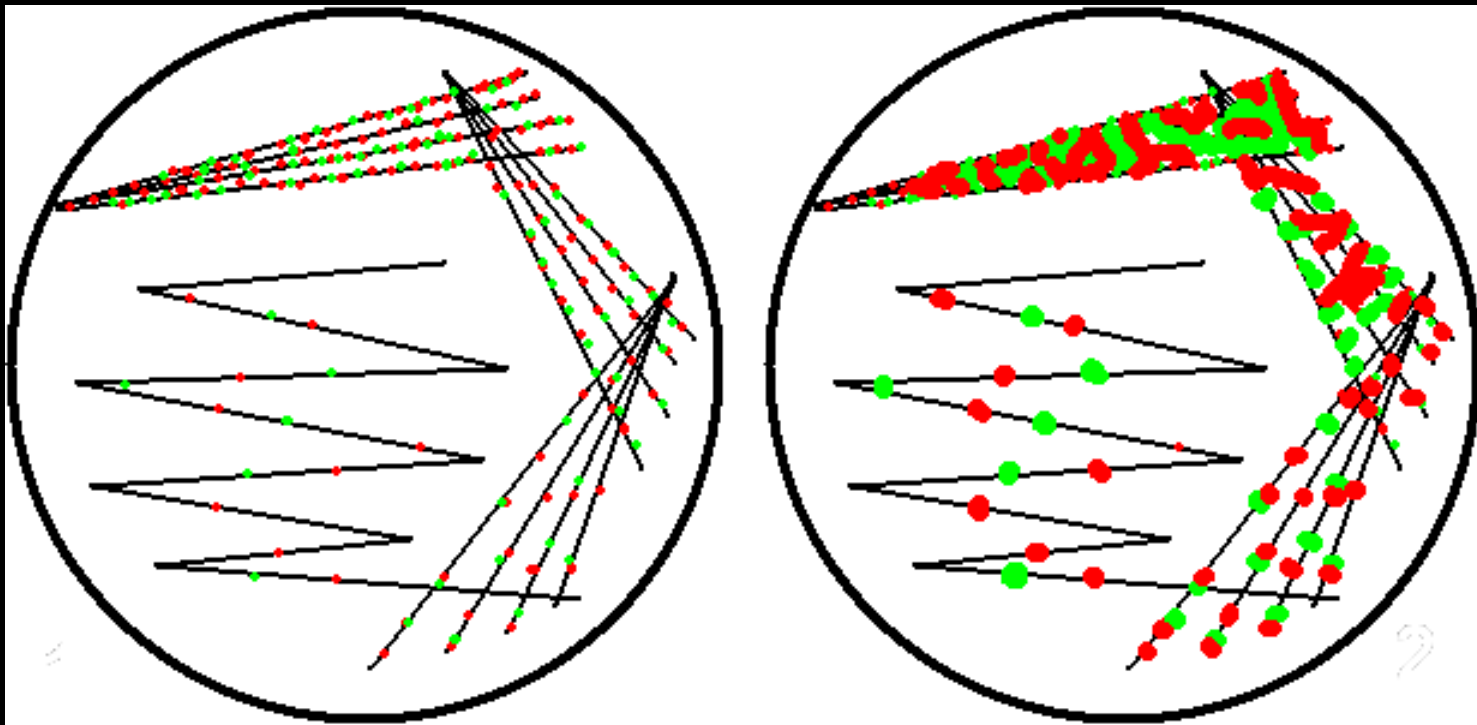
- To have all advantages, given by solid media, we have both the specimen *musíme* specimen (cultivation specimen → strain), but also strain (cultivation strain → strain) dilute properly at inoculation. Classical way of dilution inoculation is so named **cross inoculation**. In practice, usually e. g. one halfth of a plate is inoculated by a swab, and then diluted by a loop. Sometimes some discs and culture lines are added – not being a topic for today.

Why an isolated colony is so important

- Only so we can identify larger number of mixed pathogens
- But also because only isolated colonies enable to observe typical colony characteristics.

The best clown is not able to show you his art, when kept with many other clowns in a small cupboard.

In case of a mixture, each bacterium forms its own colonies
(at a proper dilution inoculation)



1 – inoculation of bacterial mixture (dots), 2 – result of cultivation: in first parts of inoculation a mixture, at the end – isolated colonies

How to inoculate of a specimen to a medium

Using the swab, inoculate the on a part of the agar plate (to about one third of Petri dish diameter)

