

Culturing of bacteria

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- ☞ The aims are:
 - ☞ To make pure cultures of pathogen bacteria.
 - ☞ To make isolated bacterial colonies on solid medium for further investigation and identification of bacteria.
- ☞ Requirements for growth:
 - ☞ Nutrients: e.g., salts, carbon-source, nitrogen-source, proteins, vitamins, organic growth factors
 - ☞ Water
 - ☞ Presence or absence of oxygen – obligate aerobes, facultative anaerobes, obligate anaerobes, microaerophils and aerotolerant anaerobes
 - ☞ Temperature (mesophils)
 - ☞ pH 6.5-7.5
- ☞ Culturing:
 - A. Natural media: milk, bile, potato
 - B. Complex media
 1. Liquid media
 2. Semi-solid media
 3. Solid media

1. Liquid media

- ☞ **Peptone water**
 - ☞ distilled water, NaCl, peptone
 - ☞ examination of bacterial biochemical activity
- ☞ **Broth (bouillon):**
 - ☞ meat soup
 - ☞ enrichment medium or biochemical examination
 - ☞ solidified bouillon – nutrient agar
- ☞ **Bouillon with meat:**
 - ☞ with meat, liver, brain etc.
 - ☞ if is boiled and paraffin-covered, anaerobe environment can be done
 - ☞ enrichment medium, maintain the culture of a bacteria



2. Semi-solid media

- ☞ **Motility examination:**
 - ☞ Medium is made from bouillon (0.5% agar)
 - ☞ Positive: determined by turbidity in semi-solid media
E. coli, *E. cloacae*, *P. aeruginosa*
 - ☞ Non motile: demonstrated by growth only in the site of inoculation
S. sonnei, *K. pneumoniae*



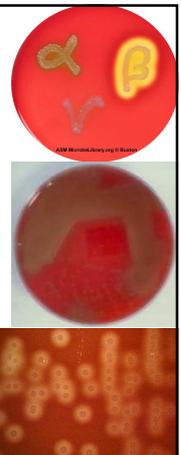
3. Solid media

- ☞ **Simple media:** agar is a polymer made up of subunits of the sugar galactose. Agar polysaccharides serve as the primary structural support for the algae's cell walls.
 - ☞ **Nutrient agar** (pigment production)
 - ☞ **Blood agar** with 5 % native (defibrinated) cattle or sheep blood. Hemolysis can be seen on it.
 - ☞ **Chocolate agar** is done with boiled blood. Mainly in case of fastidious bacteria (e.g. Haemophilus). Contains some growth factors (NAD, hemin).



Hemolysis

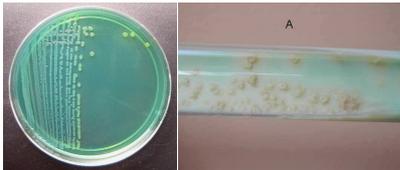
- ☞ **α-hemolysis:** incomplete (partial) hemolysis.
 - ☞ greenish colour around the colonies on the medium (blood agar, chocolate agar).
 - ☞ red blood cells don't break down, haemoglobin decomposed into greenish compound (biliverdin).
 - ☞ *Streptococcus pneumoniae*.
- ☞ **β-hemolysis:** complete hemolysis.
 - ☞ colourless area around the colonies on the blood agar, not on the chocolate.
 - ☞ red blood cells break down, haemoglobin fully decomposed
 - ☞ can not be investigated on chocolate agar (absence of intact RBC)
 - ☞ *Staphylococcus aureus*, *Streptococcus pyogenes*



3. Solid media

☞ Selective media:

- ☞ inhibition of unwanted microbes with salts, dyes or other chemicals
- ☞ allow growth only of the desired bacteria
- ☞ TCBS: *Vibrio cholerae*
- ☞ E67: *Enterococcus faecalis* has dark blue/black colonies
- ☞ Löwenstein-Jensen (slent) agar: *Mycobacteria*; malachite green is responsible for selectivity.



