Cultivation in Bacteriology

Cultivation of Bacteria

- **Aim: Isolation of pure culture**

- **Culture medium – chemically defined (synthetic) – e.g. aminoacids, proteins, salts, growth factors, glucose, glycerol**

- **Enrichment with biological ingredients - blood, heated blood, serum, yeast extract**

Nutrients – constituents of bacteriological culture medium

- **Amino-nitrogen base (protein) ……..pepton**
- **Growth factors ..blood, serum, yeast extract**
- **Energy source ……sugars, carbohydrates**
- **Buffer source ……phosphate, citrate**
- **Mineral source……calcium,magnesium,iron**
- **Selective agents ……chemicals,dyes, ATB**
- **Indicators ……phenol red**
- **Gelling agent (solid medium)……..agar**

Liquid culture medium

- **multiplication of bacteria – aerobic, anaerobic growth - sediment, cloud, granules, turbidity**

a/ basic liquid culture media

→ nutrient broth (peptone, meat extract, NaCl), enriched with liver, glucose, yeast extract

b/selective liquid culture media

→selenite broth (*Salmonella*)

→Loeffler medium (*Corynebacterium diphtheriae*)

→Middlebrook medium (*M. TBC*)

→Broth for anaerobe cultivation – Vf medium

Bacterial colony

- **group of cells originating from a single original cell, formed by it´s multiplication**

- **seen by naked eye on the surface of solid cultivation medium**

1 colony = cca 10^{11} CFU

Cultivation of bacteria

- **aeration – role of oxygen as hydrogen acceptor**
- **Obligate aerobes – oxygen as hydrogene acceptor**
- **Facultative anaerobes – aerobic or anaerobic**
- **Obligate anaerobes – oxygen exclusion**
- **CO_2 tension – CO_2 termostate**
- **illumination – dark, light (mycobacteria)**
- **incubation time – usually 12 – 24 (48) hours**
- **temperature: human pathogens - +35^oC (+4 - 44^oC)**
- **mesophilic – 30 - 37^oC – majority of human pathogens**
- **pH neutral (*Vibrio cholerae* - alkaline - 9)**

Cultivation of bacteria

Temperature:
human pathogens - +35°C (+4 - 44°C)

- **psychrophilic – 15 - 20°C**
- **mesophilic – 30 - 37°C – majority of medically important bacteriae**
- **thermophilic – 50 – 60°C** *(Bacillus thermophilus)*
- **wide temperature range** 4 - 45 °C !

(*Campylobacter jejuni 42* °C, *Enterococcus* 25 – 45°C, *Bacilus anthracis* 12 – 45 °C (optimum 25°C), *Listeria monocytogenes* 4 – 42°C (selective cultivation of listeria in refrigerator)

pH neutral *(Vibrio cholerae* - alkaline - 9)

Moist – sufficient moist, bacteriae multiply best in liquid culture medium (broth)

Culture medium

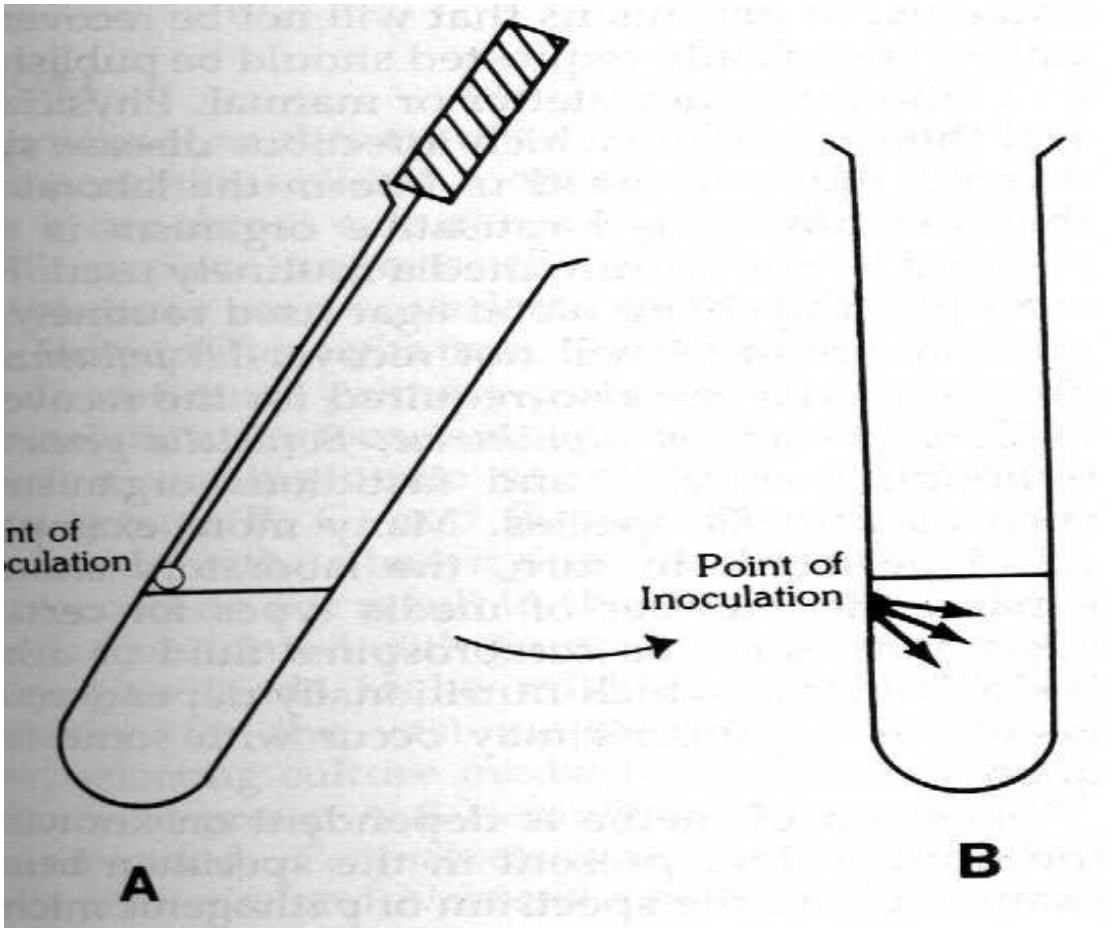
- **Basic culture medium**
- **Selective culture medium – enriched, increases growth of wanted and suppresses growth of unwanted species**
- **Differential culture medium – distinguishing one species from another (special nutrient ingredient added)**
- **Selective and differential culture medium**

Agar base

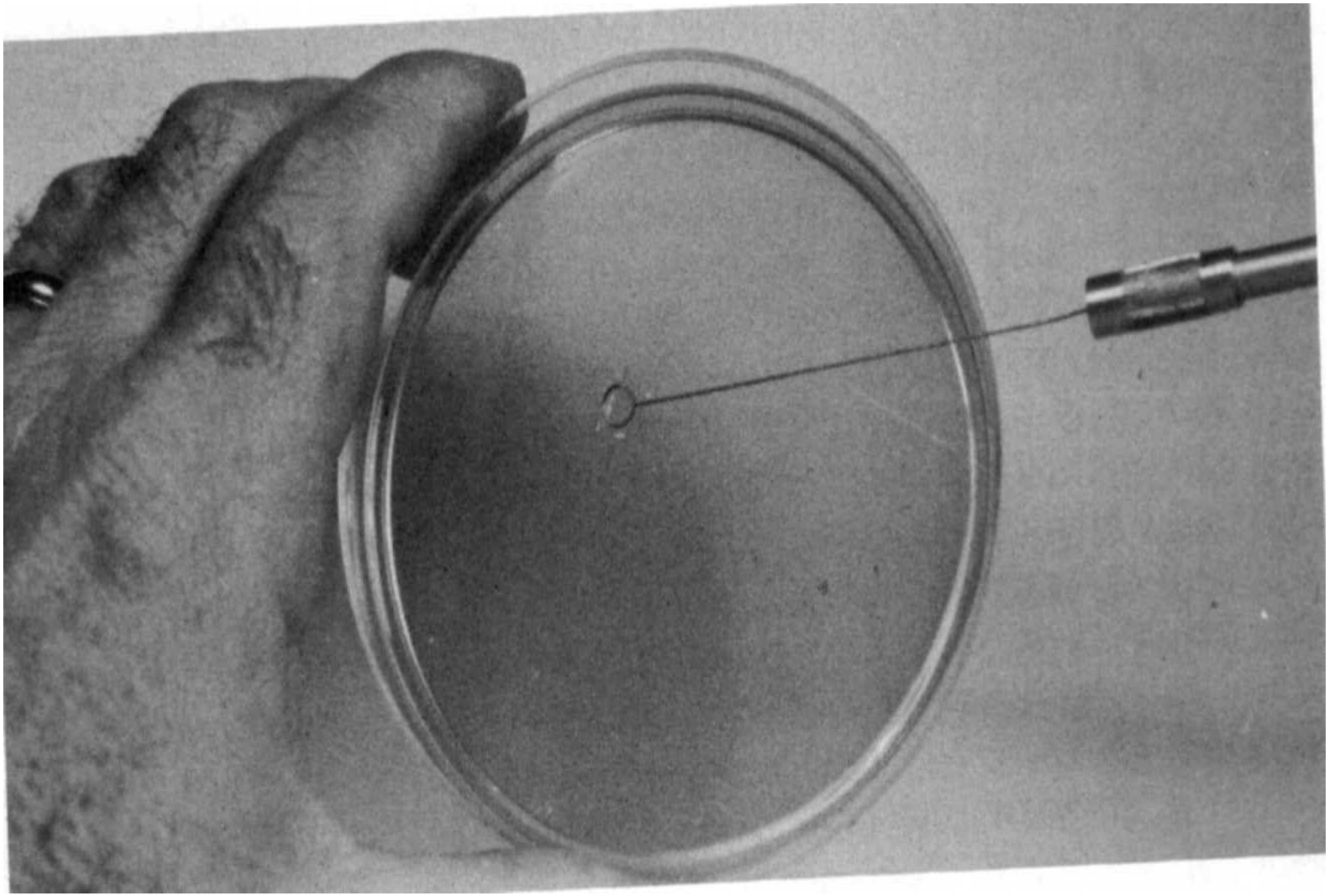

DIFCO
223220
Tryptose Blood Agar Base
Base for Use with Blood in the Isolation, Cultivation and Determination of the Hemolytic Reactions of Fastidious Microorganisms
BECTON DICKINSON
DIFCO
279240
Columbia Blood Agar Base
Base for the Cultivation of Fastidious Microorganisms with or without the Addition of Blood
BECTON DICKINSON
OXOID
Code CM375
500 grams
BRAIN HEART INFUSION AGAR
FOR IN VITRO DIAGNOSTIC USE
500 grams makes 10.6 litres
BASINGSTOKE, HAMPSHIRE, ENG
14. 11.

