

In vitro culture

Cultivation

- Multiplication of bacteria and fungi in laboratory conditions
 - artificial media
 - Usually lost some properties of metabolic activities, virulence, change genetic information
- Biological material - streaked on the plate or inoculated to the cultivation medium (solid or liquid) - for multiplication - thick, dense growth
- - for isolation - isolated colonies on solid plate media

Practical 7 - Physiology of bacteria

- Knowledges of physiology - enable to prepare required conditions for laboratory detection:
- oxygen - O₂, CO₂, without O₂, defined atmosphere - (Campylobacter, Helicobacter)
- temperature - usually 37°C, 42°C - Campylobacter, 22°C - Pasteurella, different look according to the temperature - motility, fungi - molds/yeast
- blood - animal blood - sheep, horse - blood agar, denaturated blood - chocolatised agar
- nutrition factors - vitamins, aminoacids, detoxification materials.....

Cultivation media

- Basic (multiplication, transport)
 - solid: blood agar, Muller Hinton agar,
 - liquid: bouillon, Muller Hinton bouillon,
- Diagnostic - visualise certain kind of bacteria - the color of colonies of different kind of bacteria is different
- Selective - contain substances that enable growth of one kind of bacteria and inhibits other bacteria * ATB (broad spectrum) - eliminate susceptible bacteria , concentration of NaCl,.....
- Special - defined - detection of some properties
- Combination

- Diagnostic - visualise certain kind of bacteria - the color of colonies of different kind of bacteria is different - (biochemical properties) - DC agar, Endo, Hajn agar, urea agar, esculin agar, SC agar
- Selective - contain substances that enable growth of one kind of bacteria and inhibits other bacteria
ATB (broad spectrum) - eliminate susceptible bacteria , concentration of NaCl,.....
 - manit salt (combination)
 - chocolatised agar
 - McConckey
 - Karmali
 - for M. tbc - Lowenstein, Ogawa,

Cultivation media - Classification based on consistency

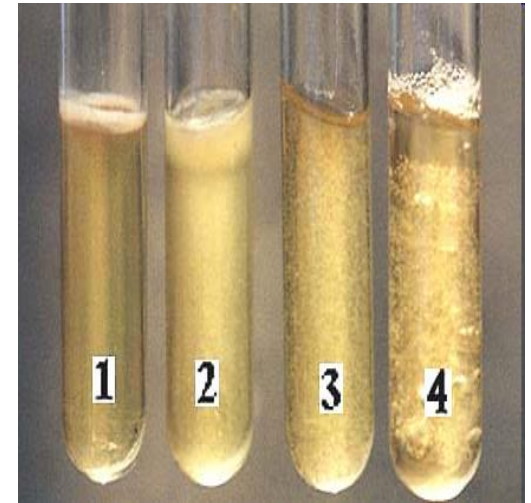
- solid: blood agar, Muller Hinton agar,
- liquid: bouillon, Muller Hinton bouillon

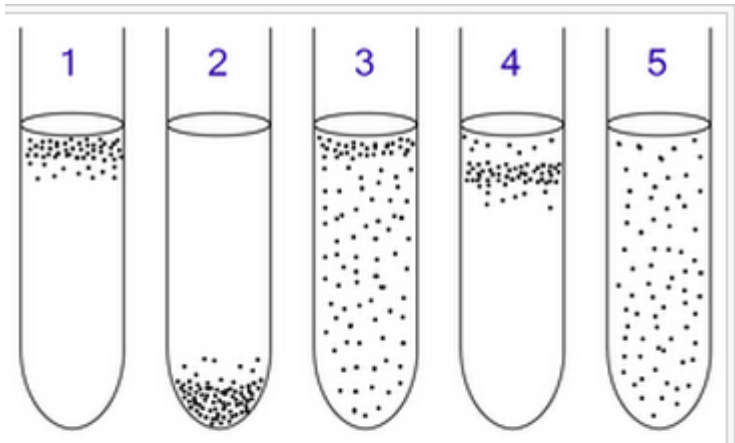
Differential media

- *Differential media* or *indicator media* distinguish one microorganism type from another growing on the same media. This type of media uses the biochemical characteristics of a microorganism growing in the presence of specific nutrients or indicators (such as [neutral red](#), [phenol red](#), [eosin y](#), or [methylene blue](#)) added to the medium to visibly indicate the defining characteristics of a microorganism. This type of media is used for the detection of microorganisms and by molecular biologists to detect recombinant strains of bacteria.
- Examples of differential media include:
 - [blood agar](#) (used in [strep](#) tests), which contains bovine heart blood that becomes transparent in the presence of hemolytic [Streptococcus](#)
 - [eosin methylene blue](#) (EMB), which is differential for lactose and sucrose fermentation
 - [MacConkey](#) (MCK), which is differential for lactose fermentation
 - [mannitol salt agar](#) (MSA), which is differential for mannitol fermentation
 - [X-gal](#) plates, which are differential for [lac operon](#) mutants

Identification of colonies

- **Description of growth on liquid medium:**
- turbidity, density
- sediment
- surface membrane

