Culture media and their preparation

- Medium (media pl.) is the substance which provides nutrients for the growth of microorganisms.
- The nutrients on which microorganisms are cultivated called *culture medium* (pl. culture media).
- A culture medium (Pl. media) is a solid or liquid preparation containing all the nutrients required by microorganisms for growth.
- Microbes can use the nutrients of culture media as their food is necessary for cultivating them in vitro.
- Culture media vary in their form and composition determined by the species to be cultivated. There is no single medium which can support the growth of majority of microbes
- It is used to grow, transport and store microorganisms.

- Liquid culture medium is called *broth*.
- It can be solidified by adding solidifying agent agar-agar in the ratio of 1.5 – 2.0% for complete solid agar and less than 1% for semi-solid medium.
- Agar-agar is a sulphonated mucopolysaccharide containing mainly D-galactose, D-glucuronic acid and 3,6 anhydro L-galactose.
- It is derived from red sea weed e.g., Gelidium and was introduced to microbiologists by *Fannie Hesse*, wife of *Wather Hesse*.

Based on chemical composition, media can be classified into.

1) Natural
2) Semi-synthetic
3) Synthetic.

1. Natural medium: Culture media of which, the exact chemical composition is not known is called natural or empirical culture media. Examples- Milk, urine, diluted blood, vegetable juices, meat extracts, beef and tomato juice, blood etc

2. Semi-synthetic: Culture media, the chemical components of which are partially known and partially obscure are termed as semi-synthetic culture media. Examples- Potato dextrose agar (PDA), Czapek-Dox agar, oat meal agar (OMA), corn meal agar (CMA), beef peptone agar and nutrient agar.

3. Synthetic medium: Such media are composed of the substances that are chemically known. These media are very useful in studying the physiology, metabolic nature and nutritional requirements of microbes. Both autotrophs and heterotrophs can be grown in these media. Examples- Mineral glucose medium, Richard's solution, Raulins medium etc.

Based on consistency the media are of three types as

1) Liquid

2) Semisolid

3) Solid medium

1. Liquid medium: Agar is not added or used while preparing the medium. After inoculation and later incubation, the growth of cells becomes visible in the form of small mass on the top of the broth. eg. Nutrient broth

2. Semi-solid medium: **H**alf quantity of agar is added This type of medium may be selective which promote the growth of one organism and retards the growth of the other organism.

3. Solid medium: If agar is added to a nutrient broth, it becomes solid medium. It is used for isolating microbes and to determine characteristics of colonies. It remains solid on incubation and not destroyed by proteolytic bacteria.

Based on application or function, media can be classified as follows.

1. **Selective media**: Used for a selected species. Provide nutrients that enhance the growth and predominance of particular microbe and don't enhance or may inhibit other types of organisms that may be present.
2. **Differential media:** It allows differentiation among morphologically and biochemically related group of organisms. It contains certain ingredients which are changed because of microbial metabolism and this change can be seen in form of change in opacity of agar, change in pH or change in colour of media or colonies.
3. **Assay media:** Media of prescribed composition are used for the assay of vitamins, amino acids, antibiotics etc.
4. **Enumeration media:** Specific kinds of media are used for determining the bacterial population in milk, water, soil and food etc.
5. **Maintenance media:** It is used for satisfactory maintenance of viability and physiological characteristics of microorganism.

Potato Dextrose Agar

Potato	200g
Dextrose	20g
Agar Agar	20g
Distilled Water	1 lit

Czapek's Medium

$NaNO_3$	2.0 g	❑Heat the full chemical solution without K_2HPO_4 & sucrose in a water-bath for 15 min. add sucrose after heating ❑Dissolve and autoclave K_2HPO_4 separately ❑Mix both the solution just before autoclaving
K_2HPO_4	1.0 g	
$MgSO_4.7H_2O$	0.5 g	
KCl	0.5 g	
$FeSO_4.7H_2O$	0.01 g	
Sucrose	30 g	
Distt. water	Makeup 1 lit	

Richards solution

KNO_3	10 g	❑Heat the full chemical solution without KH_2PO_4 & sucrose in a water-bath for 15 min. add sucrose after heating ❑Dissolve and autoclave KH_2PO_4 separately ❑Mix both the solution just before autoclaving
KH_2PO_4	5.0 g	
$MgSO_4.7H_2O$	2.5 g	
$FeCl_3$	0.02 g	
Sucrose	50 g	
Distt. water	Makeup 1 lit	

Corn Meal sand medium
(Oomycetes and predaceous fungi)