Solid and liquid culture media

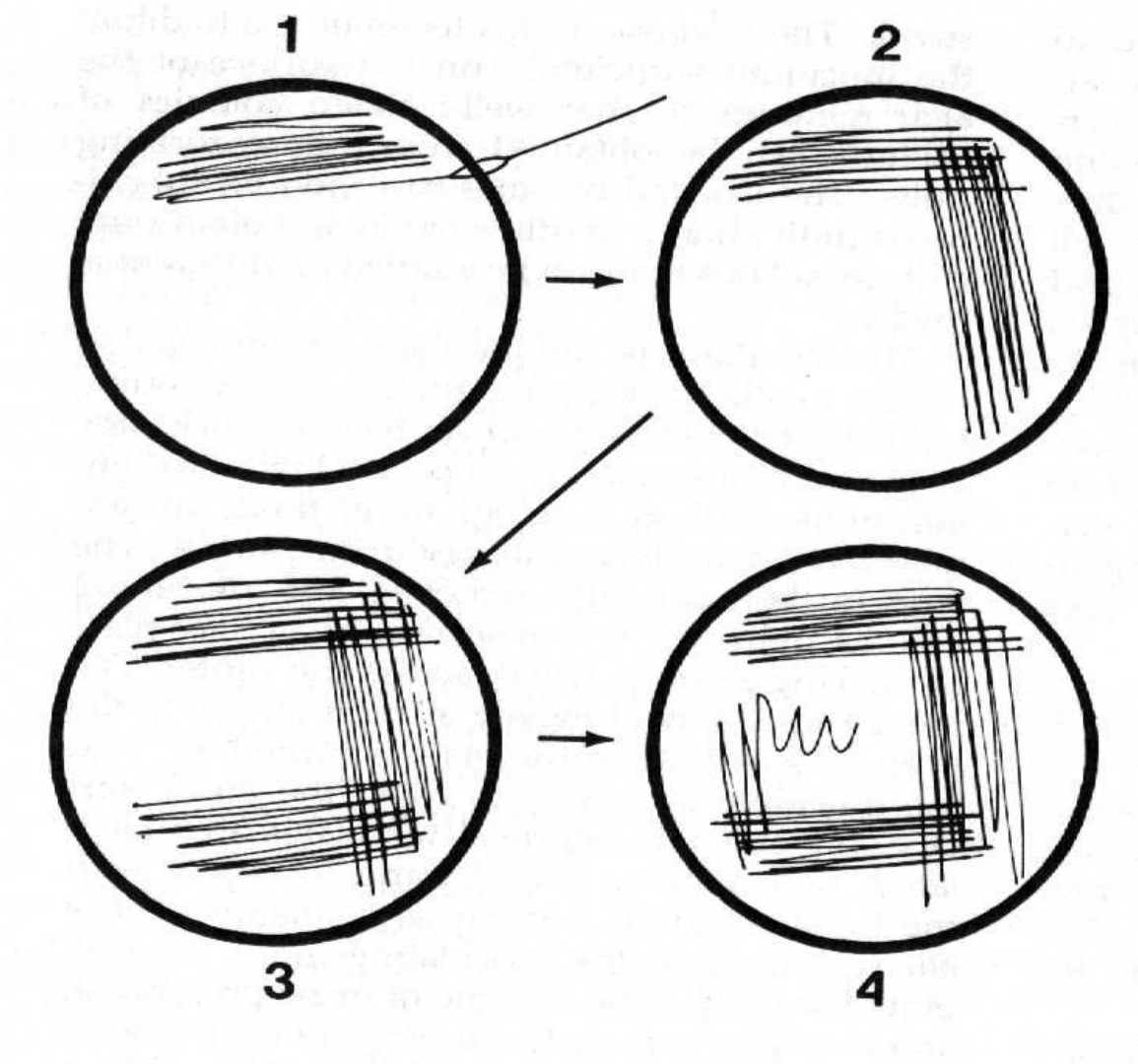
Inoculation into liquid culture medium (multiplication of bacteria) – beef broth + pepton + NaCl



Streaking with bacteriological loop onto solid culture medium

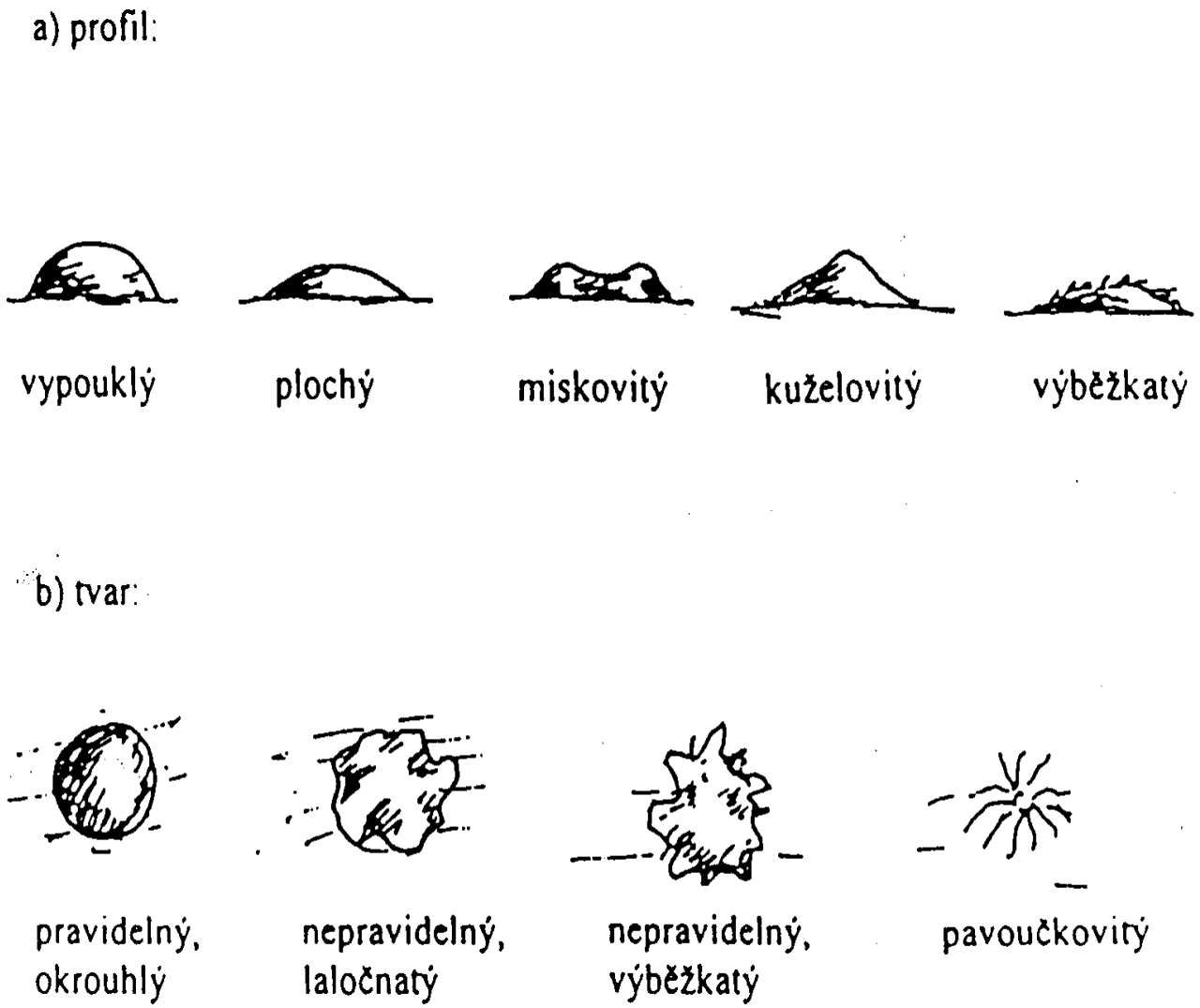


Solid culture media – inoculation and streaking (dilution of inoculum)



Bacterial colony

- **size**
- **shape**
- **profile**
- **margins**
- **surface**
- **consistence**
- **transparence**
- **color**
- **surroundings**
- **odor**



Solid bacterial culture media

A/ Basic

→Nutrient agar (nutrient broth , 1 – 2% agar)

→Blood agar (nutrient agar, 5 – 10% defibrinated blood (sheep, horse) → hemolysis

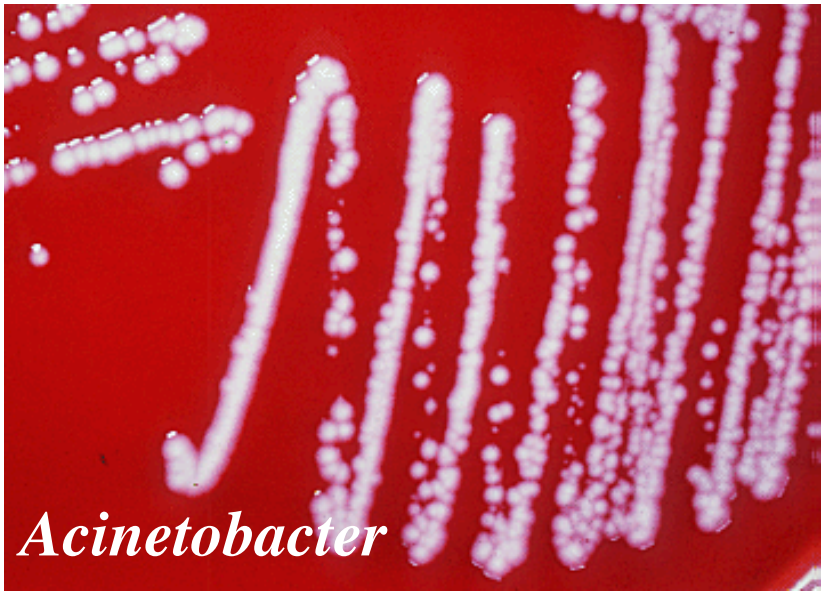
beta hemolysis (*Streptococcus pyogenes*),

alpha hemolysis – viridation (*Streptococcus pneumoniae, alpha streptococcus*)

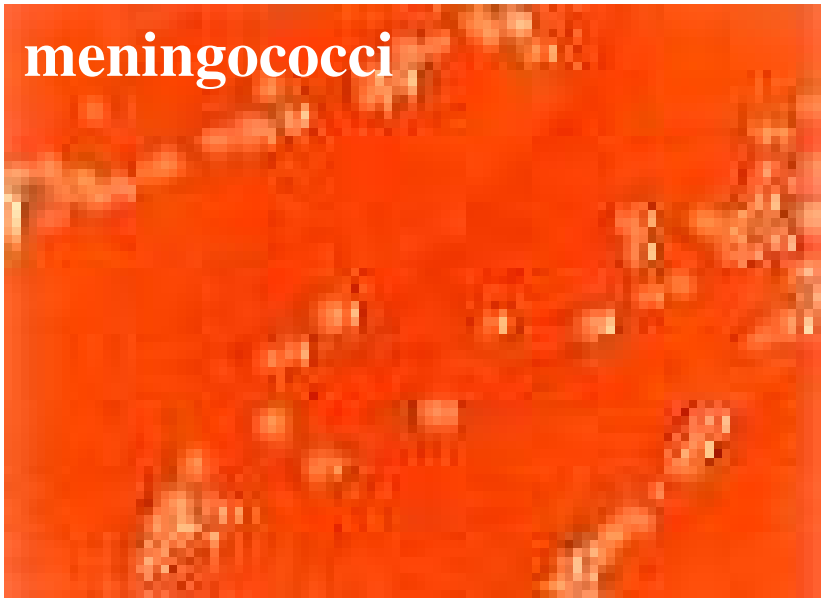
→Chocolate agar (blood agar with blood heated – *Haemophilus influenzae*)