Sterilize your loop

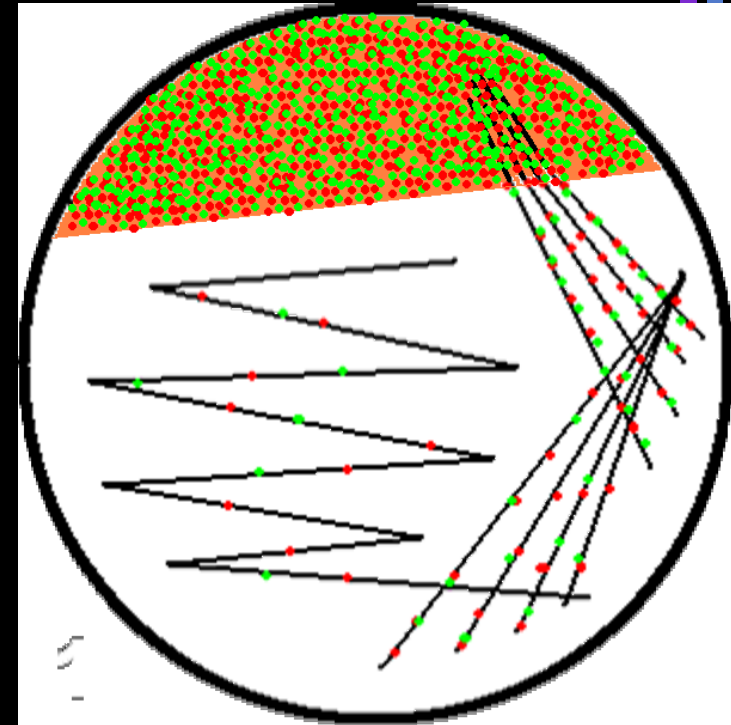
Dilute from part with specimen, making the second part of inoculation

Sterilize your loop

Dilute from lines inoculated in the second phase (not touching the part inoculated by the swab)

Sterilize your loop

Inoculate the „serpent“ on the remaining part of the plate



How to reinoculate of an agar culture

Sterilize your loop

Take the strain

Inoculate first phase

Sterilize your loop

Do not take the strain again

Inoculate second phase

Sterilize your loop

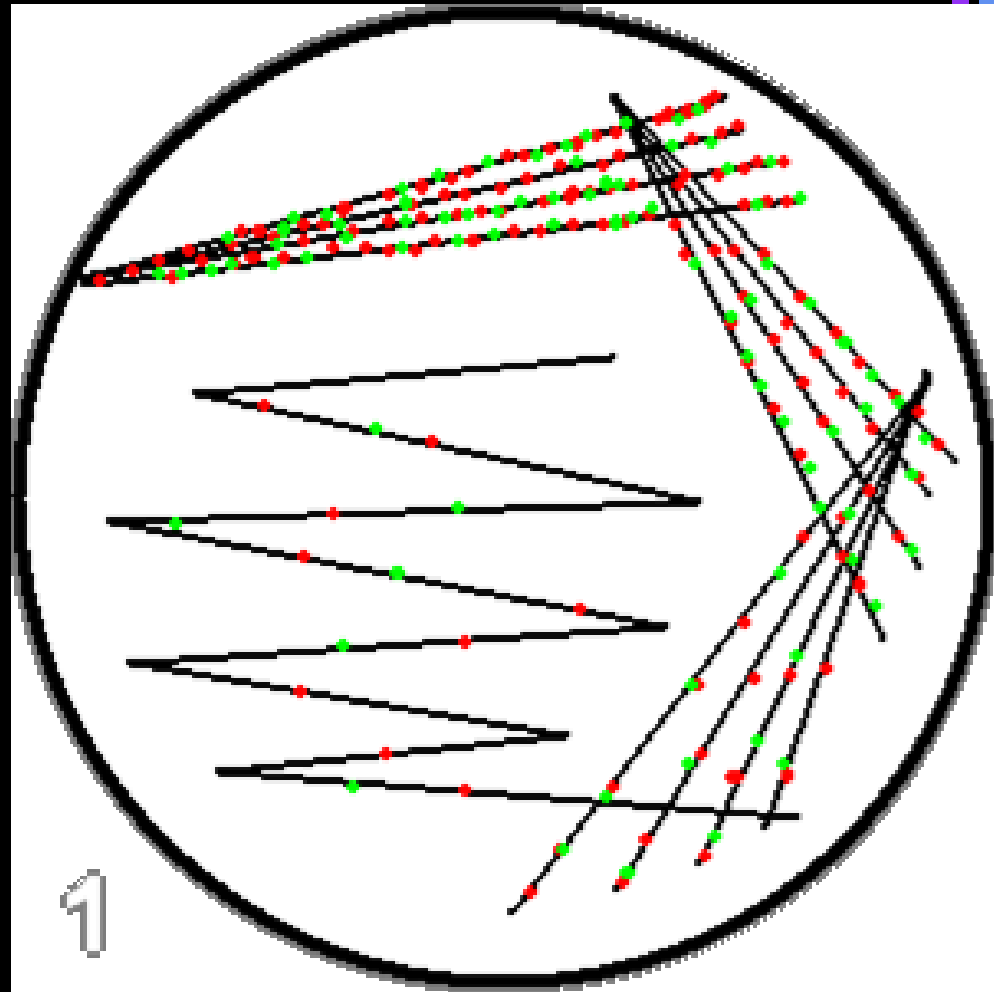
Do not take the strain again

Inoculate third phase

Sterilize your loop

Do not take the strain again

Inoculate the „serpent“



What to describe at colonies

- Size
- Colour
- Shape (round...)
- Forfile (convex...)
- Edges
- Surface (smooth, rough...)
- Consistence (dry...)
- Transparency
- Smell
- Colony surroundings*