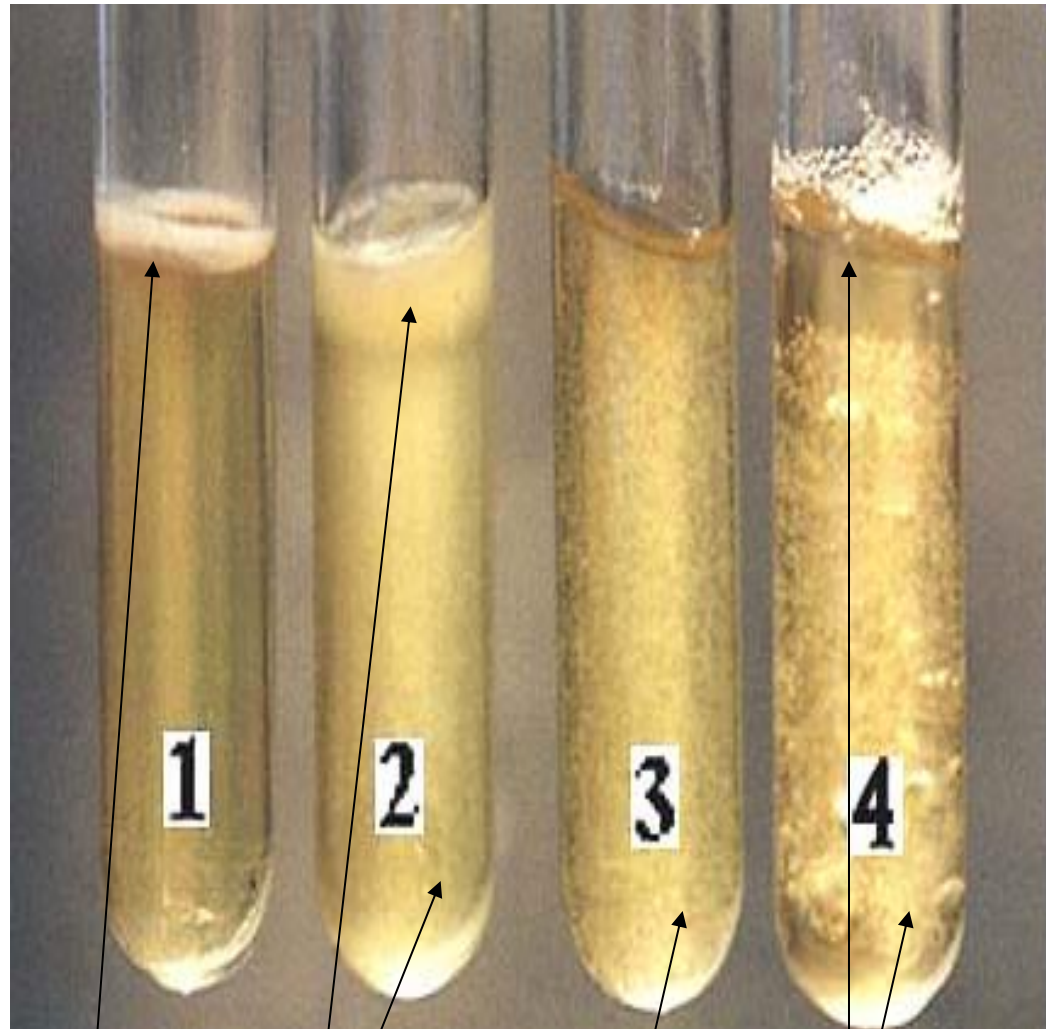




Aerobic and anaerobic bacteria can be identified by growing them in a liquid culture:

- 1: Obligate aerobic bacteria gather at the top of the test tube in order to absorb maximal amount of oxygen.
- 2: Obligate anaerobic bacteria gather at the bottom to avoid oxygen.
- 3: Facultative bacteria gather mostly at the top, since aerobic respiration is the most beneficial one; but as lack of oxygen does not hurt them, they can be found all along the test tube.
- 4: Microaerophiles gather at the upper part of the test tube but not at the top. They require oxygen but at a low concentration.
- 5: Aerotolerant bacteria are not affected at all by oxygen, and they are evenly spread along the test tube.

Oxygen relationship designation



STRICT
(OBLIGATE)
AEROBE

FACULTATIVE
ANAEROBE

AEROTOLERANT
ANAEROBE

STRICT
(OBLIGATE)
ANAEROBE

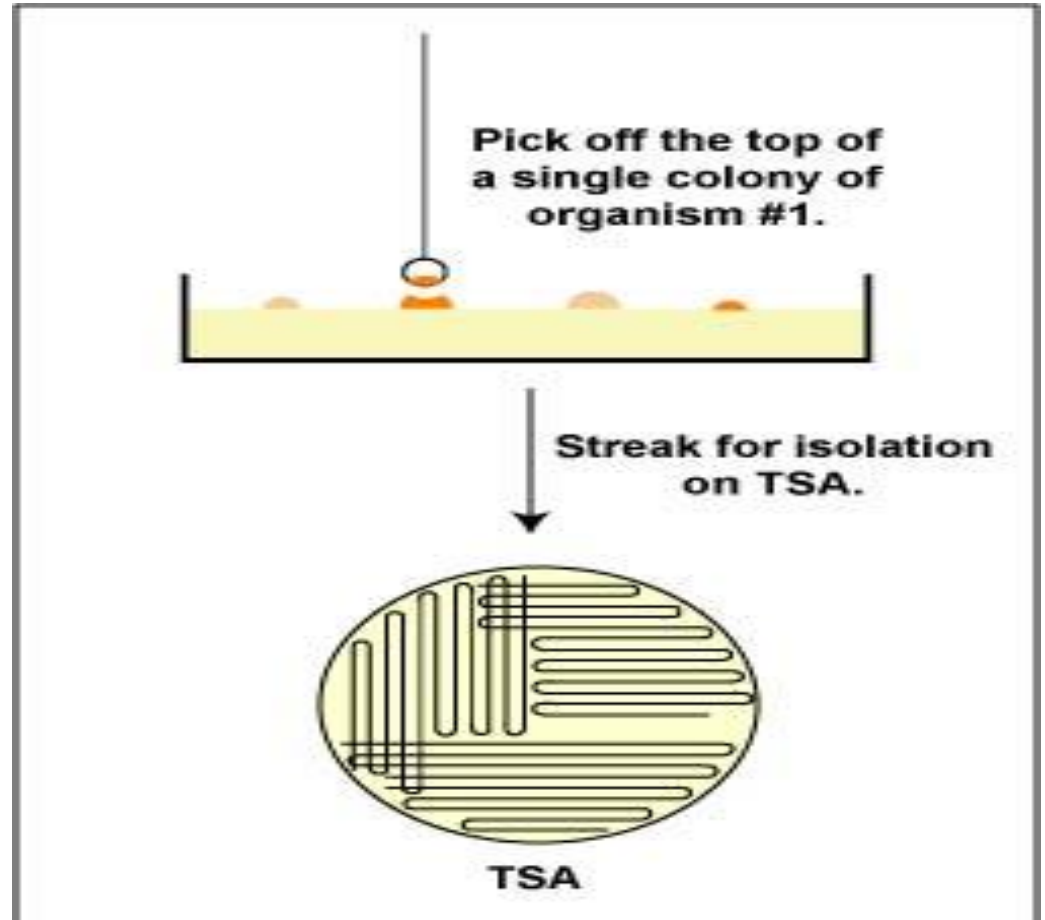
Colony

- Colony arises from a CFU - colony forming unit - a piece of biological material from which a certain quantity grow on the same place: 1 colony = thousands of bacteria (microscopy)

CULTIVATION

Colony —Picking

- Sterile needle or loop is touched to surface of colony and transferred to fresh, sterile media
- Incubation for another 24 hours



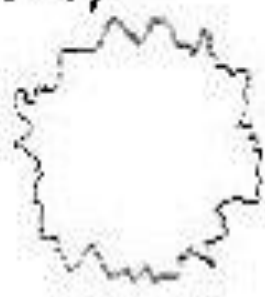
- **Description of growth on solid media**
- shape, look (dry, mucous), color- pigment, smell, colony edges, spread of colonies, localisation of colonies, change of environment, consistence,