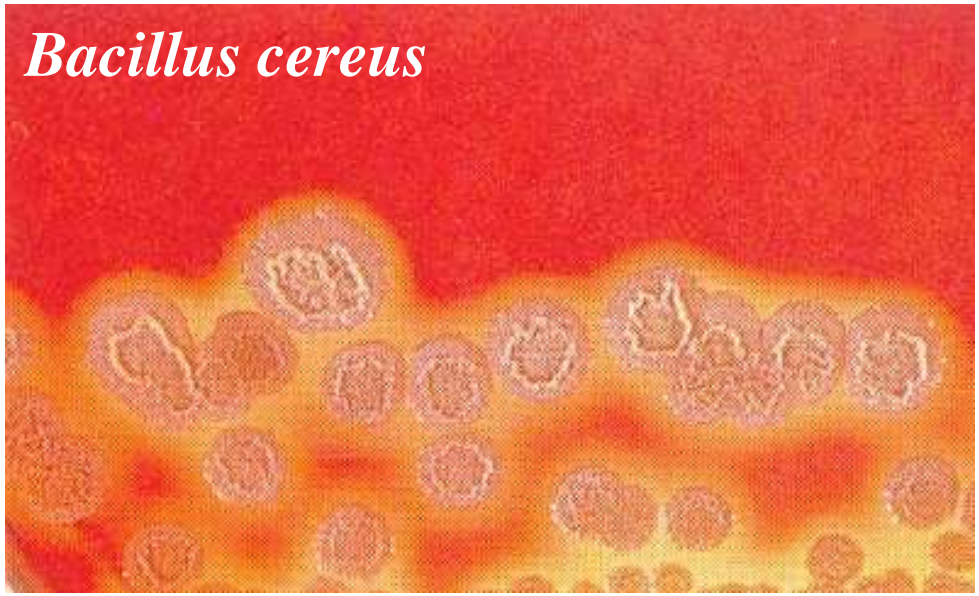
→Mueller-Hinton agar – for ATB susceptibility testing