Corn meal	1.0 kg	❑Mix the ingredients and steam in a large open pan for about 1 hr. ❑Stir well and after cooling breaks into small lumps and fill into the flask ❑Autoclave at 21lb psi for one and half hr.
Washed white sand	1 .0 kg	
Distt. water	Makeup 1 lit	

Corn meal medium

Corn meal	25.0 g
Peptone	20.0 g
Agar-agar	20.0 g
Glucose	20 g
Distt. water	Makeup 1 lit

Malt extract medium (basidiomycetous fungi)

Malt extract	20.0 g
Agar- agar	20.0 g
Distt. water	Makeup 1 lit

Oat meal medium (Oomycetes and Coelomycetes)

Oat meal	40.0 g
Agar- agar	20.0 g
Distt. water	Makeup 1 lit

NUTRIENT AGAR MEDIUM (**BACTERIA**)

Peptone	5.0 g
Beef extract	3.0 g
Agar-Agar	15.0 g
Glucose	2.5 g
Tap water	Makeup 1 lit.
pH	7.2

Actinomycetes (soil dilution 10^{-3}-10^{-4})

Starch ammonium agar medium (Actinomycetes)

Agar-agar	:	20.0g	$MgSO_4$	:	1.0g
Starch	:	10.0g	$CaCO_3$	:	3.0g
$(NH_4)_2 SO_4$	:	1.0g	Distt. water	:	Makeup 1lit.

Bacteria (soil dilution 10^{-5}-10^{-6})

Soil extract peptone agar medium (**Bacteria**)

Peptone	: 1.0 g	$MgCl_2$	: 0.1 g
Yeast extract	: 1.0 g	$Fecl_3$	: 0.1 g
Soil extract	: 250 ml	$CaCl_2$	: 0.1 g
K_2HPO_4	: 0.4 g	Agar-agar	: 15.0 g
$(NH_4)_2 HPO_4$	: 0.5 g	Tap water	: Makeup1 lit.
$MgSO_4.7H_2O$	: 0.05 g	pH	: 7.4

Preparation soil extract:

- **Take 1.0 kg garden soil and add 1 lit. of Tap water, Mix it properly and leave for 20-30 minutes. Supernatant separated and autoclave at 21 lb for 30 min.**
- **Add 0.5g $CaCO_3$ leave for 20-30 minutes and filter through a double layer of filter paper.**

Fungi (soil dilution 10^{-4}-10^{-5})

Peptone dextrose rose Bengal agar medium (Fungi)

Peptone	: 5.0 g	Chloramphenicol	0.1 g
Glucose	: 10.0 g	Rose bengal	: 0.025 g
Agar-agar	: 20.0 g	Dichloran	: 0.002 g
KH_2PO_4	1.0g	Distt. water	: Makeup 1 lit.
$MgSO_4.7H_2O$	: 0.5 g	pH	6.0-6.5

Modified *Trichoderma* Selective Medium

$MgSo_4.7H_2o$	0.2 g	Captan	0.05 g
		Vitavax	0.05 g
K_2HPO_4	0.9 g	OR	
		PCNB	0.1 g
KCl	0.15 g		
NH_4NO_3	3.0 g	Rose Bangal	0.15 g
Glucose	3.0 g	Agar-agar	20.0g
Chloromphenicol	0.5 g	Distt.Water	makeup1 lit.
Apron (metalaxyl)	0.5 g		

Malachite green agar medium *Fusarium*

$FeSO_4.H_2O$	0.01 g
$MgSO_4.\ 7H_2O$	1.0 g
K_2HPO_4	1.0g
KCl	0.5 g
$NaNO_3$	2.0 g
Sucrose	30.0 g
captan	1.0 g
Dicrysticin	1.0 g
Malachite green	0.05 g
Agar-agar	20.0 g
Yeast extract	2.0 g
Distilled Water	Make up1L

Inhibitors and antibiotics must be added to the medium after autoclaving and cooling to about 45^0C

Pythium

KH_2PO_4	1.0g
$MgSO_4.\ 7H_2O$	0.5g
Peptone	5.0
Dextrose	10.0g
Rose bengal	0.05g
diacrysticin	0.5g
Benlet	0.02g
Mycostatin	1.0g
Agar	20g
Water	1L

Antibiotics, fungicides and rose bengal must be added after autoclaving

.

