

## Microbial growth requirements

### Definition of growth

Orderly increase in the sum of all the components of an organism, cell multiplication is a sequence of growth; in unicellular organism, growth leads to an increase in the number of individuals making up a population or culture.

### Nutritional requirements for growth

Bacteria differ widely in their nutritional requirements, some bacteria can synthesize the entire requirement from the simplest elements.

Other including the most pathogenic bacteria are unable to do this, they need a ready made supply of some organic compound required for their growth.

### Elements

Bacterial structural components and the macromolecules for cell metabolism are synthesized from elements \*table

For the most part, organic matter is macromolecules formed by anhydride bonds between building blocks synthesis of these compounds needs energy provided by ATP (adenosine triphosphate). And additional energy required to maintain the cytoplasm composition during growth which derived from proton motive force (is an electrochemical gradient with two

components a difference in pH (hydrogen ion concentration) and difference in ionic charge

### Sources of metabolic energy

The three major mechanisms for generating metabolic energy are

- Fermentation
- Respiration
- Photosynthesis

### Fermentation

Fermentation is characterized by a substrate phosphorylation, the phosphorylated intermediates are formed by metabolic rearrangement of a fermentable substrate such as glucose, lactose, or arginine.

Respiration is analogous to the coupling of an energy-dependent process to the discharge of a battery

Photosynthesis is similar to respiration in that the reduction of an oxidant via a specific series of electron carriers establishes a proton motive force. The difference in the two processes is that in photosynthesis the reductant and oxidant are created photochemically by light energy absorbed by pigment in the membrane; it can continue as long as the source of sunlight

## Nutrition

All organism require source of energy, some rely on chemical compounds for there energy and called as **Chemotrophes**.

Other utilize radiant energy (light) are called **phototrophs**

All require source of electrons for there metabolism, by reducing inorganic compounds as electron donors **chemolithotrophic**

Or using organic compounds as electron donors and called **chemo-organotrophs**

### Carbon source

All organism require carbon in synthesizing cell component.

All organism at least small amount of  $\text{CO}_2$ . , those organism using  $\text{CO}_2$  as a major source know as autorophs. Other require organic compounds as there carbon source and called as **heterotrophs**

### Nitrogen source

Nitrogen is a major component of proteins and nucleic acid about 10 % of dry weight, nitrogen may be supplied in a number different forms and microorganism vary in their ability to assimilate nitrogen The end products of all pathways for nitrogen assimilation is the most reduced form of the elements ammonium ion ( $\text{NH}_4^+$ ).

Many microorganism possess the ability to assimilate nitrate ( $\text{NO}_3^-$ ) and nitrite ( $\text{NO}_2^-$ ) reductively by conversion these ions to ammonia ( $\text{NH}_3$ )

The ability to assimilate nitrogen gas reductively by ammonia which his called nitrogen fixation is properties unique to

prokaryotes. Most microorganisms can use  $\text{NH}_4^+$  as a source for nitrogen source and many organism posses ability t o produce ammonium ions from amines or amino acids.

#### Sulfur source

like nitrogen is component of many inorganic cells substances it forms part of several coenzymes most of microorganism can uses sulfate and sulfur source reducing sulfate to level of hydrogen sulfide ( $\text{H}_2\text{S}$ )

#### Phosphorus source

Phosphate is required as a component of ATP nucleic acids and such as coenzyme NAD NADP and flavines, metabolites lipid, cell wall, capsular polysaccharides. It are always assimilated as a free inorganic phosphate

#### Mineral sources

numerous minerals are required for enzyme function as, metal ions  $\text{K}^+$ ,  $\text{Ca}^+$ ,  $\text{Mg}^+$ ,  $\text{Fe}^+$  and others trace elements.