blood agar

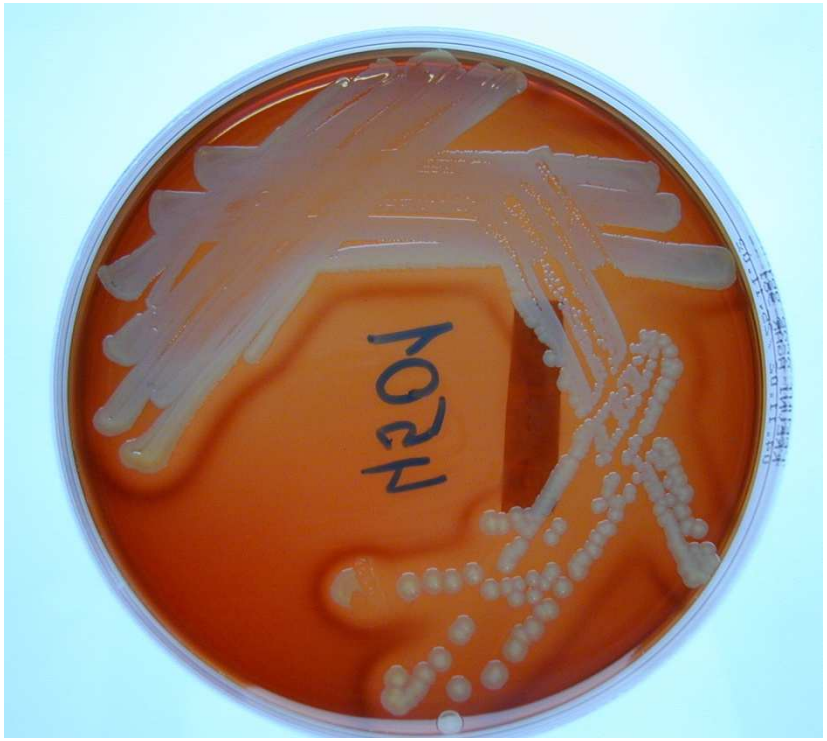

Acinetobacter


Bacillus anthracis

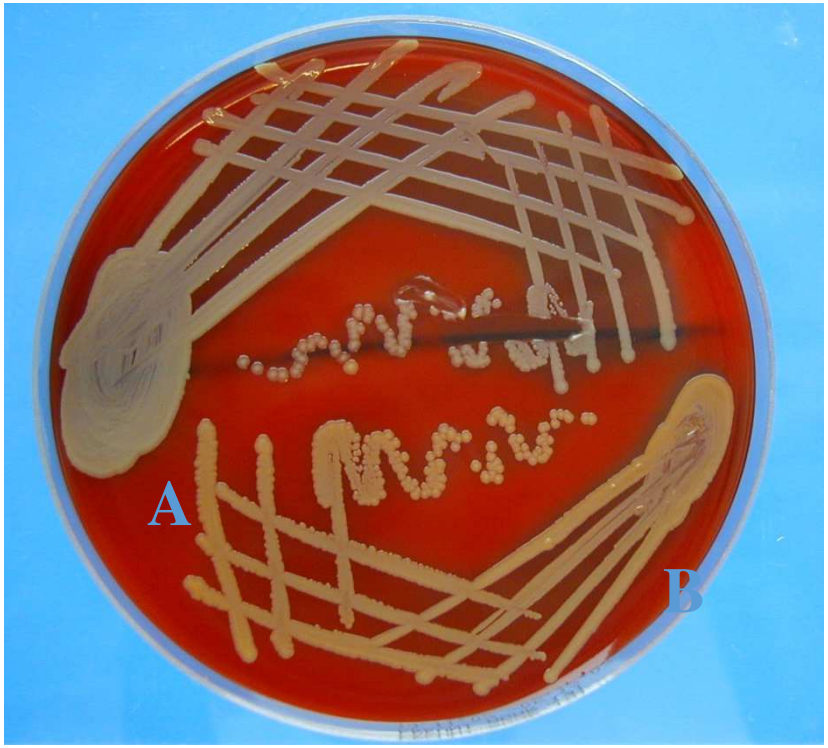

meningococci


Bacillus cereus

S. aureus - growth on blood agar

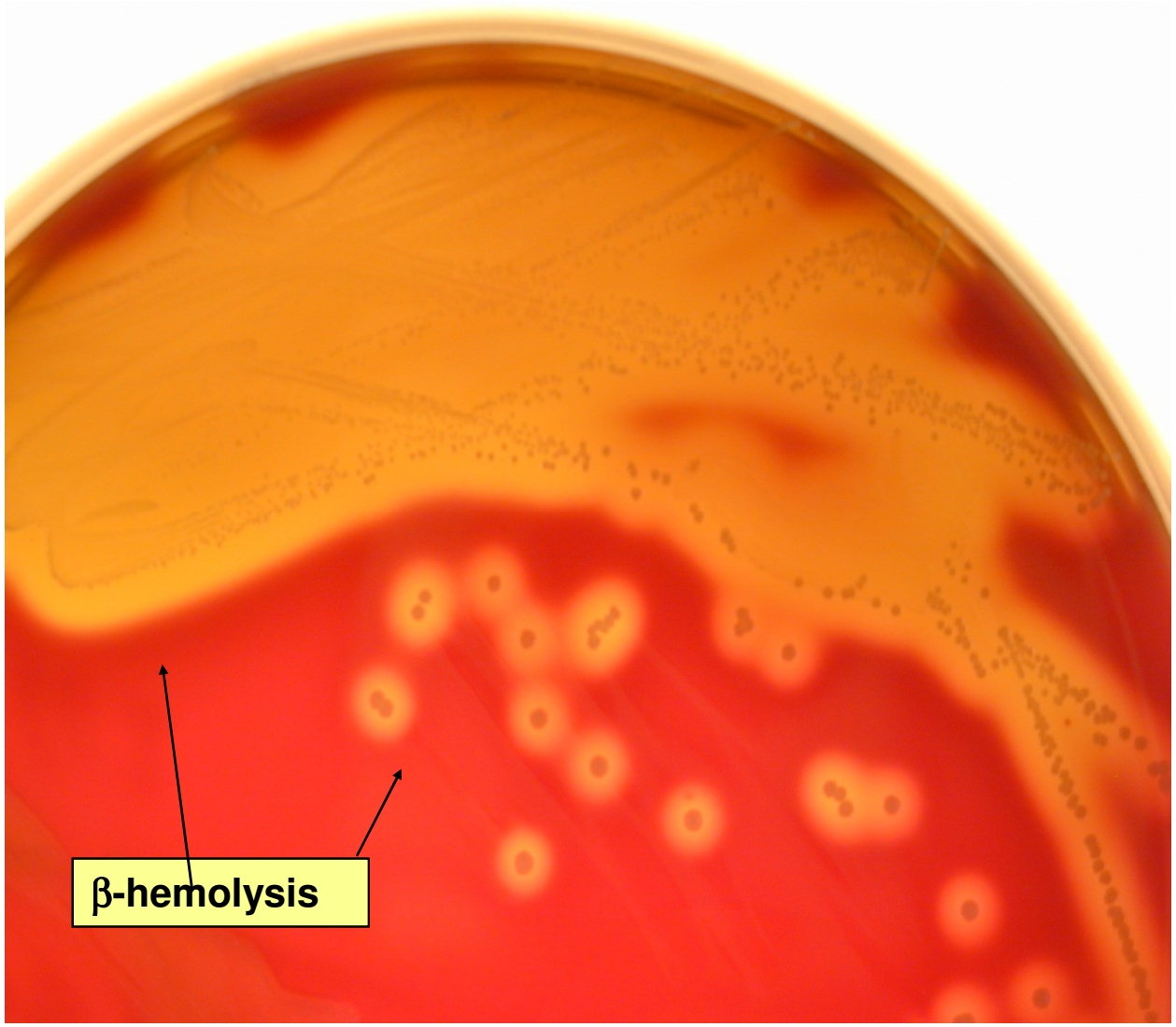


β- hemolysis



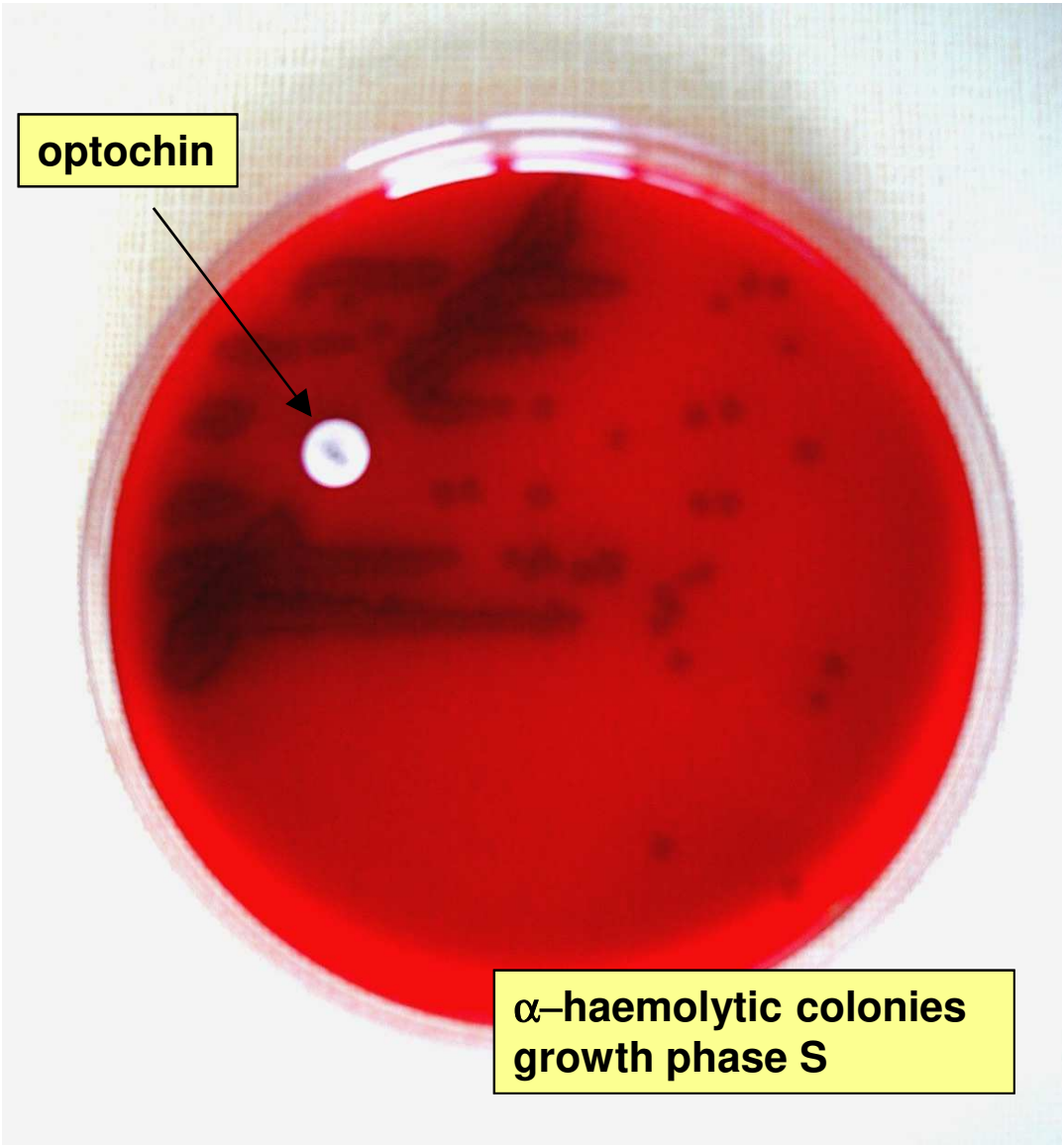
A - *S. epidermidis*
B - *S. aureus*

S. pyogenes gr. A on blood agar

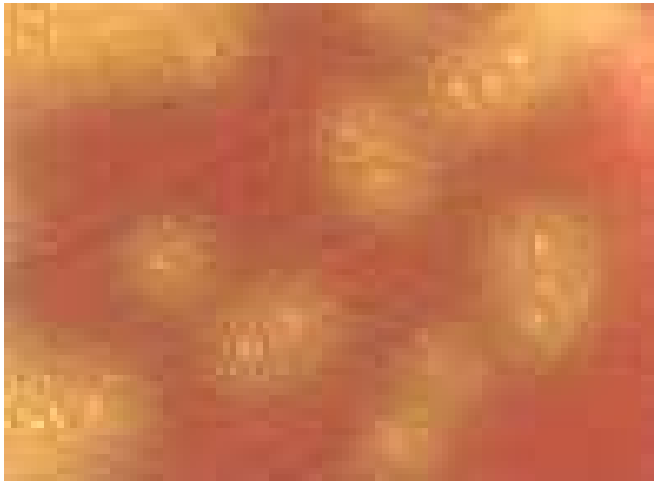


S. pneumoniae

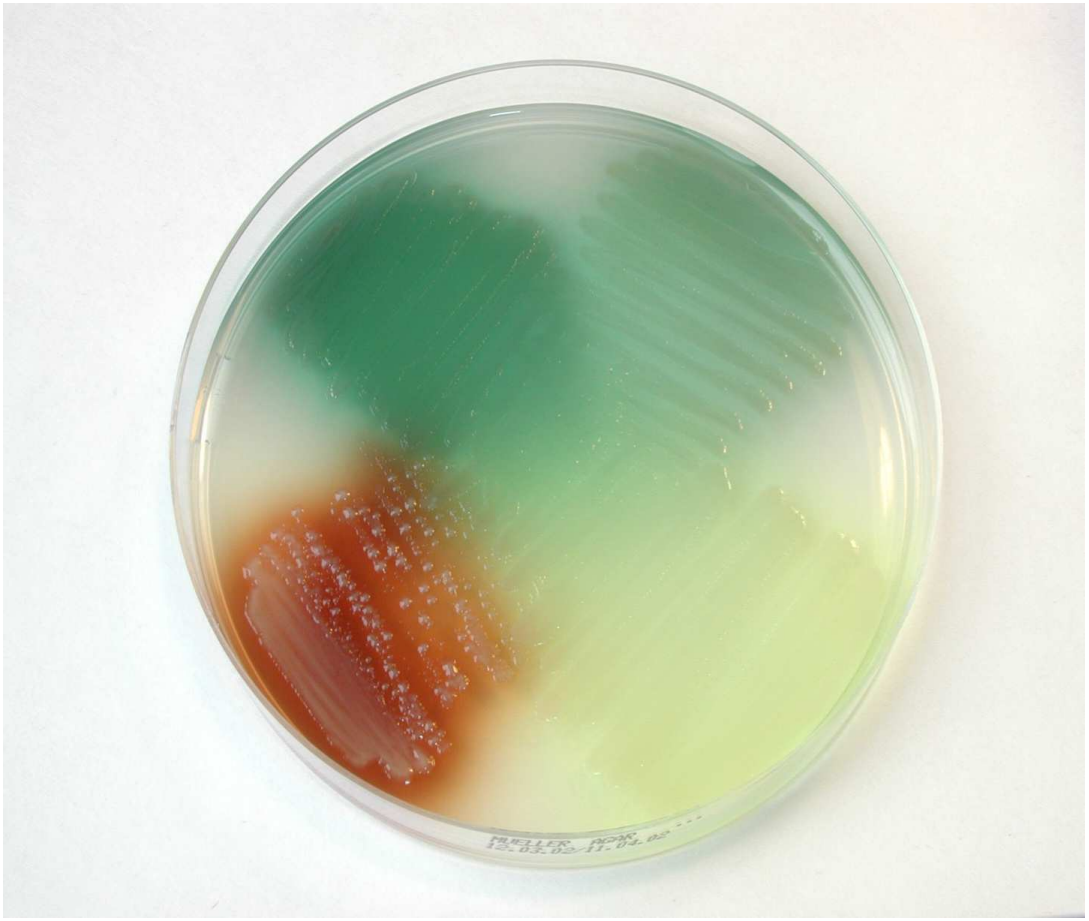
cultivation on BA



chocolate agar



Pseudomonas aeruginosa – nutrient agar



Differential and selective media

→Endo agar (agar, lactose, sodium sulphate, fuchsin – inhibition of growth of g+ bacteria, differentiation of lacose fermenting (pink) and non-fermenting (colorless) bacteria – *Enterobacteriaceae*

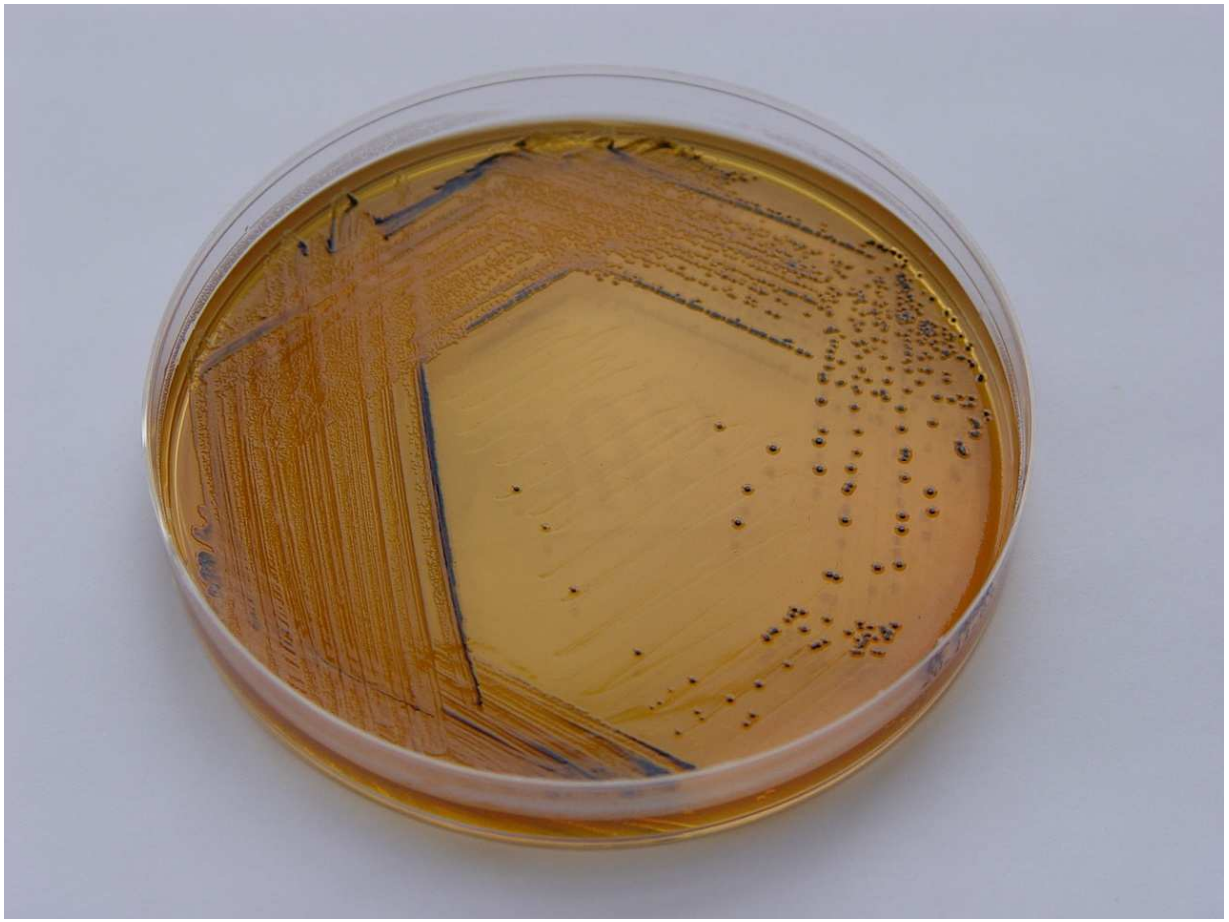
→MacConkey agar (agar, lactose, bile salts, neutral red) - *Enterobacterales*