**Definition is related to the medium used. For example, haemolysis is observed around some bacteria grown on media with RBCs.*

Difference between shape and profile

Shape



round



irregullar

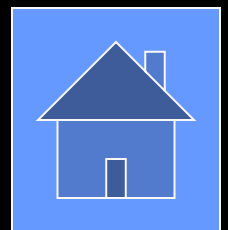
Profile



convex

flat

dish shaped



Solid media: classification and examples

Solid selective media

- They have to **select (separate)** from a **bacterial mixture** only one of several groups of genera
- An example is **blood agar with 10 % NaCl** used for **stafylococci**
- Sometimes, selectivity is reached by an antibiotic addition. **Blood agar with amikacin** is selective for streptococci and enterococci

Diagnostic media

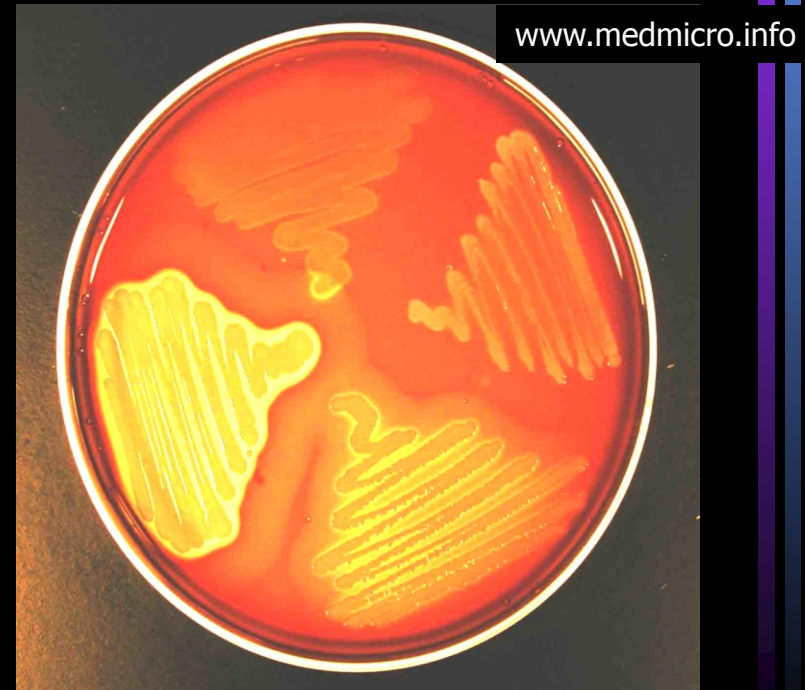
- They do not suppress growth of any microbe
- On the other hand, their composition enable them to **differentiate microbes according to some properties**
- An example is **blood agar** to observe haemolytical properties, and **VL blood agar** (similar, but to anaerobes)
- Special case are chromogenic and fluorogenic media



Changes on blood agar

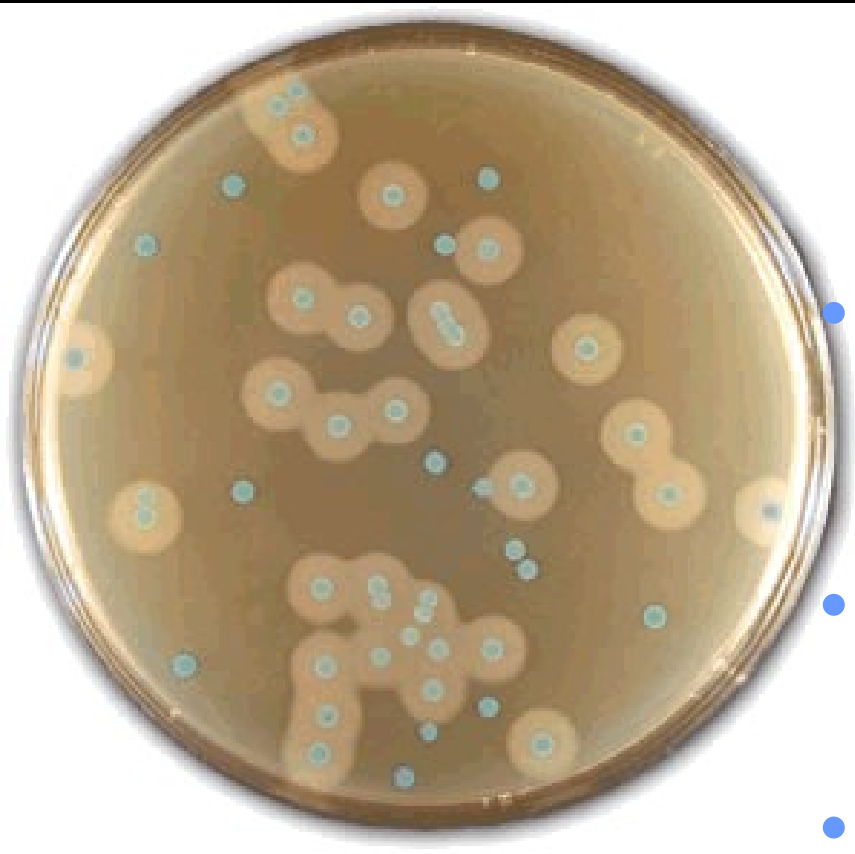
- All media with RBCs (blood agar, VL bloodní agar, agar with washed RBCc etc. –but not blood agar with 10 % NaCl, where RBCs are lyzed) enable us to see:

- Total haemolysis
- Partial haemolysis
- Absence of haemolysis
- Viridation (green)



Chromogenic and fluorogenic media

- **Chromogenic** media contain a dye with bound specific substrate → it loses its colour, it is no more a dye, but a **chromogen**
- bacteria able to breakdown the specific substrate **change the chromogen** against to the original dye
- The medium may contain more chromogens (for more species)
- **Fluorogenic** media: similar, with a **fluorescent dye**



Chromogenic media for yeasts



Four various yeasts that grow in typical colonies – one in green, one in blue, one in dry pink and one in smooth pink. Other yeasts are white on this medium



Properties of Endo agar

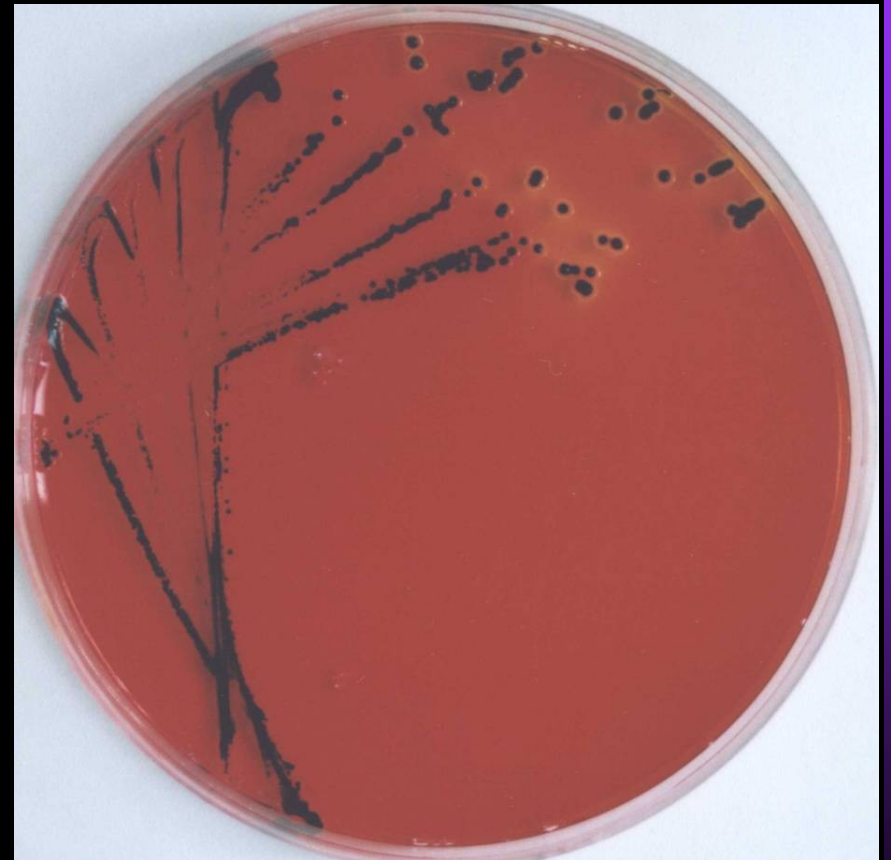
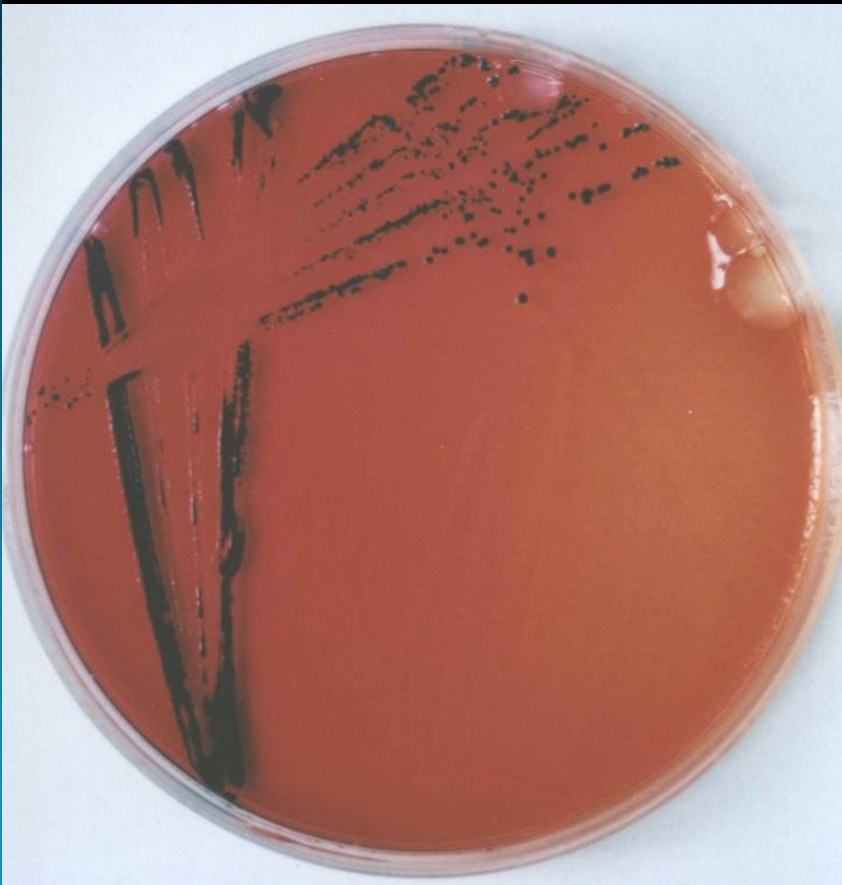
- Endo agar enable growth of **G- bacteria only**, and even not all of them (selectivity)
- The growing bacteria can be differentiated into **lactose positive** (red) and **negative** (pale).



