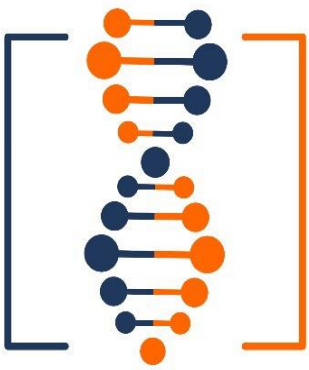


DESTINATION PHARMAGENS

Nutritional requirement for bacterial growth :

Source of energy

Energy source	Known as	Example
sunlight	Phototrophs	Rhodispirillum rubum
Chemical rexn	chemotrophs	E. Coli

Source of electrons

Generally electrons derived from metabolism.

Electron source	Known as		Example	
Inorganic compound	lithotrops		Pseudomonas pseudoflova	
	Photolithotrops (H2S)	Chemo-lithotrops	Chromatium okini	Nitrosomas europea
Organic compound	organotrophs		E. Coli	
	Photoorganotrophs (Fatty acids and esters)	Chemo-organotrophs	Rhodispirillum rubum	E. Coli

Source of carbon:

CO2 – autotrophs eg Chromatium okini

Organic compound – heterotrophs eg E. Coli

Nitrogen : nitrogen can be obtained from nitrates, nitrites, ammonium compound or other organic compounds such as amino acid

Sulphur: from organic and inorganic compounds elewmental sulphur derived, needed for cellular processed and synthesis of sulphur containing amino acid

Phosphorus : from organic and inorganic compounds, needed for nucleotides, nucleic acid and phospholipids.

Minerals salt:

Sodium, potassium, iron, calcium

Growth factor or bacterial vitamins

S aureus _ thiamine, nicotinic acid

Water: major essential nutrient

Bacteriological media:

Water

- For preparation of media
- For flow of nutrients
- Copper distilled water cant be used since it inhibits the growth of bacteria

Peptone

- It is a mixture of partially digested proteins obtained from various source\
- Its constituent are proteins, peptides, amino acids, inorganic salts, growth factors (nicotinic acid, riboflavin)
- Hygroscopic in nature and kept in tightly closed container
- **Function: mainly supply nitrogen and act as a buffer**

Yeast extract :

- Preapared from bakers yeast or saccheromyces
- Contains carbohydrate amino acids, inorganic salts, growth factors (nicotin, thiamine, riboflavin, pantpothenic acids)

Meat extrtact:

- Prepared from freash lean meat by hot extraction process
- contains gelatin, amino acid, proteins, peptides, amino acid, growth factors

Agar:

- Long chain polysaccherid obtained from see weedalgae called as agarophytes (such as gelidium, gracilaria, hyphae, gelidiela, ceramium)
- Agar is a mixture of two polysaccharide **agarose (70 %) and agaropectose (30 %)**
- **Act as solidifying agent at conc 2%**

Types of culture media :

Classification of basis	Class	% of agar	Example/ purpose
Physical state	Solid media/basal	1.5-2.5	Nutrient agar,
	Semisolid media	0.2-0.5	Nutrient broth
	Liquid media	0	Fluid thioglycolate
Oxygen requirement	Aerobic media		MacConkeys agar
	Anaerobic media		Robertson cooked meat media
Chemical composition	Simple or basal		peptone water, Nutrient broth.
	Synthetic or defined		Used for special purpose and metabolic studies
	Non-synthetic or undefined or complex		Used for growth of unknown bacteria
Functional type	Enriched meida		Used for fastidious medium , having blood and serum , egg in it (eg blood agar)
	Enrichment media		Specific substance added in liquid form to inhibit the growth of unwanted bacteria (eg tetra thionate broth)
	Selective media:		Specific substance added in solid form to inhibit the growth of unwanted bacteria and favors that of wanted in form of colonies 1. macConkeys agar for ecoli (contains inhibitor sodium taurocholate) 2. ddeoxycholate citrate agar for for salmonella and shigella sp.
	Indiactor media		Wilson blair media (s typhi)
	Differential media		macConkeys agar media , blood agar medium
	Sugar media (1% media)		Durhams tube
	Transport media		Stuarts transport media
	Assay media (hving known composition of growth factors)		
	Storage media		Dorset egg media, nutruient agar slabs

Physical conditions required for growth :

1. **temperature**

Table no 5.2 pg 5.7 kokare micro book

2. PH

Class	pH	example
ACIDOPHILES	1-6	lactobacillus acidophillus (5.8-6.6)
NEUTROPHILES	6.5-7.5	E COLI and all pathogenic bacterias
ALKALOPHILES	7.5-14	Vibrio choler

3. Gaseous requirement

Aerobic microbes – need oxygen for growth eg e.coli , s.aureus

Anaerobic microbes – don't need oxygen eg clostridium sp

Facultative anaerobic bacteria: don't require oxygen but if available used for energy source eg pseudomonas