3. Solid media

- ☞ **Differential media:** Differential media include an indicator that cause visible, easily detectable changes in the appearance of the agar gel or bacterial colonies.
- ☞ **Eosin-methylene blue agar:** to distinguish species of *Enterobacteriaceae* family on the basis of lactose fermentation, only for Gram negatives.
 - ☞ Lactose + : blue/pink (*E.coli*, *Klebsiella*)
 - ☞ Lactose - : colourless (*Proteus*, *Shigella*, *Salmonella*)
- ☞ MacConkey agar
 - ☞ for Gram-negatives
 - ☞ lactose fermentation



3. Solid media

- ☞ **Selective-differential media:** for isolation and differentiation of enteric pathogens
- ☞ **Brilliant green agar:**
 - ☞ lactose, fuchsine, brilliant green:
 - ☞ inhibit growing of *E. coli* and swarming of *Proteus*.
 - ☞ *Salmonella* and *Shigella* have colourless colonies.
- ☞ **Bismuth-sulphite agar:**
 - ☞ glucose, Na-bisulphite, Bi-sulphate, brilliant green.
 - ☞ *Salmonella* has black colonies because of Bi-salt.
- ☞ **XLD agar**
 - ☞ xylose lysine deoxycholate agar
 - ☞ *Salmonella*, *Shigella*



☞ Deoxycholate-citrate agar:

- ☞ Lactose, Na-citrate, Na-deoxycholate, Pb-acetate, neutral red indicator.
- ☞ *E. coli*: red colonies because of lactose fermentation, *Salmonella* and *Proteus*: brown colonies because of H₂S production (Pb-sulphide), *Shigella* has colourless colonies.

☞ Clauberg-agar:

- ☞ K-tellurit, cysteine.
- ☞ Difference between the three colony types of *Corynebacterium diphtheriae*.
- ☞ Gravis, mitis, intermedius.

3. Solid media

☞ **Special media:** for fastidious bacteria

- ☞ Löffler medium: *Corynebacterium diphtheriae*.
- ☞ Bordet-Gengou agar: *Bordetella pertussis*.
- ☞ Francis agar: *Francisella tularensis*.
- ☞ CYE agar: *Legionella pneumophila*.

☞ **Synthetical media:**

- ☞ Citrate media: to distinguish *E. coli* (-) and *Klebsiella* (+).

Anaerobic culture

- ☞ **Physical:** in the anaerostat: air is removed and CO₂ and N₂ gases are added.

☞ **Chemical:**

- ☞ Alcalic pyrogallol: pyrogallic acid and NaCO₃ are added, which binds oxygen, and Petri dish is closed.
- ☞ Organ bouillons: bouillon is made with meat, liver, brain, is boiled and paraffin is added, and so closed from oxygen.
- ☞ Thioglycolate medium: sodium-thioglycolate is added, which decreases redox-potential.

☞ **Biological:**

- ☞ Fortner-method: there is a strong O₂ user bacteria (*Serratia marcescens*) on the half of the medium, too, near the anaerobe.

Macroscopic morphology of bacterial colonies

- ☞ Shape
- ☞ Edge
- ☞ Elevation
- ☞ Surface
- ☞ Opacity (e.g. transparent (clear), opaque, translucent (almost clear), iridescent (changing colours in reflected light)).
- ☞ Pigment production (e.g. white, green, red, purple, yellow)

<p>Whole colony:</p> <p>Punctiform </p> <p>Circular </p> <p>Rhizoid </p> <p>Irregular </p> <p>Filamentous </p>	<p>Edge:</p> <p>Entire </p> <p>Undulate </p> <p>Lobate </p> <p>Filamentous </p> <p>Curled </p>
<p>Surface:</p> <p>Smooth, glistening</p> <p>Rough</p> <p>Wrinkled</p> <p>Dry, powdery</p>	<p>Elevation:</p> <p>Flat </p> <p>Raised </p> <p>Convex </p> <p>Pulvinate </p> <p>Umbonate </p>