From the point of view of medical microbiology, it is important: lactose positive bacteria are usually milder pathogens than lactose negative bacteria

- A similar is **McConkey medium**, more common in world (but not used in OUR laboratory)
- Selective diagnostic are also **XLD**, **CIN** media etc.

XLD and MAL media for *Salmonella*



From our
chamber
refrigerator



Selective, diagnostic and selective diagnostic media – review

Selective medium	Strain A does not grow	Strain B grows	
Diagnostic medium	Strain C grows, colonies ■	Strain D grows, colonies ●	
Selective diagnostic medium	Strain E does not grow	Strain F grows, colonies ■	Strain G grows, colonies ●

Enriched and selective enriched media

- For bacteria with specific need for nutrients
- They are enriched by different chemicals
- **Even blood agar is an enriched medium**, although shown as a diagnostic medium (it may be considered a member of both groups).
- An example of „pure enriched medium“ is **chocolat and Levinthal agar for pathogenous Neisseriae** and hemophili (that do not grow even on blood agar)
- Media may be **selective enriched** (e. g. **GC agar**, – chocolat agar with antibiotics for culture of *Neisseria gonorrhoeae*)

Chocolate agar



Special use media – 1

Observation of virulence factors

- Picture shows a medium with Congo red for staphylococcal biofilm
- or e. g. **yolk agar** for histotoxic clostridia

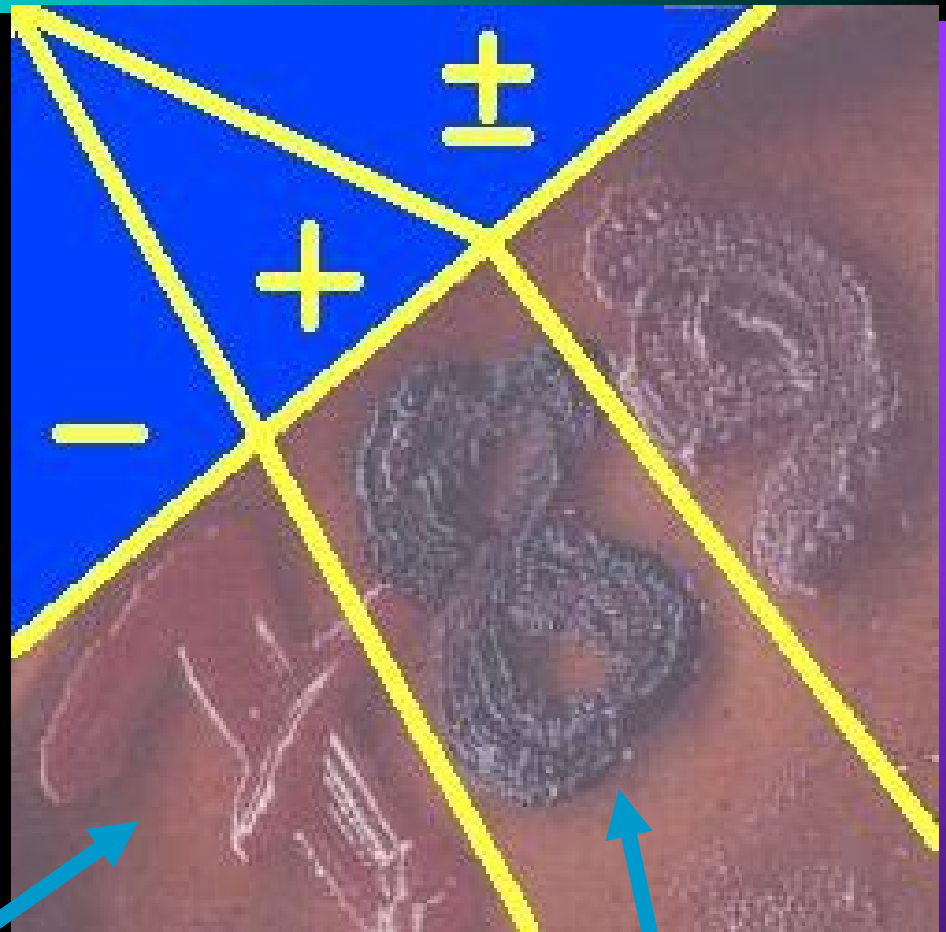


Photo: O. Z.