COLONIAL MORPHOLOGY

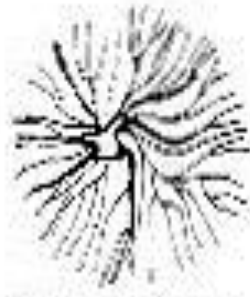
SHAPE OF COLONY



round



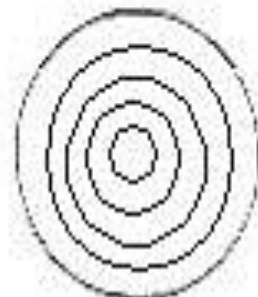
irregular



filamentous



rhizoid



curled

EDGE



entire



filamentous

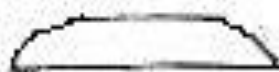


undulate

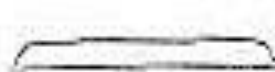
lobate

irregular

ELEVATION



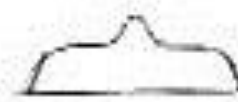
raised



flat



convex



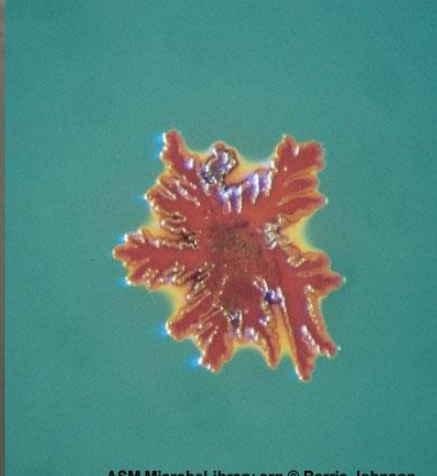
umbonate



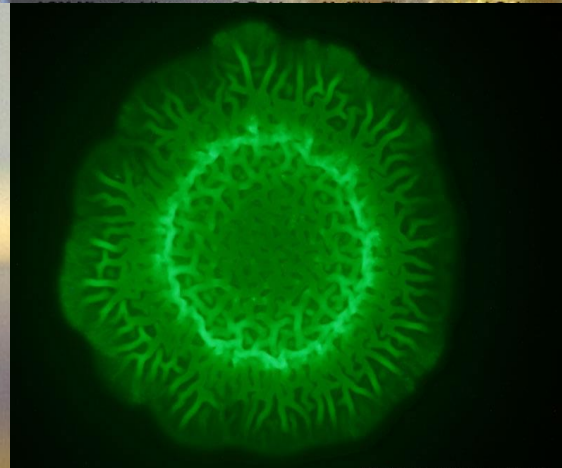
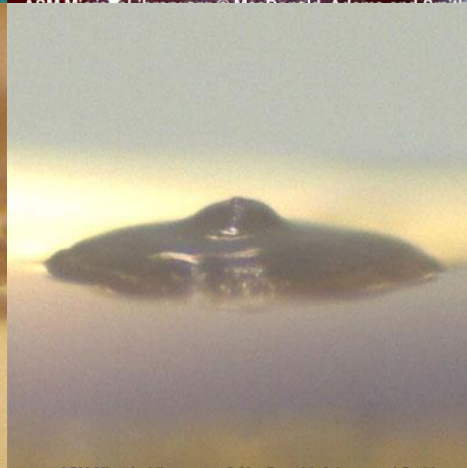
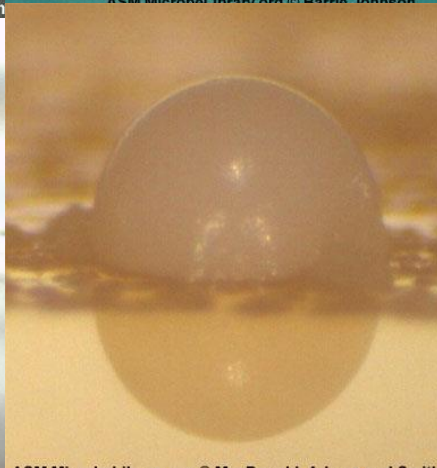
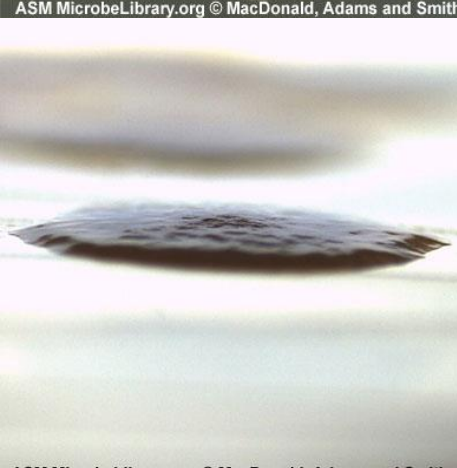
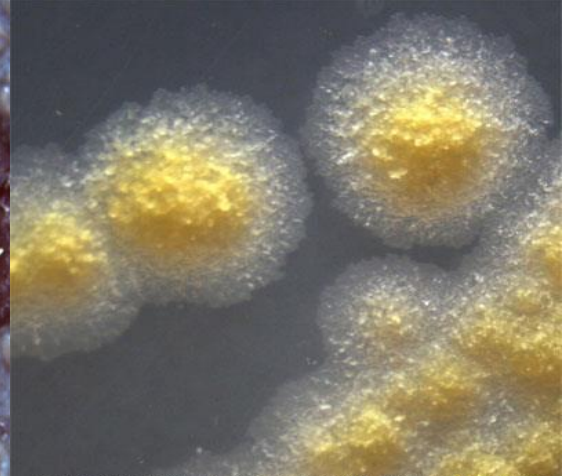
growth into
medium