Rhizoctonia solani

K_2HPO_4	1.0g
$MgSO_4.\ 7H_2O$	0.5g
KCl	0.5g
$FeSO_4.H_2O$	0.01g
$NaNO_3$	0.2g
Gallic acid	0.4g
Dexon	0.09g
Chloramphenicol	0.05g
Streptomycine sulphate	0.05g
Agar	20g
Water	1L

CMRA medium *Macrophomina*

Chloroneb	300mg
$HgCl_2$	7mg
Rose Bengal	90mg
Streptomycin	40mg
Penicillin G.	60mg
Rice agar of medium	1L

The best results are obtained if chloroneb and rose Bengal are dissolved first in sterile cool water and $HgCl_2$ in sterile hot water prior to adding to the cool, molten medium.

King's medium B *Pseudomonas* spp.

Protease peptone	20.0g	Adjust pH to 7.2 before autoclaving the medium
Glycerol	15 ml	
K_2HPO_4	1.5 g	
$MgSO_4\ 7H_2O$	1.5 g	
Agar	20.0 g	
pH	7.2-7.4	
Distilled water	1L	

Incubation period for enumerating microbial population (at 26±1^{0}C (fungi & actinomycetes) or 28±1^{0}C (bacteria))

Total fungal count	**:**	**4-5 days**
Total bacterial count	**:**	**5-7 days**
Total Actinomycetes count	**:**	**7-9 days**
Pythium	**:**	**2 days**
Trichoderma	**:**	**3-5 days**
Fusarium	**:**	**4 days**

Plates containing spreading types of bacteria, fungi or large clear zones of antibiosis should be discarded.