→Desoxycholate citrate agar (agar, lactose, bile salts, neutral red) – *Enterobacterales,* H_2S positive bacteria – central black dot – *Salmonella, Citrobacter, Proteus*

E. coli – MacConkey agar

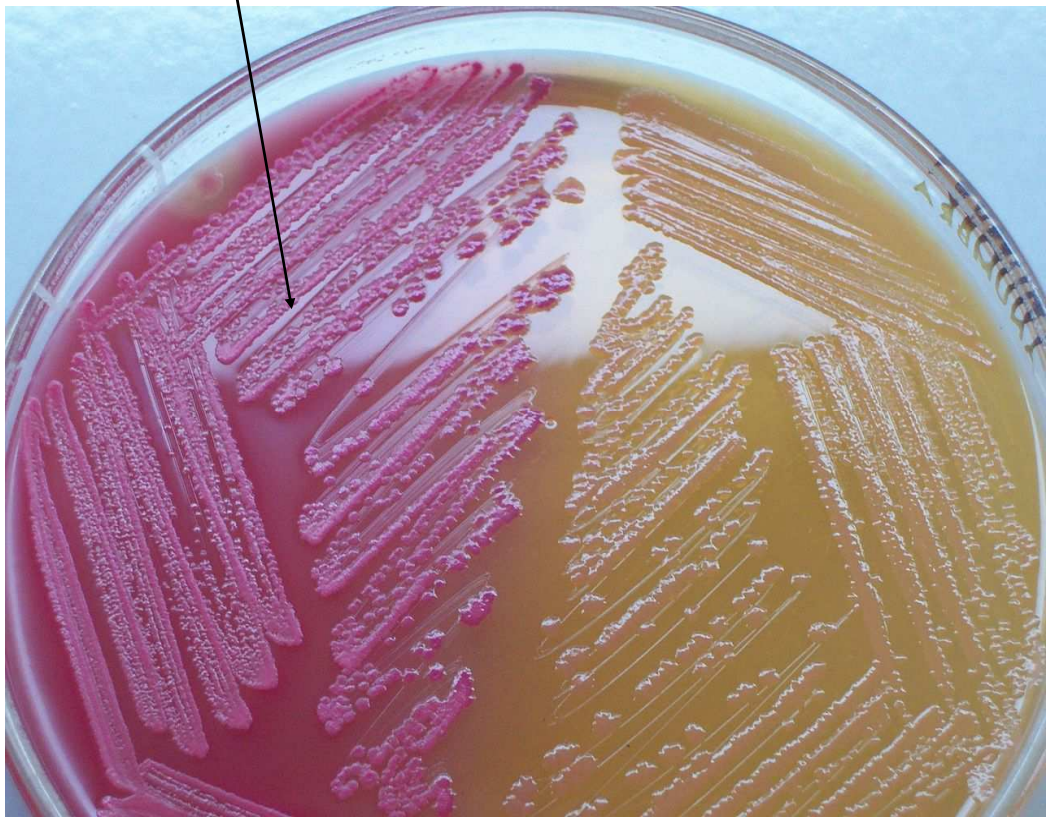
E. Coli – Endo agar

Salmonella –DC agar

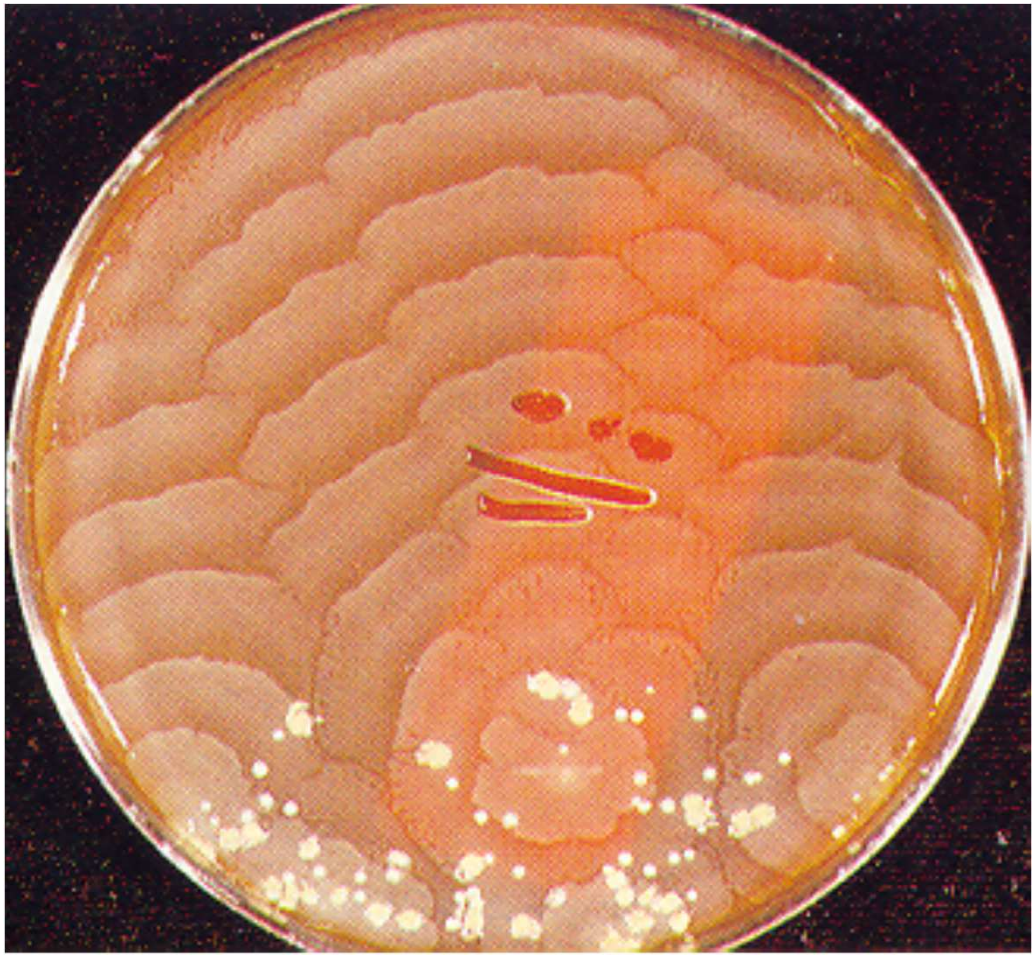


MacConkey agar – lactose fermentation and nonfermentation

Escherichia coli ***Enterobacter***

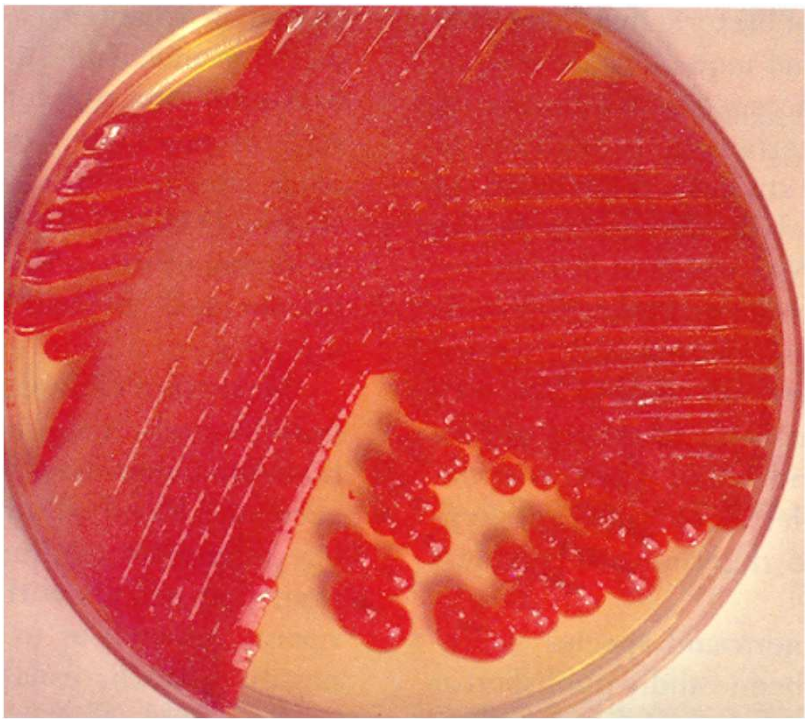


MacConkey agar - swarming growth *of* ***Proteus*** spp.