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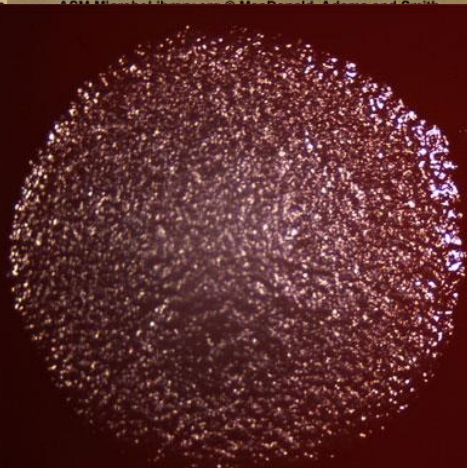
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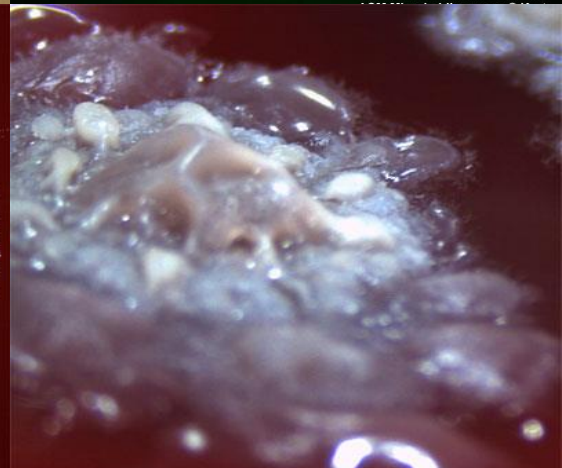
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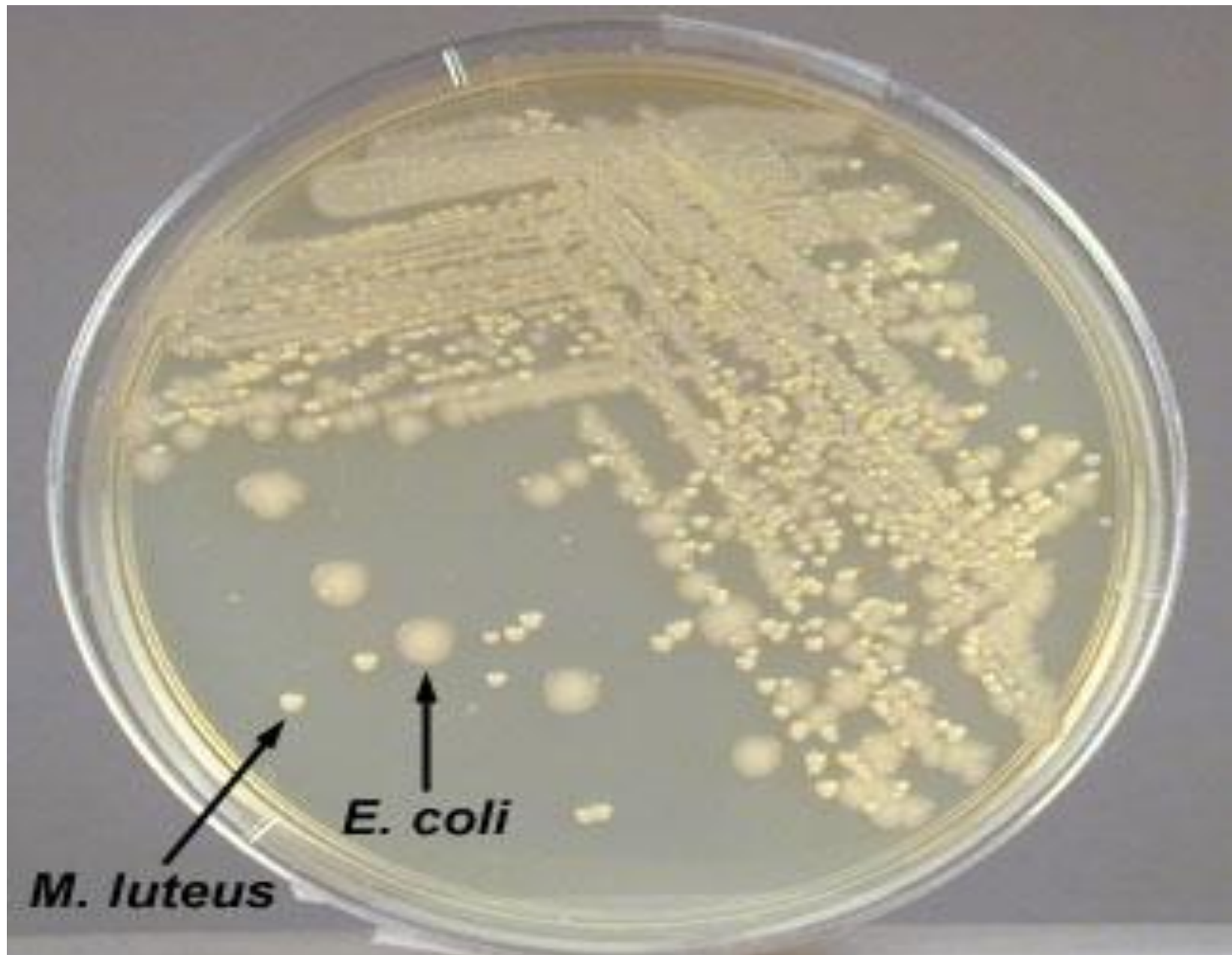
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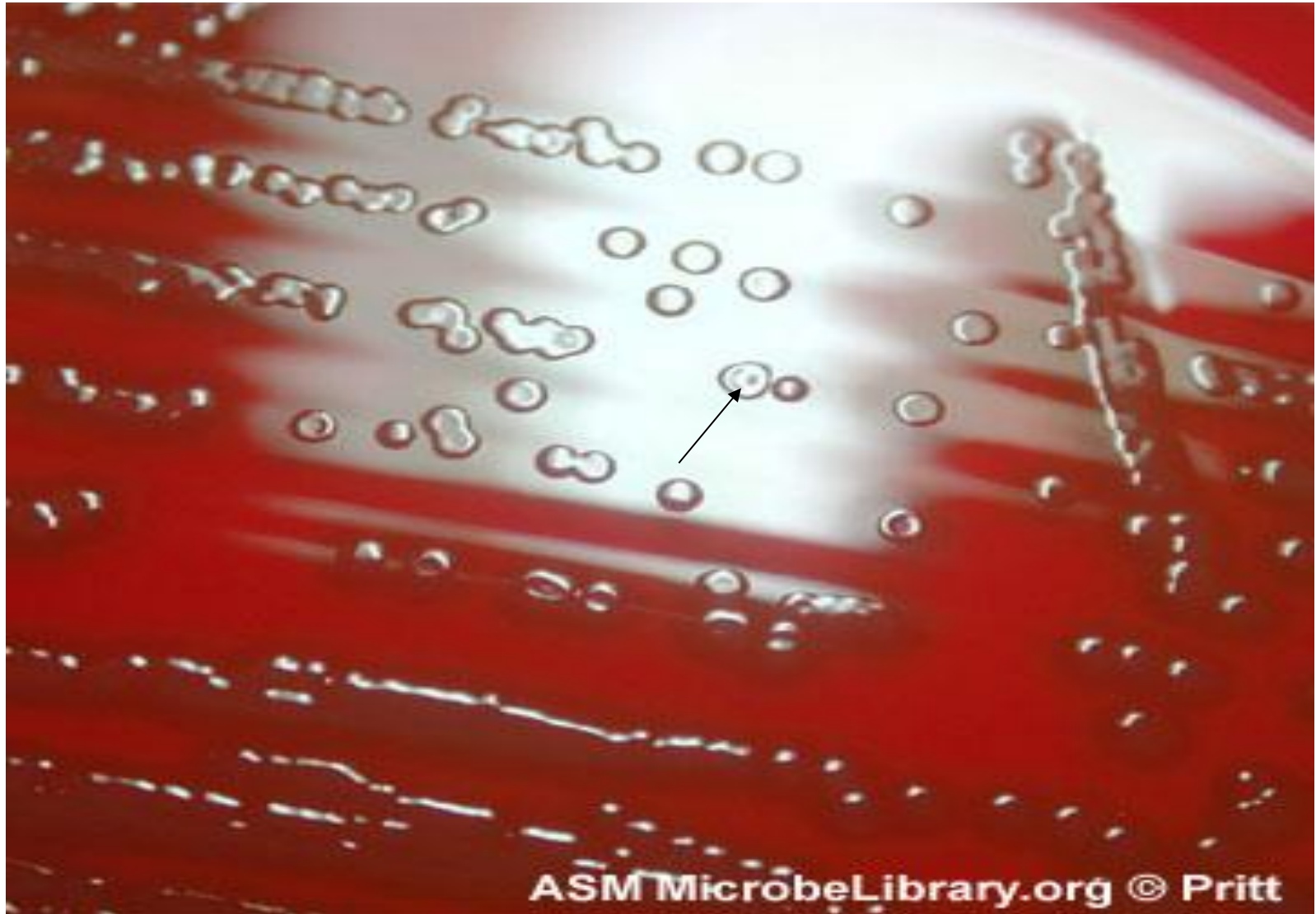
M. luteus

E. coli

Mucous colonies - *Str. pneumoniae*,
Klebsiella pneumoniae



Str. pneumoniae- convex in the center , umbilical.