Microaerophilic bacteria: require very low levels of oxygen

Bacterial reproduction techniques

- Binary fission
- Budding
- Fragmentation
- Formation of conidospores or sporangioispores

Growth curve of bacteria:

- Lag phase
- Log phase / exponential phase
- stationary phase
- death or decline phase

Methods of bacteria growth measurement

1) **determination of cell number**
   a) total count or direct method
      i) direct microscopic/ breed method
      ii) counting chamber or heamocytometric method
      iii) proportional count method
      iv) electronic counter method
   b) viable count/indirect method
      i) plate count method
      ii) membrane filter count method
2) **determination of cell mass:**
   a) direct method
      i) dry weigth measurement
      ii) measurement of cell nireogen
   b) indirect – turbidimetric method
3) **determination of cell activity** -indirtect – measurement of biochemical activity

Pure culture techniques

1. Streak plate method

   Sample is streaked in such a way as to provide dilution

2. Pour plate method

   Mixed culture is diluted directly into the tubes of liquid cooled agar medium and liquid media maintained at 45 °C, which later allowed to cool solidified.

   Eg Loop dilution technique, Serial dilution technique

3. Spread plate method

   Mixed culture is not diluted in culture medium but it is diluted in series of tubes containing sterile water and saline solutions

4. Micromanipulator method

   Device which picks up single cell from the culture and transferred into large drop of sterile media

5. Roll tube method

   Used for isolation of stringent anaerobs.

Preservation techniques:

1. Periodic transfer to fresh media
2. Storage at low temperature
3. Storage in sterile soil
4. Preservation by overlying culture with mineral oil
5. Lyophilization or freeze drying

Chemical test for detection and differentiation of microbes

Technique	Stain used	Mechanism	Color produced
Simple staining – for elucidation of morphology and arrangement of bacterial cells	**Basic (cationic) stains** used such as methylene blue, crystal violet, carbol fuschin, safranin	Bacterial nucleic acid and certain cell wall have negative charge and this makes basic cationic chromagens able to bind to cell	Depend on stain used
Negative staining- for bacterial capsule and cell morphology	**Acidic stains** are used such as eosin, nigrosin (**negatively charged)**	Bacterial nucleic acid and certain cell wall have negative charge and so such cells remains colorless,which can be easily detected against colored background.	Depend on stain used
Gram staining- differentiation staining technique	**Basic stain**: crystal violet stain **mordant** Iodine: pot iodide- **Decoloriser:** Alcohol+ acetone- **Counter stain:** Safranin	Basic stain produces violet color ; mordant fixes this color over cell wall ; decoloroiser removes color but only gram negative loses it, which is confirmed by counter stain.	Gram positive blue Gram negative pink
Acid fast staining- differential staining technique	**Basic stain** zheil neilson carbol fuschin **Decolorizer** alcohol+acetone **Counter stain** methylene blue	Basic stain produces color ; decoloroiser removes color but only acid fast negative loses it, which is confirmed by counter stain.	Acid fast pink Non-acid fast blue
Spore staining- for detection of spore carrying bacteria	**Primary stain**- malachite green **Mordent** purpose heat **Decolorizer**- tap water **Counter stain** safranin	Bacterial cells do not easily accepts primary stain for which heating is done Decolorized and vegetative cells confirmed by counter stain	
Cytoplasmic inclusion staining- for detection of intracellular deposits of starch other cellular materials			
Capsule staining for detection of capsule			
Flagella staining for detection of flagella and its arrangement			

Biochemical test for detection and differentiation of microbes

Test & conditions	Principle	Reagent /color
Sugar fermentation	Based on ability of microbes to fermant variety of sugars, suitable indicator added which gives color.	
Litmus milk reaction	Based on ability of microbes to convert milk sugar (lactose) milk protein (casein, lacto albumin, lactoglobulin) depending upon enzymes present	
Indole production: **Temp:** 37 °C & **Duration:** 48-96 Hrs **Media:** peptone water	If bacteria contains tryptophanase, which degrades amino acids into indole, pyruvic acid, ammonia at	On addition of kovacs reagent red color produced
METHYL RED TEST **Media - Glucose phosphate broth** **Temp:** 37 **°C** **Duration:** 2-5 days	Used to detect production o facid during the fermentation of glucose	Positive- red color Negative - yellow color
VOGES – POROSKAUER TEST **Media**-GLUCOSE PHOSPHATE broth **Temp –** 37° C **DURATION**- 48 HRS	Many bacteria fermant the carbohydrate with the production of acetyl methyl carbinol (acetoin)	PEPTONE (form the media)- pink
Citrate utilization test **Media –** kosers citrate agar media	Based on ability of microbe to utilize citrate as a source of C-atoms	Turbidity – indicates positive
Nitrate reduction test **Media-** KNO_3/PEPTONE/WATER **Temp**- 37 °C **Duration-** 96 Hrs **Indicator –** 5N acetic acid	Test detects the production of enzyme nitrate reductase, which reduces nitrite.	Positive- red color develops
Hydrogen sulphide reduction **Media –** ferric ammonium citrate or lead acetate or ferrous acetate	Based on ability of micro-organism to produce H2S from amino acid	Positive – black or brown
Potassium cyanide test **Media-** buffed peptone with 1/13000 KCN **TEMP:** 37 °C **DURATION –** 24 Hrs	Based on ability of microorganism to grow in the presence of pot cynide.	POSITIVE – TURBIDITY
Catalase production test **MEDIA-** nutrient agar **Indicator-** H_2O_2	Based on ability of microorganism to produce catalase in presence of H2O2	Positive – production of gas bubbles
Urease test **Media –** christensens media **Indicator –** phenol red **Temp**- 37 °C **DURATION**- ONE DAY + 4 HRS	Based on ability of microorganism to produce urease	POSITIVE – Purple red