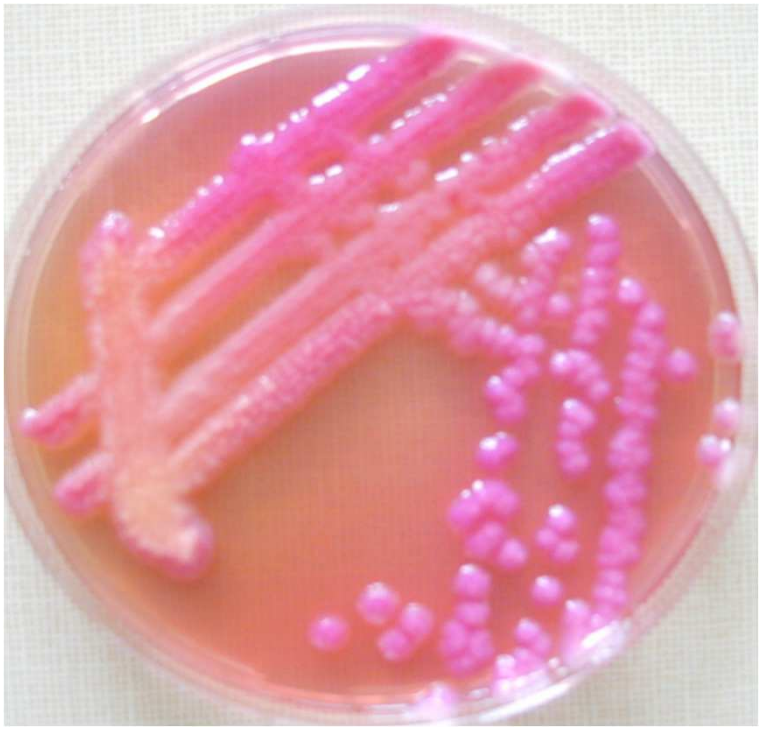
Pseudomonas aeruginosa – DC agar

Beef extract agar



Serratia marcescens

MacConkey agar



Klebsiella pneumoniae

Differential and selective media

→ Sabouraud dextrose agar – for *yeasts* and *fungi,* low pH inhibits most bacteria

→ Löwenstein-Jensen agar, Ogawa agar, Middlebrook medium - glycerol, malachite green (inhibitors) - *Mycobacterium TBC,* mycobacteria

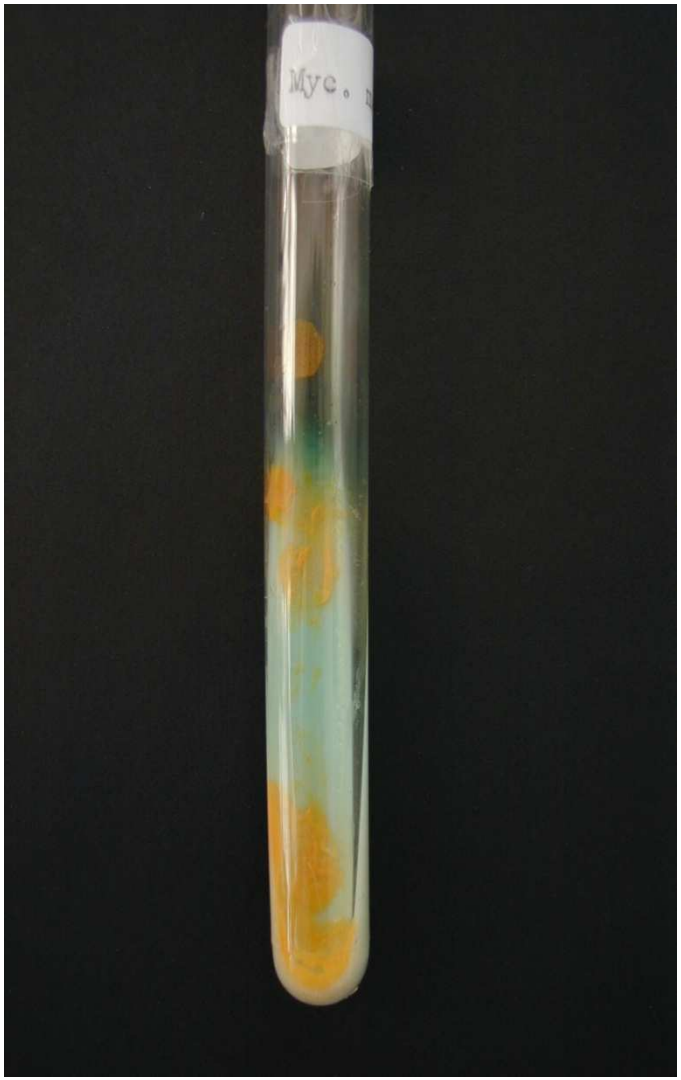
Culture media for *Mycobacterium TBC* and *Mycobacterium spp.*

Löwenstein-Jensen egg medium

Ogawa egg medium

MGIT
Middlebrook medium

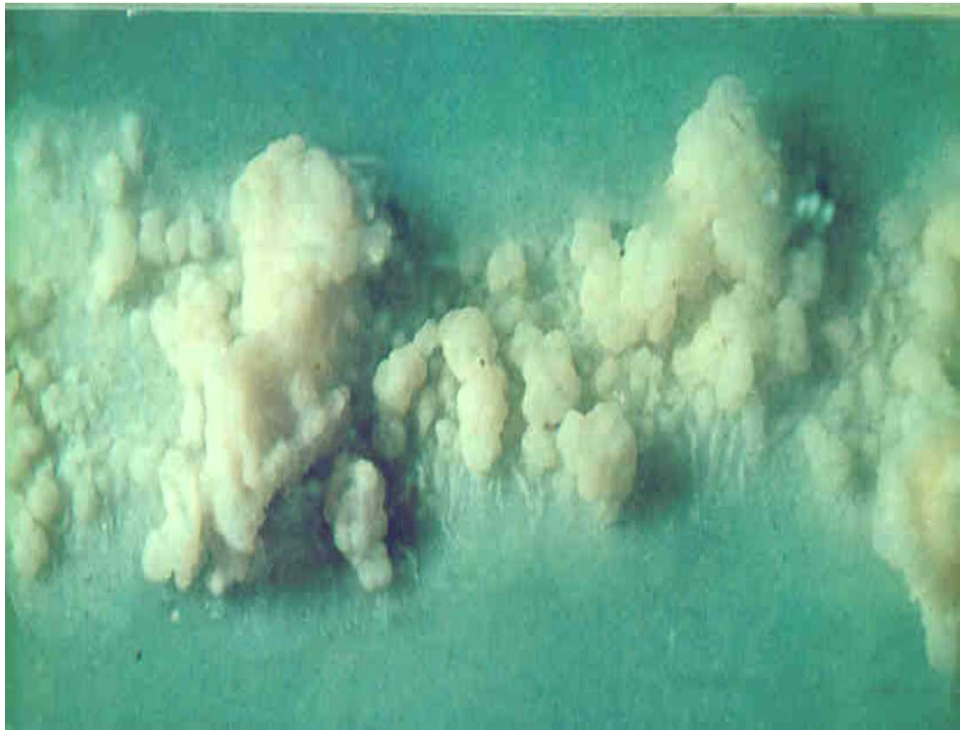
M. marinum

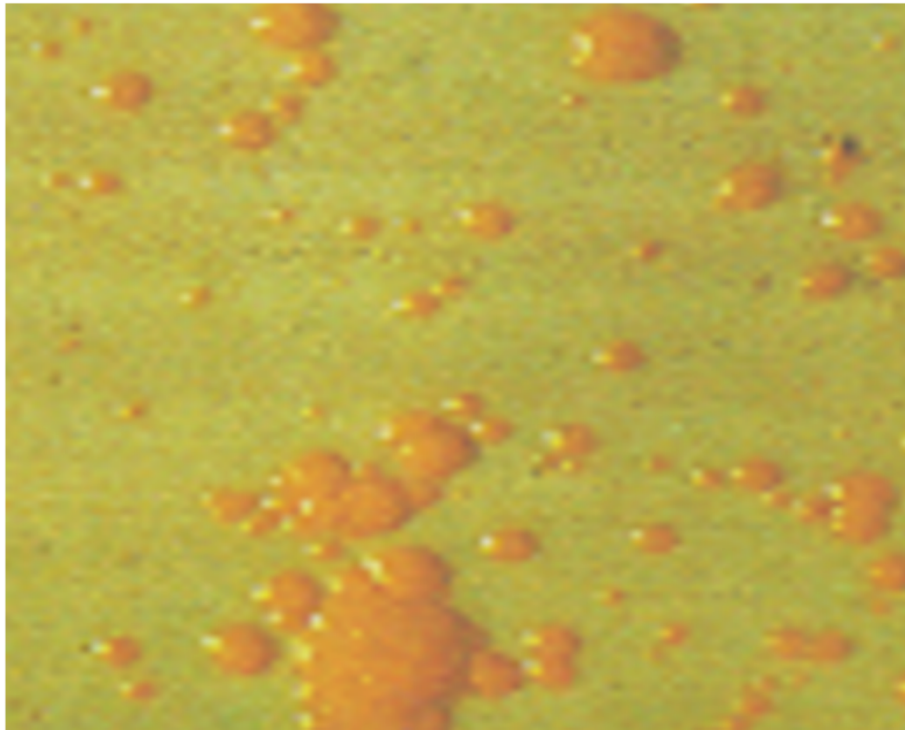


M. gordonae

Mycobacterial colonies after 6 weeks on Löwenstein – Jensen, Ogawa

Mycobacterium TBC ***Mycobacterium kansasii***





Middlebrook medium for metabolic cultivation of mycobacteria

Selective culture medium

→ Wilson-Blair agar (bismuth sulphite agar, -brillant green) – *Salmonella*

→ Mannitol salt agar (mannitol, salt, phenol red)- *Staphylococcus* species differentiation

→ Clauberg, Mansula medium - tellurite salts medium (*Corynebacterium diphtheriae*)

→ Alkaline pepton water (*Vibrio cholerae*)

→ Chromogen media (selective+diagnostic) – selected bacteria grow in special colour

Chromogen agar - *Staphylococcus aureus*

CHROMOGENIC MEDIA FOR DETECTION OF PATHOGENS IN CYSTIC FIBROSIS PATIENTS

chromID S. aureus

Incubation: 24 hours
S. aureus ATCC® 25923

S. aureus (green colonies), *S. saprophyticus* (pink colonies), *S. xylosus* (mauve colonies)

S. epidermidis (white colonies)

20

chromID P. aeruginosa

Incubation: 24 hours
P. aeruginosa ATCC® 27853

P. aeruginosa morphotypes in cystic fibrosis specimens

Visualisation of golden-metallic coloured *P. aeruginosa* colonies zoom x3

chromID™ S. aureus

For the the direct identification of *S. aureus*

Direct identification of *S. aureus* is based on the spontaneous green colouration of glucosidase-producing colonies (patent pending).

Rapidity with the immediate identification of *S. aureus* = Green colonies (reading between 18 and 24 hours).

- Excellent performance for the culture of *S. aureus* in terms of nutrient capacity, detection sensitivity and colouration specificity.
- Optimum differentiation of mixed cultures due to the presence of a 2nd substrate.
- Orientation of identification towards Staphylococci = *S. epidermidis* (white colonies), *S. saprophyticus* (pink colonies), *S. xylosus* (mauve colonies).