Blood agar



- Contains mammalian blood (usually sheep or horse),
- to isolate fastidious organisms
- detect hemolytic activity

Hemolytic activity



- β -hemolytic - *Streptococcus pyogenes*, *Str. haemolyticus*,
Str. agalactiae
- α -hemolysis - *Streptococcus pneumoniae*, *Streptococcus viridans*
- γ -hemolysis (or *non-hemolytic*)- *Staphylococcus epidermidis*

alfa hemolysis - green - viridation

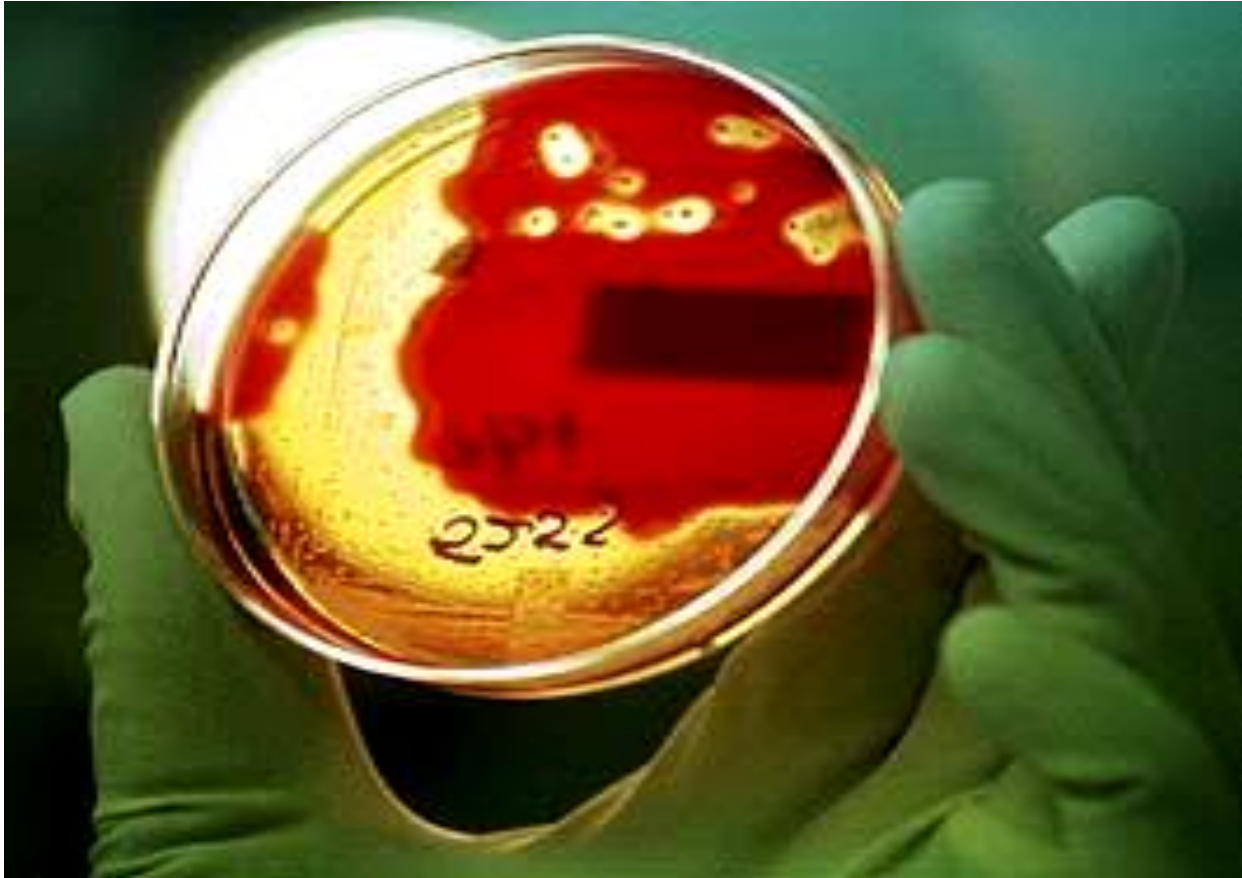
beta hemolysis - transparent - real hemolysis