Red, wet colonies mean no production of biofilm

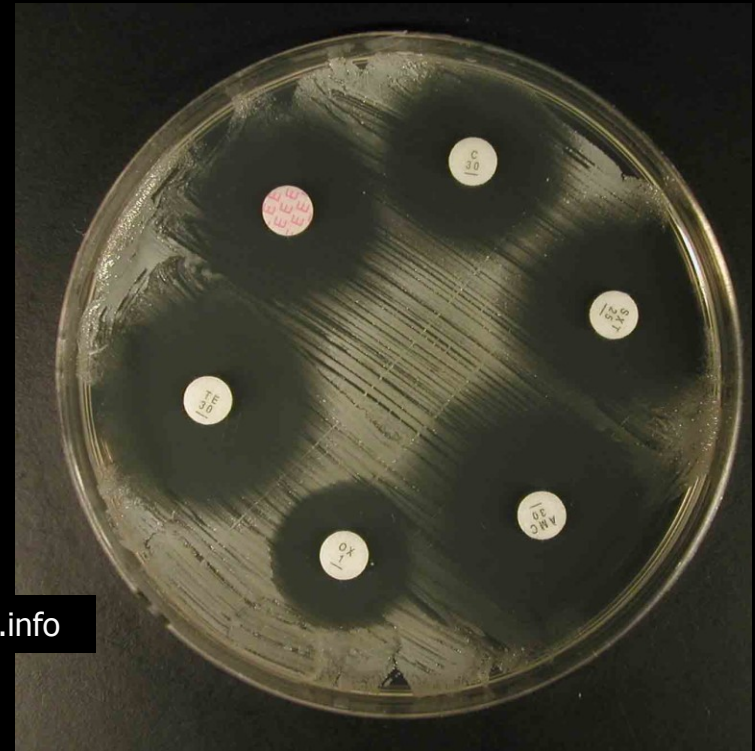
Dry, black colonies show biofilm production

Special use media – 2

In vitro antibiotic susceptibility testing: Müller-Hinton agar; also to pigments production observation



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Right, a non-pigmented Staphylococcus strain, left down a pigmented Pseudomonas strain

Note



In bacteria requiring growth factors even antibiotic testing should be performed on enriched media

Modern trends in culture



- Despite development of genetic methods, **cultivation still keeps its key role** in mostly bacterial diagnostics
- Because of standardization, laboratories **have to switch from „home made“ media to commercial products**
- **Chromogenic and fluorogenic media** start to be used more and more, despite the price

Survey of the most important media

1. Broth
2. VL-broth
3. Selenite broth
4. Sabouraud
5. Löwenstein-Jenssen
6. Blood agar (BA)
7. Endo agar
8. MH
9. BA + 10 % NaCl
10. VLA (VL BA)
11. XLD (and MAL)
12. CHA
13. Levinthal
14. Slanetz-Bartley

Survey of media – part one

*only with antibiotics

Name	Class	Colour	Type	For
broth	liquid media	yellowish	multiplying	aerobes
VL-broth		darker		anaerobes
selenite broth		pinkish	selective multiplying	<i>Salmonella</i>
Sabouraud agar	solid media in a test tube	white	selective*	fungi
Löwentein-Jensen		green	enriched	TBC
blood agar	solid media in dish	red	enriched + diagnostic	majority of bacteria
Endo agar			pink	selective diagnostic

Survey of media – part two

Name	Class	Colour	Type	For
MH	solid media on Petri dish	nearly white	special	atb suseptibility
NaCl		brown	selective	staphylococci
VL-agar		red	like BA	anaerobes
XLD (and similar MAL)		orange	selective diagnostic	<i>Salmonella</i>
chocolate agar		brown	enriched	haemophilli, neisseriae
Levinthal agar		yellowish	enriched	haemophilli
Slanetz-Bartley		pink	selective diagnostic	enterococci