Inhibition of other bacteria (Gram + and Gram -) and yeasts.

chromID S. aureus
Ref. **43 371** • kit of 20 plates

chromID MRSA / chromID S. aureus
Ref. **43 466** • kit of 20 tests

Slidex® MRSA
Ref. **73 117** • kit of 50 tests

chromID™ P. aeruginosa

For the direct identification of *Pseudomonas aeruginosa*

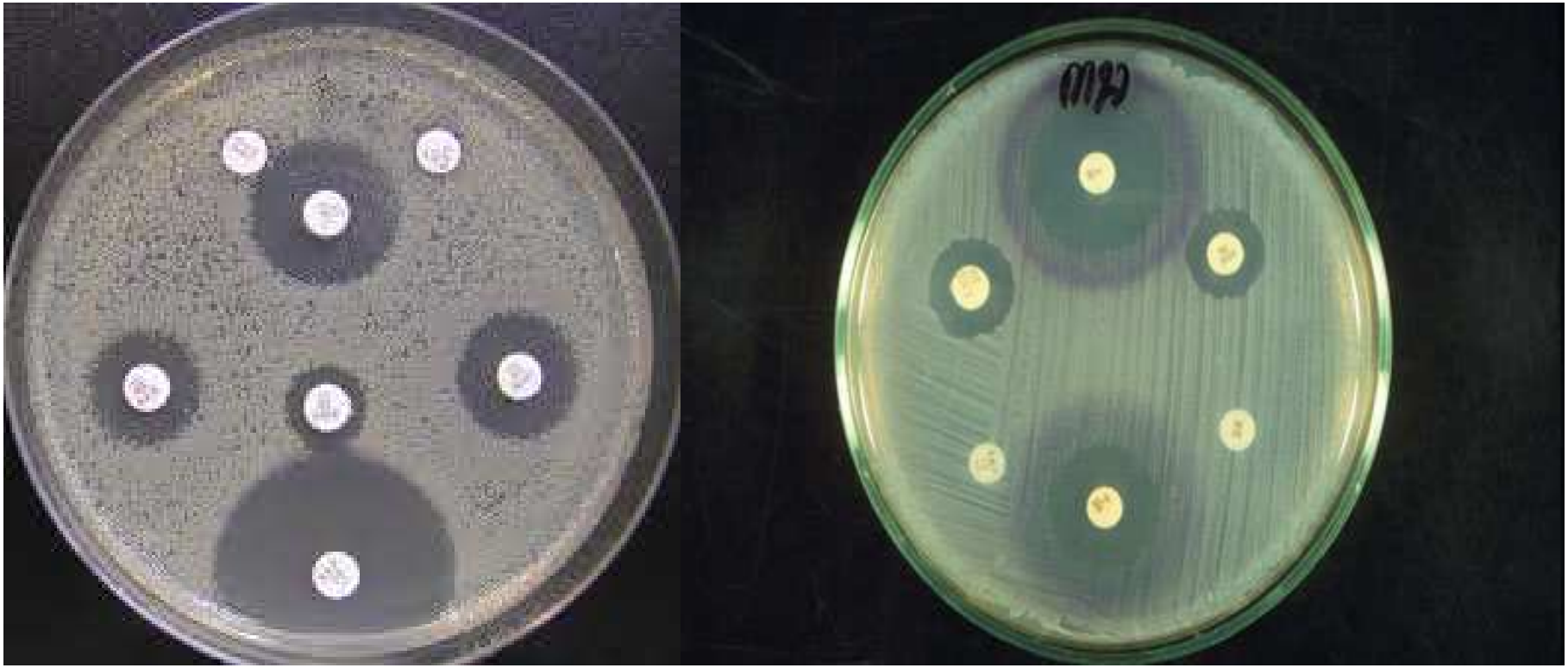
The direct identification of *P. aeruginosa* is based on the specific pink to violet coloration of aminopeptidase-producing colonies due to the chromogenic substrate ß-alanyl-resorufamine (2 bioMérieux patents).

chromID P. aeruginosa
Ref. **43 462** • kit of 20 plates

www.biomerieux.com

Mueller-Hinton agar

ATB susceptibility testing



Staphylococcus aureus - growth on salt mannitol agar

Special culture media

- **enriched, ATB added, supplemented**

- ***Neisseria meningitidis, Neisseria gonorhoae M.TBC, Corynebacterium diphtheriae, anaerobes, Vibrio cholerae, Legionella pneumophilla, Yersinia enterocolitica, Campylobacter jejuni, Helicobacter pylori, Bordetella pertussis,*** **special mycologic culture media**

Special culture media

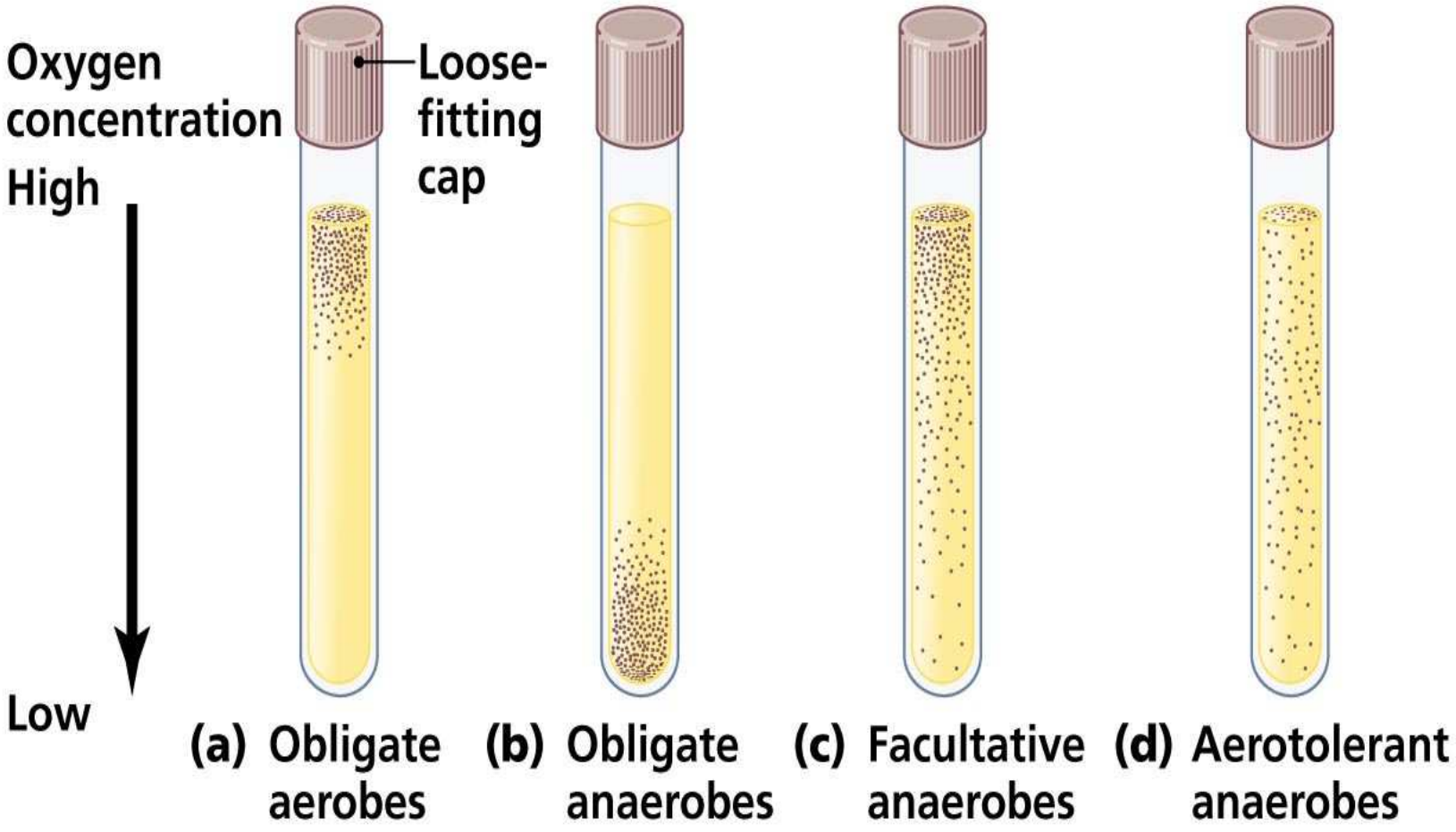
Karmali agar
Campylobacter jejuni

Clauberg Telurite agar
Corynebacterium diph.

Sabouraud agar

Cultivation of yeasts and fibrous fungi

Bacteriae - types of aeration



Anaerobic cultivation

- **Anaerobic bacteria:**
- **obligate anaerobes - obtain energy through fermentative pathways in which organic compounds serve as final electron acceptors**
  - **strict - survive O_2 conc. max 0,5%**
  - **moderate - survive O_2 conc. 2-8%**
- **aerotolerant - bacteria better growing under anaerobic conditions than in presence of O_2**

Anaerobic cultivation

- **media with low E_h - Vl broth, Vf broth**
  anaerobic BA
  cultivation minimum 1 week under anaerobic conditions at 37° C in

  - **anaerobic jar**
  - **anaerobic glove box**
  - **anaerobic disposable plastic bags**

- **hemocultures - BACTEC**
- **parallel cultivation in aerobic BA**

Anaerobic cultivation

anaerobic jar

anaerostat

Hemocultivation

- Continual cultivation + monitoring
- Incubation of bottles with blood in a special thermostat (37°C) with the growth and multiplication of bacteria detection – measurement each 10 minutes
- Incubation time up to 5 – 7 days for bacteria detection,14 days for yeasts
- The presence of microorganisms – detection of produced CO_2.
- The bottle bottom has got a sensor where CO_2 difuses, pH is reduced – result is a change of the colour
- Colorimetric detection with SW
- Light and noise alarm when bacterial multiplication is detected

Hemocultivation

Hemocultivation - Bactec

Hemocultivation - Bactec

Biochemical Identification of bacteria

- Based on bacterial enzymes, other metabolic products detection
- Identification from pure culture – tubes, microplate, etc.
- Saccharides, tryptofan, urea, indole, use of NH4 citrate, colored indicators of pH change (e.g. Phenol red)
- Time of incubation: 24 - 48 h change of colour, consistence of medium, motility, utilization of different substances

Biochemical Identification of bacteria - examples

- **Oxidase test**: oxidase detection with filter paper stripe saturated with parafenylendiamin and alfa-naftol (positive = dark blue to black colour) E.g.: *Pseudomonas*, *Neisseria*
- **Catalase test**: with 3% hydrogene peroxide

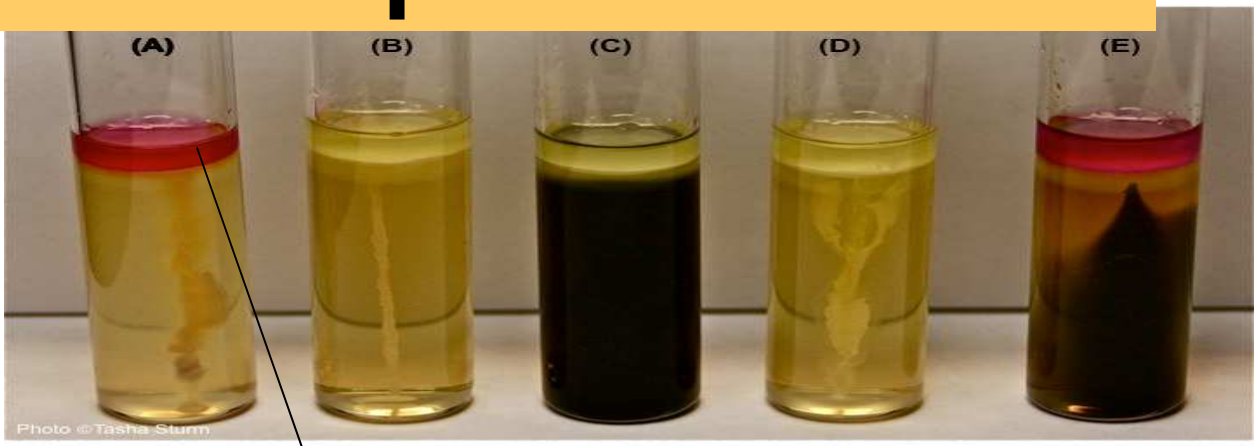
  catalaze production is determined by bubbles of O2
- **Plasmacoagulase test**: rabbit plasma, positivity = coagulation by *Staphylococcus aureus*
- **Fermentation of saccharides:** pepton + 1% saccharide, phenol red

  positivity = pH decrease, red colour of medium; for enterobacteriae identification
- **Urea hydrolization:** medium with phemol red

  urea hydrolysis into amoniak + CO_2 = red colour of the medium (*Klebsiella, Proteus)*
- **Nitrate to nitrite reduction:** agar with nitrates