gama hemolysis - no change

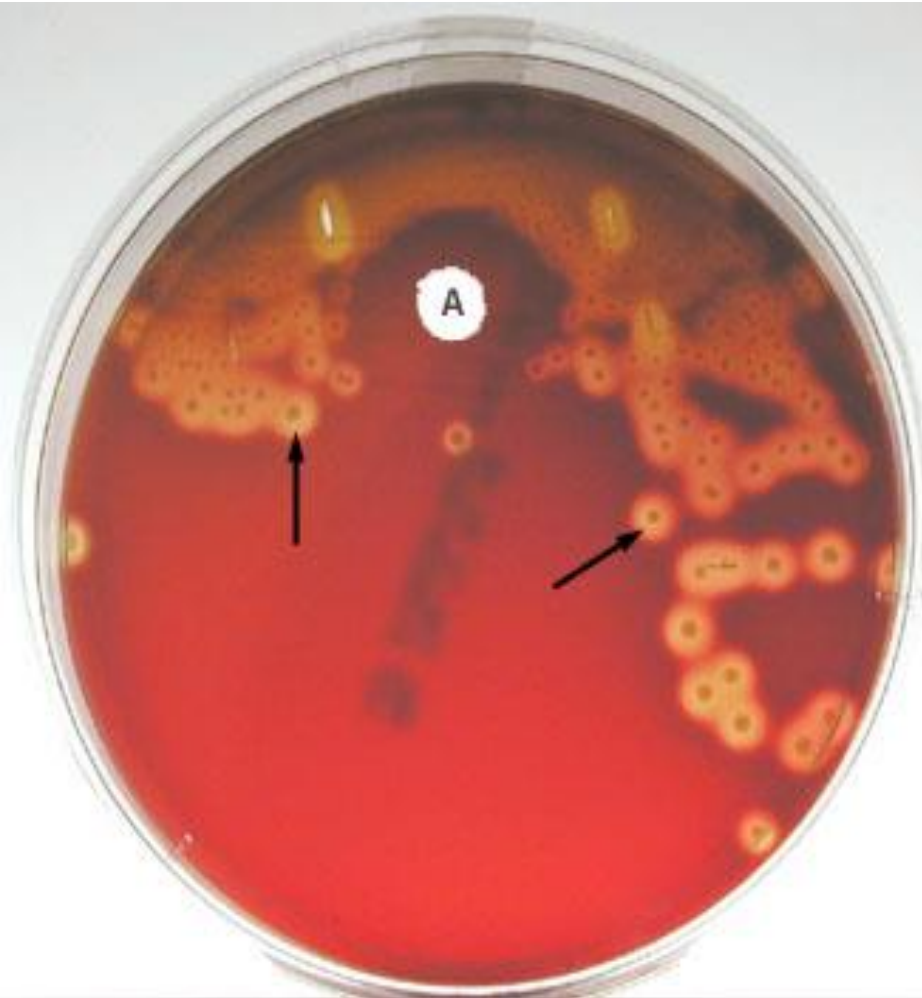


Demostration

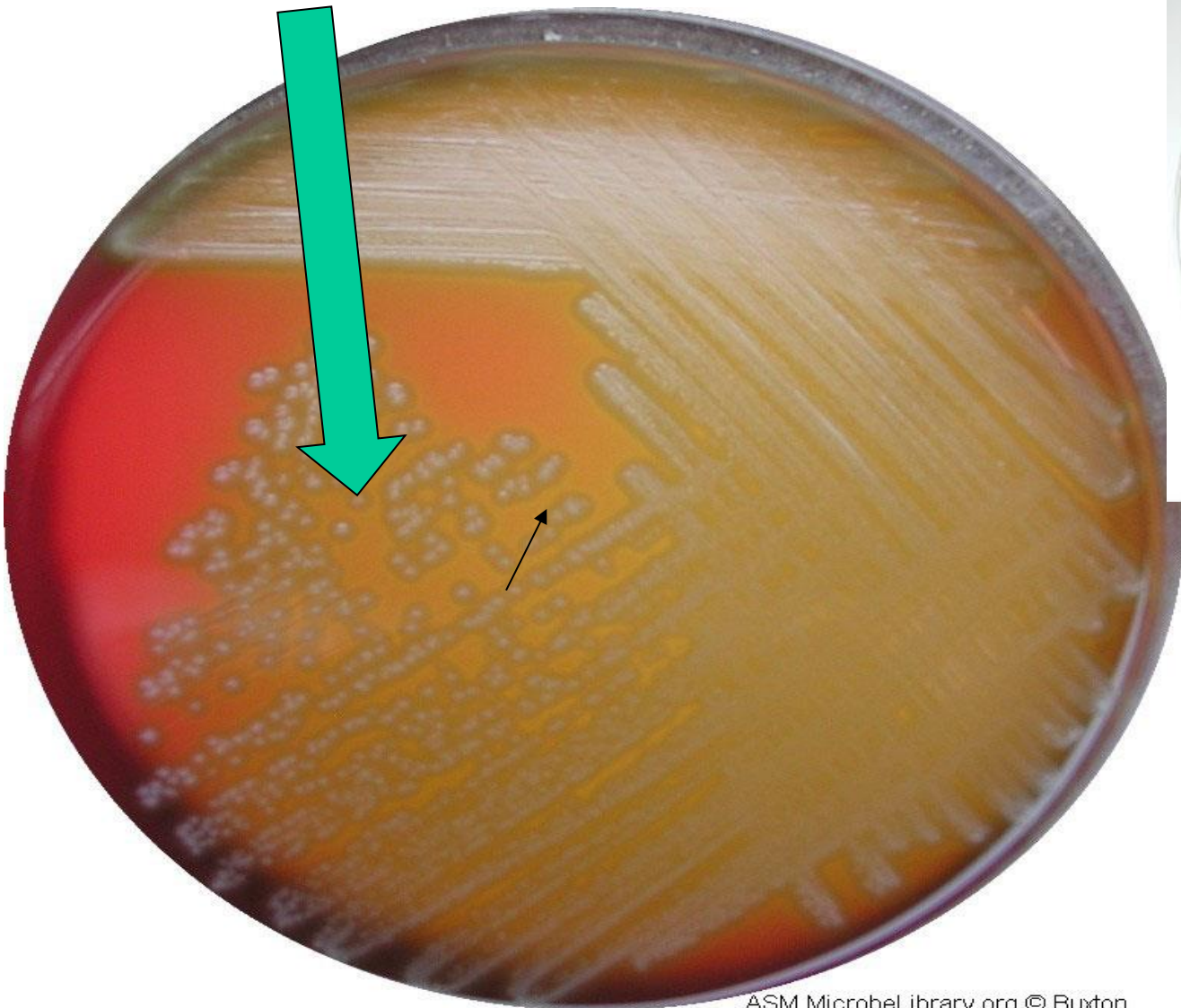
- Hemolysis - alfa - *Streptococcus pneumoniae*
 - beta - *Streptococcus agalactiae*,
 - gama - *Staphylococcus epidermidis*
- Pigment . *Staphylococcus aureus* - yellow,
Staphylococcus epidermidis - white
- Look - dry - *Staphylococcus epidermidis*
 - mucous - *Klebsiella pneumoniae*



HSA – β hemolysis



α hemolysis



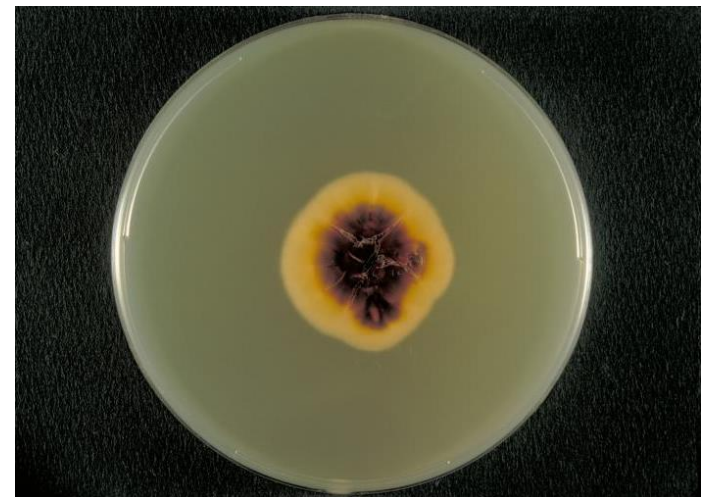
β hemolysis

TT



Sabouraud agar

- Sabouraud agar is used to culture fungi and has a low pH that inhibits the growth of most bacteria; it also contains the antibiotic gentamicin to specifically inhibit the growth of Gram-negative bacteria.



- Some of the first steps in identifying bacteria are to examine:
- the shape of the individual bacterial cells
- if the bacteria exist in specific groupings
- the colony morphology (the appearance of a colony of bacteria...a group composed of millions of bacteria growing on laboratory media).