Test & conditions	Principle	Reagent /color
Oxidase test	Test is based on presence of enzyme oxidase that catalyses the oxidation of reduced tetra methyl-p-phenyl diamine dihydrochloride (oxidizing agent)	Positive- deep purple colored colonial growth

Indole test, Methyl red tets, VP test, and citrate test are used for detection of gram negative enteric bacteria , COLLCETIVELY reffered as ***IMViC TEST***

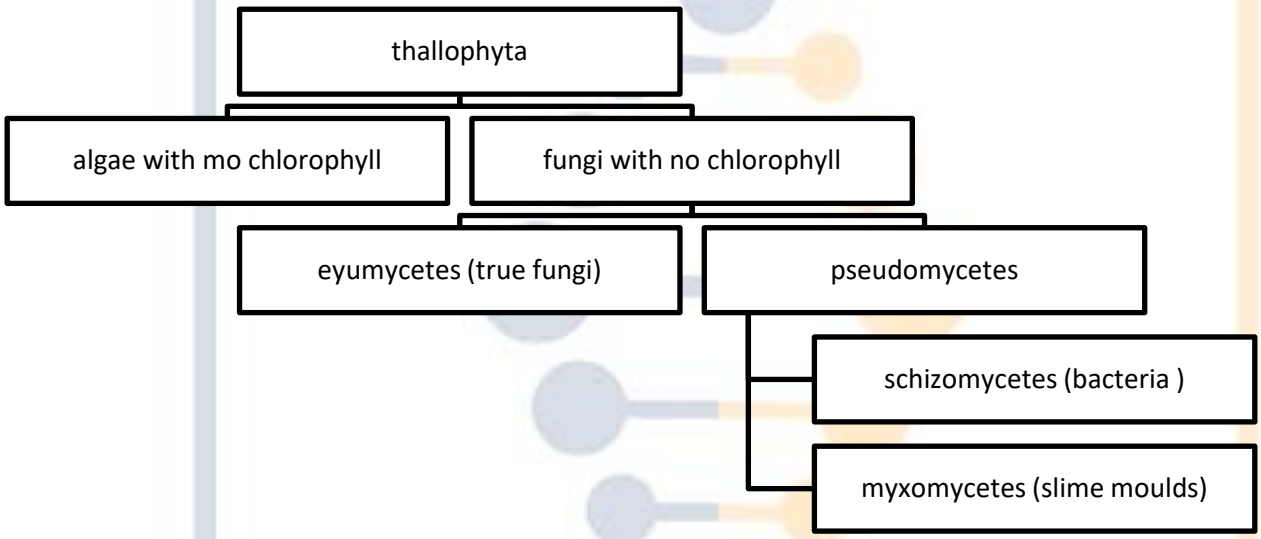
Fungi

Group of non motile eukaryotic organism which exist as a saprophytic parasites, possess differentiated nucleus surrounded by nuclear membrane and get reproduced by spores or budding.

Characters	**Fungi**	**Bacteria**
Cell type	Eukaryotic	Prokaryotic
OPTIMUM PH	4-6	6.5-7.5
Optimum temperature	25-30C (saprophytic sp.) 32-37 C (parasitic sp.)	32-37 C
CELL MEMBRANE	STEROLS PRESENT	STEROLS ABSENT

CLASSIFICATION OFR FUNGI

Fungi are placed in phylum thallophyta which contains the irregular plant mass that lack definite root, stem leaf structure.



Depending upon the cell morphology fungi can be divided into four classes

Moulds, yeasts, yeast like fungi and dimorphic fungi

Moulds and fleshy fungi:

- Form smycelium, also known as filamentous fungi
- Eg : aspergillus niger, peniclillium notatum, microsporum gypsum.