Result = red colour (enterobacteria identification)

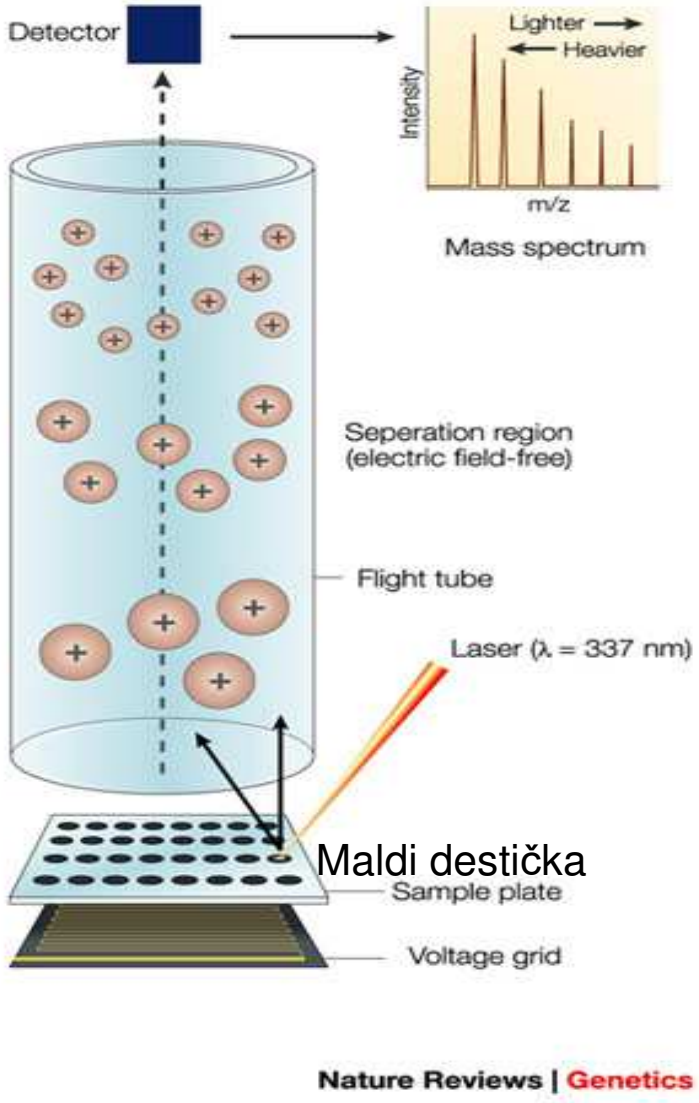
Biochemical Identification of bacteria - examples



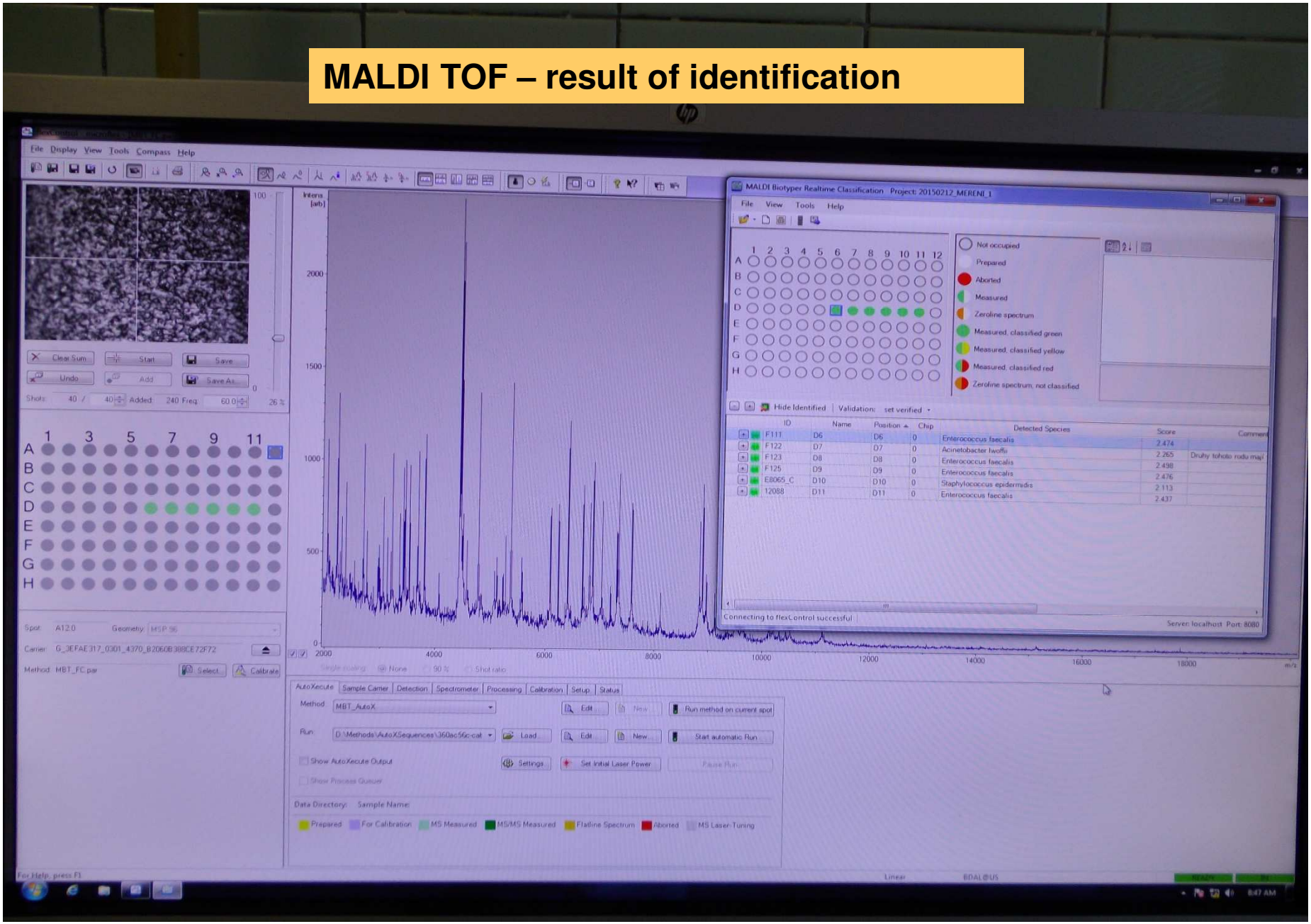
Medium	motility	H2S production	indole
A) *Escherichia coli*	+	-	+
B) *Staph. aureus*	-	-	-
C) *Salmonella arizonae*	+	+	-
D) *Enterobacter*	+	-	-
E) *Proteus vulgaris*	+	+	+

MALDI TOF

MALDI TOF - schema



MALDI TOF – result of identification



MALDI-TOF

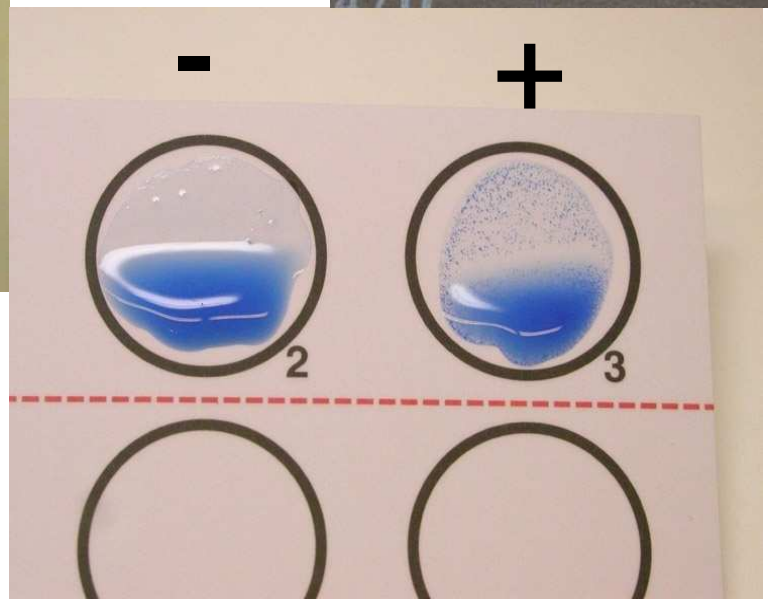
- *Matrix Assisted Laser Desorption/Ionization*, with *Time of Flight* analysis

- for microorganism adentification
  - 1 colony of microorganism putted on special MALDI-TOF plate and coverved by special matrix (hydroxo-cinnamid acid, etc)
  - This matrix is than exposed by laser
  - Matrix absorbes the laser energy and during this absorption are highly ionisated also molecules of sample with release of ionisated proteins parts from samples (mainly ribosomal proteins)
  - This ions with positive charge are accelerated by strong electrical field and than are released into detector tubes. In this detector is precisely measured the speed of each protein in absolutly vaccum. And because it is well known that the speed is full depend on mollecular weight, the MALDI-TOF device recalculated the speed profile into molecular weight profile.
  - The molecular weight profiles are higly conservative and are should be used for identification

Antigenic structure of bacteria with specific antisera - serotyping

agglutination of Antigen + Antibody

Agglutination - result



Latex agglutination (somatic antigen of O157 *Escherichia coli*

Cultivation

- Biological specimens obtained from patients are processed under aseptic conditions (laminary flow box, BSL 3,4 box in highly infectious agents)
- The type of cultivation is chosen according to susp. bacterial ethiology (the knowlege of pathogenesis of infectious diseases, processes, normal physiological microflora
- The chioce of culture media depends of the nutritional requirements of susp. bacterial agents (nutrients, moist, pH, temperature, aerobic or anaerobic, CO2 athmosphere, etc.)

Cultivation in BSL3 laminary flow box

Isolation of pure culture from mixed culture of bacteriae

Bacteriological loop and isolation of 1 pure colony from the mixed culture plating on other culture media for: ATB susceptibility testing
Identification of bacteria
Typing
PCR