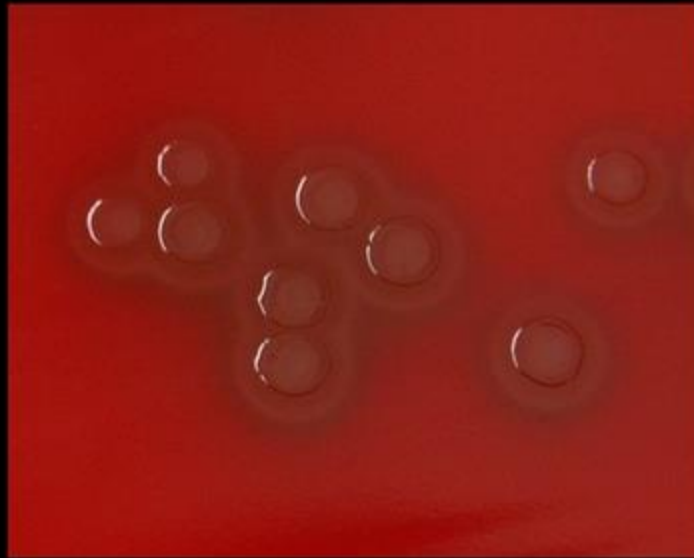
Klebsiella pneumoniae

gamma hemolysis



Enterococcus faecalis

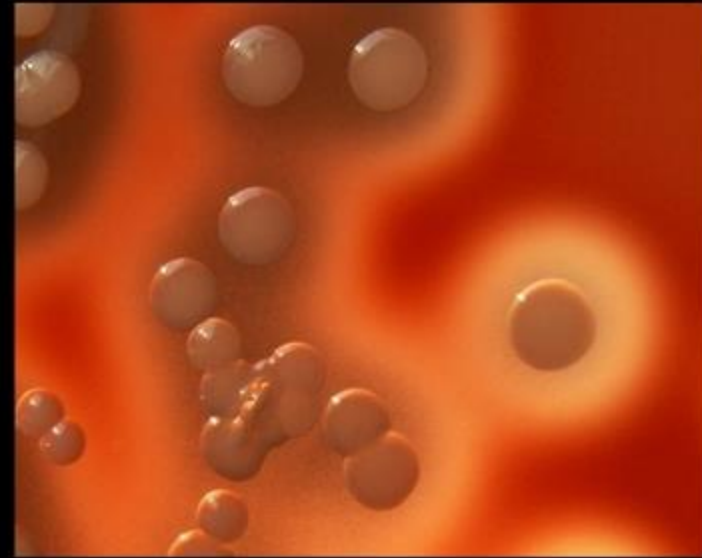
gamma hemolysis



FN

Streptococcus pneumoniae

alpha hemolysis



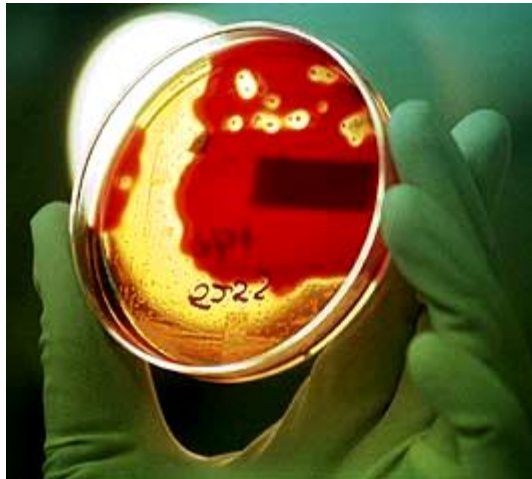
Staphylococcus aureus

beta hemolysis

1. *St. aureus*, *St. epidermidis* – blood agar



2. *Str. pyogenes*, *Str. pneumoniae*, *Str. salivarius*



**3. *M. catarrhalis*,
S-phase**



***Kl. Pneumoniae*,
M-phase**



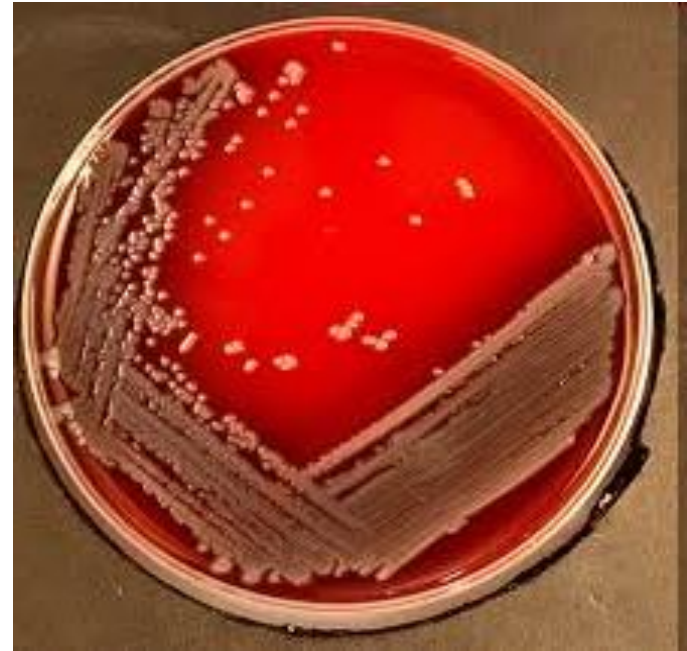
***B. cereus*
R-phase**



4. *E. coli*



Pseudomonas aeruginosa



ENDO agar



- differential
- selective
- detection of coliform and other enteric microorganisms