Yeast:

- Rouynd, oval, unicellular fungi
- Reproduced by asexual process called as budding
- Eg : saccharomyces cerevisae

Yeast like fungi

- **Reproduced by budding**
- **Eg ;** candida albicans

Dimorphic fungi

- These are yeast like funggii reproduced by budding

Dermatophytes:

These are closely related fungal sp which infects only superficial skin layers keratinized tissues.

Protozoa

Protozoa are group of microbial sp recuire large amount of moisture hence found in high moisture places only.

Classification o fp[rotozoal species:

1. **Rhizopoda –** unicellular, produces for locomotion , divided by binary fission. **Eg entamoeaba histolytica**
2. **Mastigophora-** flagella as a locomotive organ present in bacteria classified as a **zeoflagelates** and phytoflagellates. Zeoflagellates causes trichiomoniasis, leishmaniasis, trympanosomiasis, giardiasis.

Class	**Organ of locomotion**	**Eg**	**Disease**
Rhizopoda	pseudopodia	**entamoeaba histolytica**	**Amoeabiasis**
Mastigophora classified as a **zeoflagelates**** and phytoflagellates	flagella	**Trichomonia vaginisis**** **Leishmania donovani**** **Trympanosoma sp**** **Giardia lamblia ****	trichiomoniasis, leishmaniasis, trympanosomiasis, giardiasis.
Spporozoa	**None, possess littile amoeboid movement**	**P. FALCIPARUM** **P. VIVAX** **P. OVALE** **P. MALARIAE**	**MALARIA**
Cilianta	**Cilia**	**Balantidium coli**	**Dysentery**

Reckettsia

- Small gram negative baciili which have properties between bacteria and viruses
- They are primarily parasites of arthropods such as flea, lice, mites, ticks.
- These organisms infects vascular endothelium and RE cells in vertebrates including humans.

GENRAL FEATURES

- ✓ these are **cocco bacilli** and visible in **light microscope** only
- ✓ **these are obligate intracellular parasites**
- ✓ produces only **endotoxin** but no exotoxin
- ✓ require arthropods as a **vector**
- ✓ grow on **blood agar**

SUCCEPTIBILITY TO PHYSICAL AND CHEMICAL AGENTS:

- **readily inactivated by physical and chemical agents**
- **quickly destroyed by heat, drying, bacteriostatic chemicals**
- **unstable under extracellular conditions at normal environmental conditions**

DISEASE CAUSED BY RICKETTSIAL SPECIES

Group	Disease	Causative agent	Vertebrate reservoir	Arthropod
Typhus fever	**Epidemic/classical typhus fever**	**R. prowazeki**	**Man**	**Louse**
	Recrudescent / brill zinsers disease	**R. prowazeki**	**Man**	**-**
	Endemic / murine disease	**R. typhii**	**Rodent**	**-**
Scrub typhus spotted fever	**Scrub typhus fever**	**R. tsutsugamushi**	**Rodent bird**	**Mite**
	Rocky mountain fever	**R. RICKETTSIA**	**Rabbit dog frog**	**Tick**
	Rickettsial pox	**R. akari**	**Mouse**	**Tick**
	Indian tick typhus	**R. conori**	**Rodents**	**Tick**
Q fever	**Q fever**	**Coxiella burnetti**	**Rodent cattle sheep**	**Tick**
Trench fever	**Trench fever**	**Rochalimaea quinata**	**Man**	**Louse**

Actinomycetes

These bacterias with fungal morphology.

They are gram positive, non motile, non capsulated filamentous that may break into bacillary and coccoid element.

General habitats of actinomycetes:

Soil, marine water, fresh water, compost, extreme enviormental conditions.

Classification of actinomycetes

Section	Characteristics
Nocardioform actinomycetes	Aerobic bacteria, may be alcohole / acid fast bacteria occurring as a rods/cocci/branched filament. Contains mycolic acid Chemotype IV
Actinomycetes with multilocular sporangia	Aerobic or facultatively anaerobic, mycelium divides in all planes. Chemotype III
Actinoplanetes	Aerobic sporactinomycetes, nonmotile, spores may be formed, Chemotype II
Streptomycetes	Aerobic sporactinomycetes, forms exclusively branched substrate and aerial mycelium.
Maduromycetes	Aerobic sporactinomycetes Motile/non-motile Chemotype III
Thermomonospora	Aerobic sporactinomycetes, forms exclusively branched substrate and aerial mycelium. Chemotype III
Thermoactinomycetes	Stable filaments Forms endospores
Other genera	Produces aerial growth with spores in chains

Biotechnological uses of acatinomycetes : Refer newly added page - Not available now

Antibiotics production , enzyme , biosurfactants .

CLICK HERE www.destinationpharmagens.com