The End

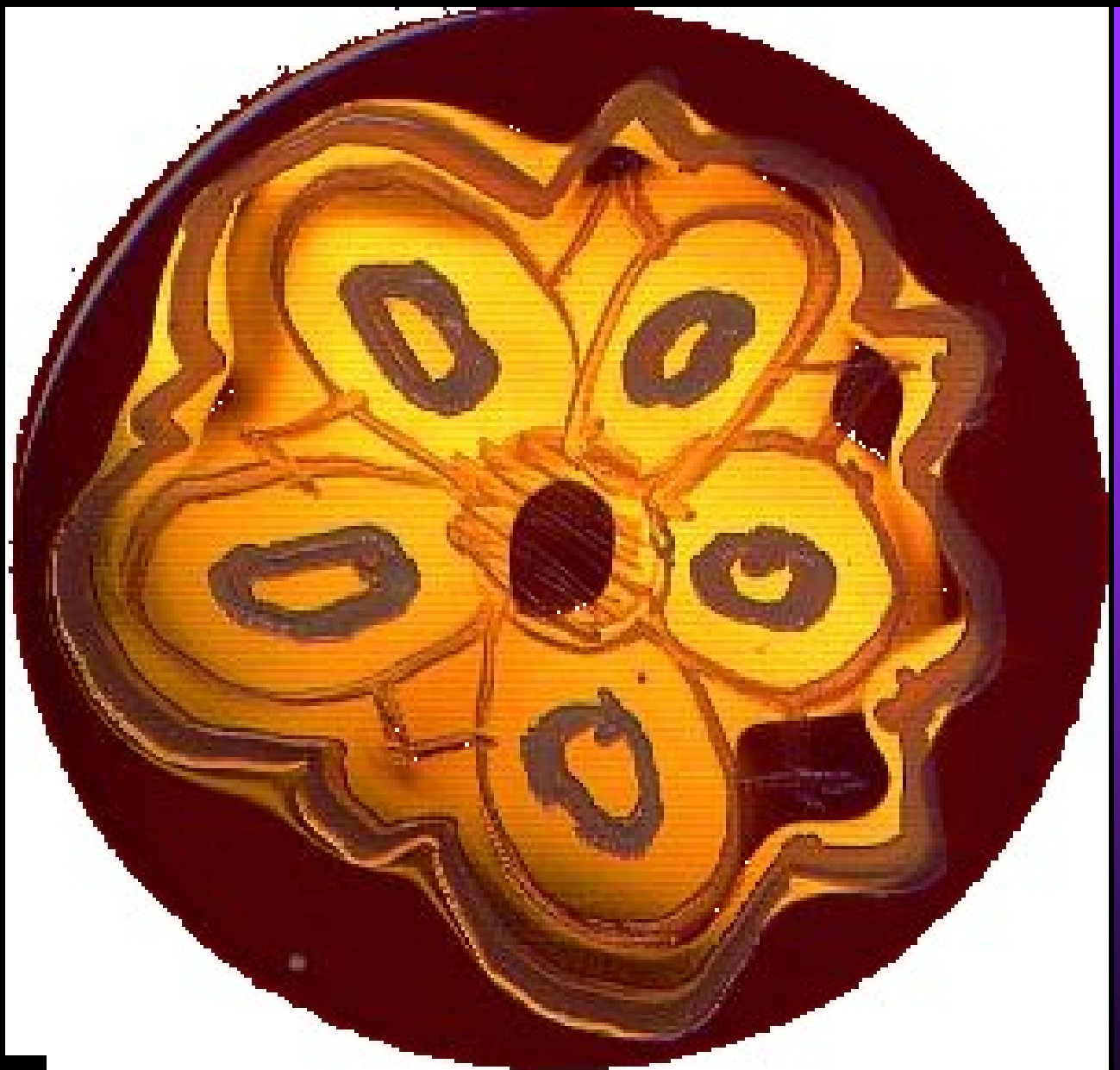
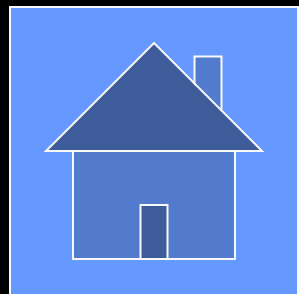
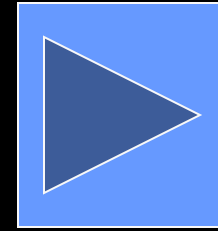


Photo O. Z.