5. ENDO agar

E. coli



Shigella dysenteriae



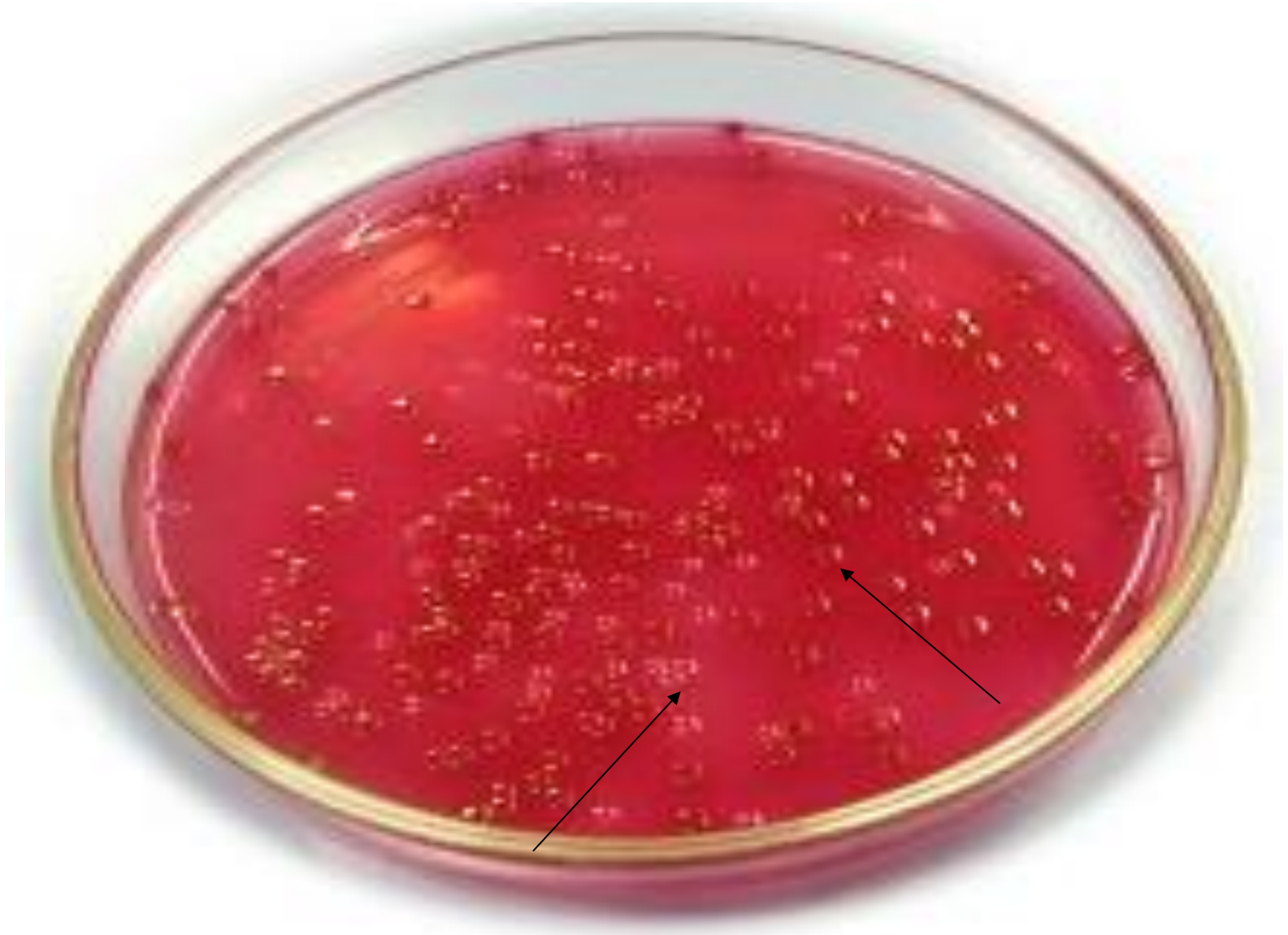
Salmonella typhi



Proteus vulgaris



Endo agar *E. coli* + *Sh. flexneri*



DC agar

E. coli

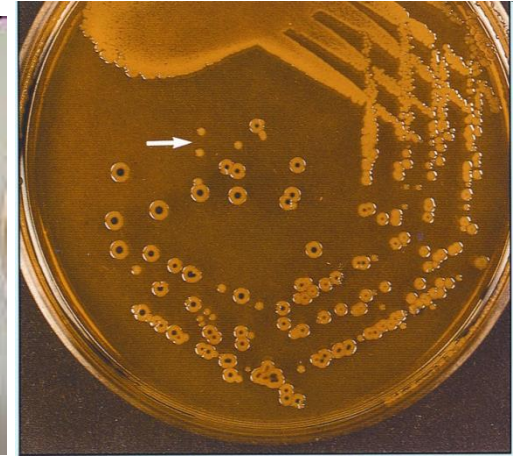
Shigella dysenteriae

Salmonella typhi

Proteus vulgaris



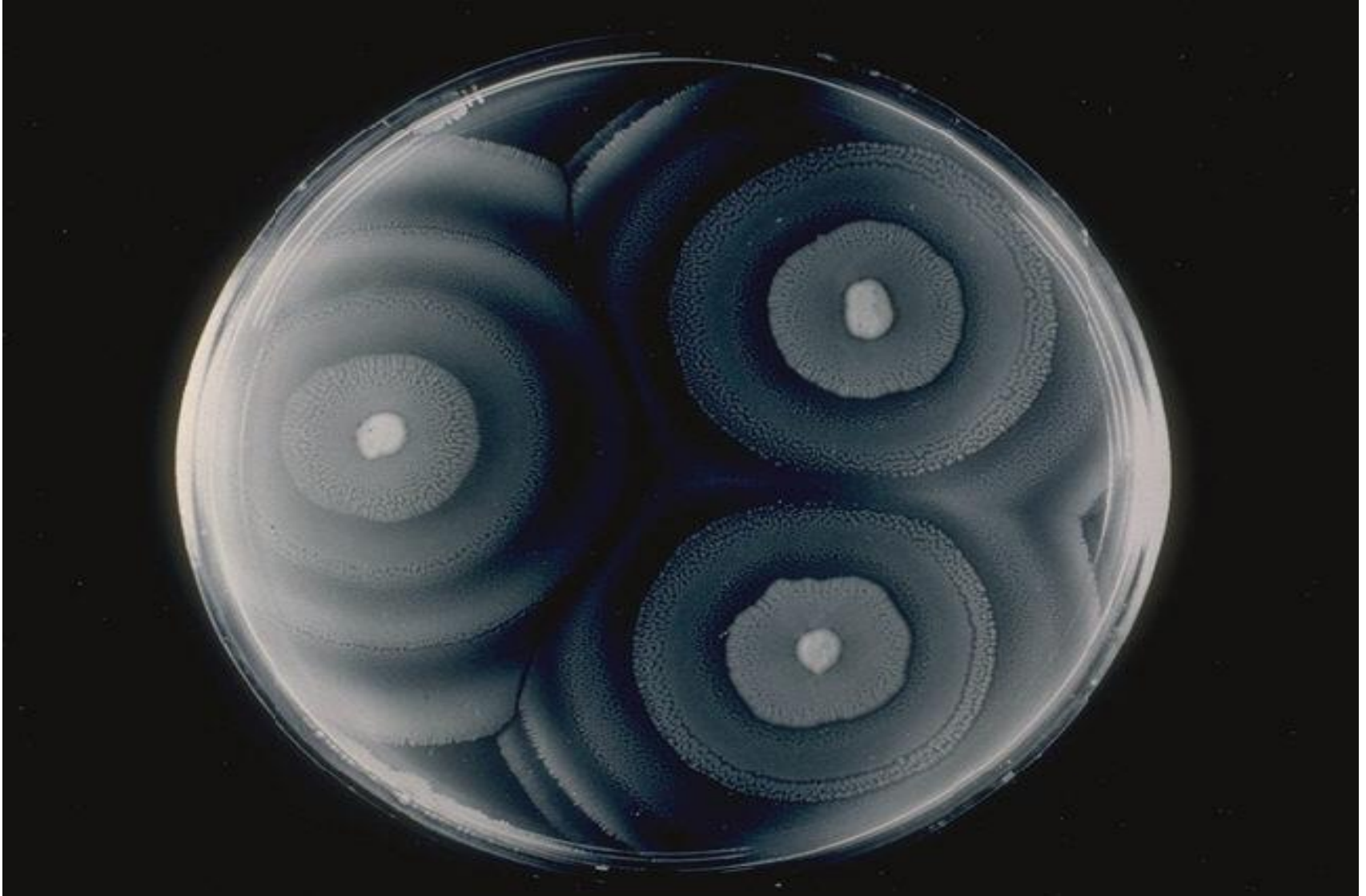
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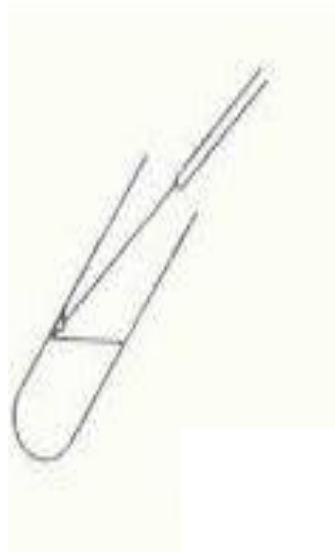
6. Sabouraud agar - *Candida albicans*



P. Mirabilis –Raussov fenomén



Inoculation – liquid medium



Innoculation

- On solid plate medium - multiplication, isolated colonies
- On solide tube medium - streaking, innoculation by one puncture