Biological clock

brain

Supra Chiasmatic nucleus

gene

TIM1, TIM2

Photosynthesis

$CO_2 + H_2O$

Chlorophyll

$C_6H_{12}O_6 + O_2$

Respiration

$C_6H_{12}O_6 + O_2$

$CO_2 + H_2O +$ ~ 686 kilocal of energy

38 ATP

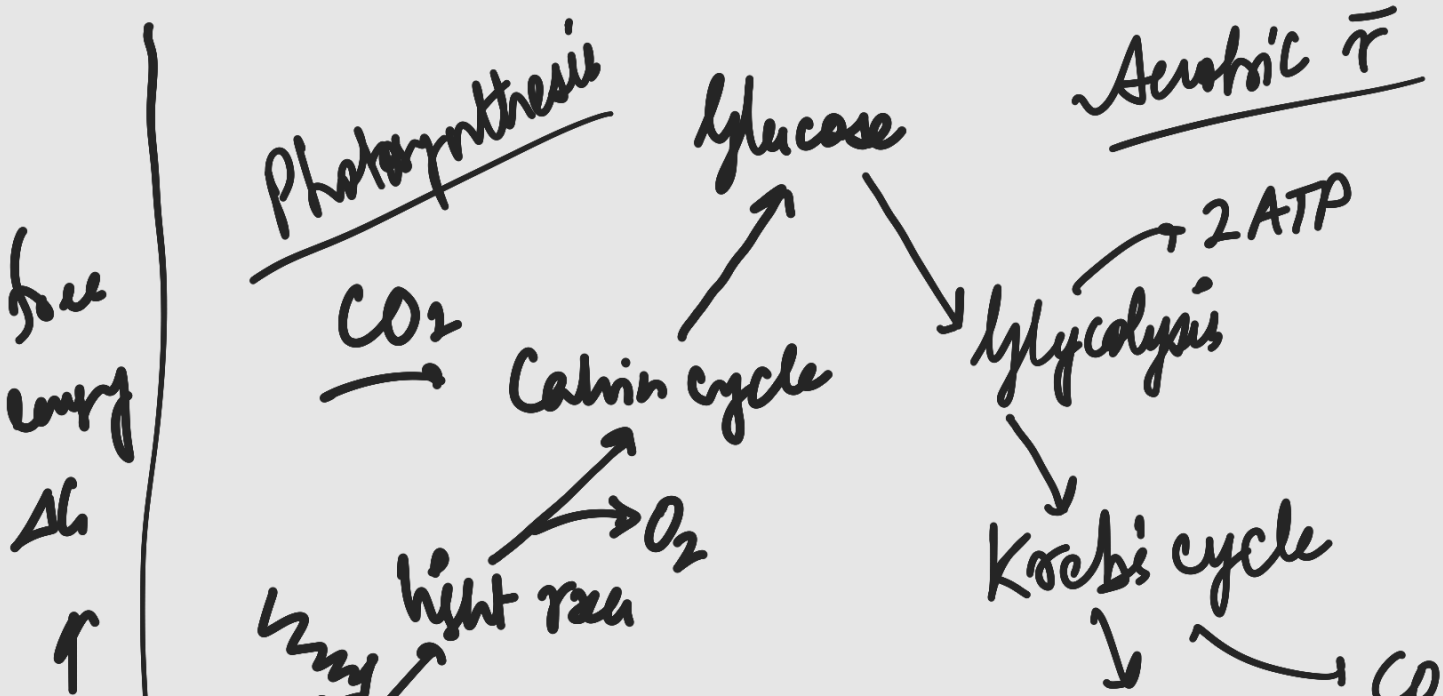
Exergonic r Endergonic r

release of Δ absorption of Δ

Standard free energy change

Gibbs free energy

$\Delta G, \Delta U, T, \Delta S$



H_2O

ETC
↓
36 ATP

Reaction progress ⟶

Low entropy ⟶ less disorder
less chaos

$\Delta S \uparrow \longrightarrow \Delta G \downarrow$

Anabolism ⟷ Catabolism

C=O	⟶	187 Kcal mol^{-1}
C=C	⟶	145 "
O-H	⟶	110 "
H-H	⟶	103 "
C-H	⟶	98 "

C-O ⟶ 78 "

C-C ⟶ 80 "

Standard biochemical free energy change

$\Delta G = \Delta_{products} - \Delta_{reactants}$

If $\Delta G < 0$.

forward reaction ✓

$\Delta G = 0$.

reactants + products

equilibrium

$\Delta G > 0$.

reactants have lower energy

than products

extra energy has to be supplied

* $\boxed{\Delta G^0 = -RT \ln K'_{eq}}$

$A \longleftrightarrow B$ $\quad \Delta G = \Delta G^{0'} + RT \ln \frac{[B]}{[A]}$

↓ $\Delta G^{0'}$

↑ K_{eq}'

formation of [B] is more favoured

$A \longleftrightarrow B$ Coupled to $ATP \longleftrightarrow ADP + P_i$

$\Delta G^0 = 3$ Kcal mol^{-1}

$\Delta G^0 \rightarrow -7.3$ Kcal mol^{-1}

Overal $\Delta G^\circ = 3 + (-7.3)$

$= -4.3$ Kcal/mol

Protein folding → Coupled

+2ATP

* The phosphoryl transfer potential of ATP is the highest among all PO_4^{3-} containing biomolecules.

* Inhibitors → uncouplers.

Microbiology

* Unicellular microbes

↓

adaptive mechanism → natural selection

- variations leading to natural selection and evolutionary adaptation
- diversity of rare forms

Species vs. Strains

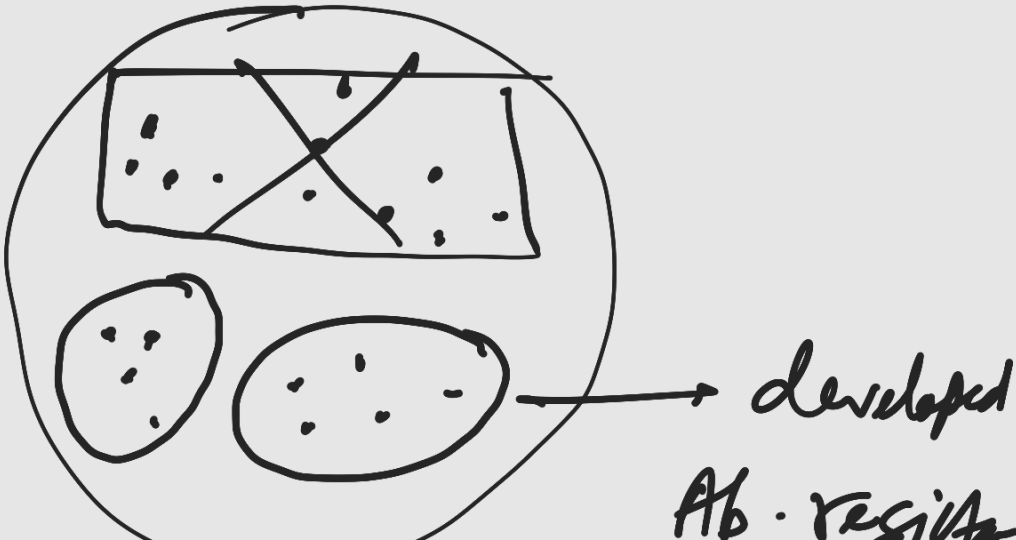
Species	Strains
develop slowly over time	develop as a result of perturbation

Plasmids, cosmids, phagemids

extra chromosomal DNA

↓

confer antibiotic resistance




Superbug
biofilms