Robert Koch

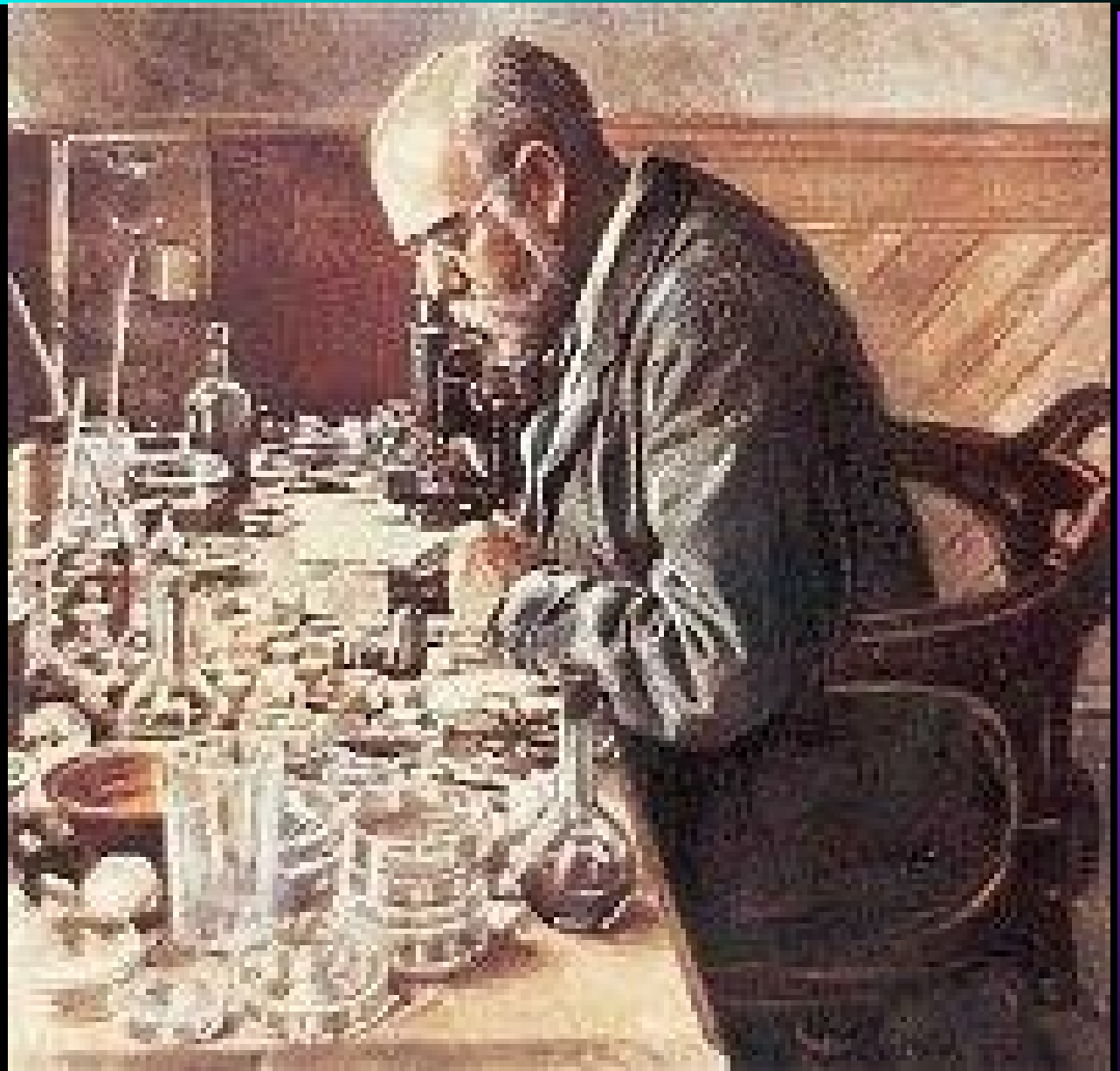


Robert Koch (1843 – 1910)

Bacteriologist Robert Koch discovered the anthrax disease cycle (1876); and the bacteria responsible for tuberculosis (1882) and cholera (1883). Koch formulated rules for the control of epidemics of cholera. "Koch's Postulates" (Kochsche Postulate, refined in 1884) are still the basic procedures used by modern epidemiologists and medical researchers: (1) Identify and specific organism, (2) obtain and pure culture of that organism, (3) reproduce the disease in experimental animals using the pure culture, and (4) recover the organism from the infected animals.

<http://www.general-anaesthesia.com/images/robert-koch.html>

Once more Robert Koch



Robert Koch in Egypt expedition during a cholera epidemics



Back

www.amuseum.de/rkoch.htm

Solid media in a test tube? Why?

Among given media, two of them are in test tubes, although they are solid. The reason is that they are used for slowly growing organisms. Both mycobacteria (**Löwenstein-Jensen**) and some molds (**Sabouraud**) grow slowly and the medium would be dry before the organism would grow on a Petri dish.

In case of **Hajna** medium (see J04) the reason is different: the medium is used for biochemical testing and the difference between the lower part (no access to oxygen) and upper part (surface of medium) is important for its function.

Löwenstein Jensen medium is also interesting as is it solid although agar is absent; it is solid because of coagulated eggs.

Blood agars

- It is possible to use blood agar with red blood cells of various organisms (horses, chicken, cattle, and even humans). Nevertheless, the **sheep RBCs** are far most used ones
- It is possible to add blood cells to various bases. For example, if you add blood to VL broth (simplified), you get VL agar (VL blood agar)
- For haemolytical interaction testing (e. g. CAMP test, see P02) it is recommended to use agar with washed red blood cells.

Back

Endo agar and its principle

- Endo agar contains **lactose** as a substrate
- It also contains **basic fuchsin**
- This fuchsin acts as **factor of selectivity**
- The same fuchsin (together with **Na₂SO₃**) also acts as **indicator (Schiff reagent)**. Bacteria forming lactaldehyde from lactose are visualised by purple colour

Endo agar should be kept away from light, otherwise it becomes purple without bacteria.