Water: all living microorganism require water

## Growth factor

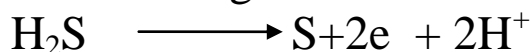
Growth factor is an organic compound (amino acids, purines and pyrimidines, vitamins) which a cell must contain in order to grow but which is unable to synthesize.

many micro-organisms when provided with the listed above are able to synthesize all of the building blocks for macromolecules (amino acid, purine, pyrimidine, and pentose) all are metabolic precursors for the nucleic acids then incorporated into DNA, additional cho. precursors for polysaccharides and fatty acids

When an organism undergoes a gene mutation so the chain is broken and no longer there is a product, so the organism must obtain that compound from the environment the compound has become as a growth factor for the organism

## Nutritional types of bacteria

Phototrophs: uses inorganic compounds as their source of electrons e.g. *Chromatium okenii* uses



As an electron donor oxidizing it to elemental sulfur

Others use organic compounds such as fatty acid and alcohols as electron donors

Chemotrophs that use inorganic compounds as their source of electrons e.g. ammonia as their electron source obtaining their energy by oxidizing ammonia to nitrite

Autotrophic and heterotrophic

Organism can utilize and uses for example carbohydrates and  $\text{CO}_2$  as their source of carbon

Obligate parasite those bacteria which cannot be cultivated artificially on artificial media

Environmental factors affecting growth

A suitable growth medium must contain all the nutrients required by the organism to be cultivated \*

Nutrient

The following must be provided

Hydrogen donors

Carbon source

Nitrogen source

Minerals sulfur and phosphorus

Growth factors

## Hydrogen ion concentration (pH)

Most organisms have optimal narrow pH. Most organisms are neutrophils and grow best at pH 6.0-8.0.

Other acidophiles grow at low pH 3.

Alkaliphiles grow at high pH 10.5.

## Temperature

Different microbial species vary widely in their optimal temperature ranges for growth:

Psychrophilic forms grow best at low temperatures (15-20 °C)

Mesophilic grow best at (30-37 °C)\*

Thermophilic grow at (50-60 °C)

Heat shock response: when organisms are exposed to a sudden rise in temperature above growth optimal, these proteins appear to be unusually heat resistant to stabilize the heat-sensitive proteins.

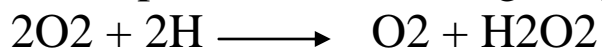
Cold shock; a number of compounds protect cells from either freezing or cold shock (glycerol and dimethylsulphoxide are most common used).

## Aeration

Many of organism are obligate aerobic others are facultative aerobic and anaerobic.

The natural products of aerobic metabolism are the reactive compound hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), And superperoxide (O<sub>2</sub>)

These products can damage any biological macromolecules



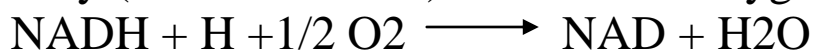
Many aerobes and anaerobic are protected from these products by the presences of superperoxidisedismutase enzyme that catalysis the reaction (Catalase enz).



Some fermentation organism doesn't contains either of enz.

Oxygen is not reduced therefore there will be no products

For anaerobic organism have a considerable tolerance to oxygen as a result of their ability to produce high level of an enzy (NADH oxidase) that reduces oxygen to water



Hydrogen peroxide owes much of its toxicity to the damage it causes to DNA

Obligates anaerobic present a problem in oxygen exclusion using reducing agent such as thioglycolate can be added to liquid medium or the medium sealed with a layer of petrolatum and paraffin



Ionic strength and osmotic pressure

Organism require high salt concentration know as **hallophilic**

Those requiring high osmotic pressure are called **osmophilic**

Most of bacteria are able to tolerate external osmotic pressure and ionic strength because of their ability to regulate internal osmolality and ion concentration

Culturing of microorganism

Culture teachnique used to isolate pathogens in pure culture so that they can be identified , and ifindicated , testedfor thiere sensitivity (Susceptibility to antimicrobials)

Most of bacteria can be cultured artificially providing:

- The culture medium contains the required nutrients in the correct amounts and the osmotic pressure and pH of the medium also correct
- The microorganism are incubated in atmosphere and temperature most suited to there metabolism

Microbial growth requirement

Approximately 80% of the living weight of bacterial cell is water and the rest is of dry weight 2-5% is phosphorus , minerals oxygen and hydrogen inorganic compounds

So the media should contains water, source of nitrogen, carbon, minerals , and essential vitamins. Other substances may be included according to the species requirements.

## Common ingredient of culture media

### Peptone:

This is a general term for the water soluble products obtained from the breakdown (hydrolysis) of animal or plant proteins.

The proteins are commonly those from **meat, milk, and soya bean meal**. They **are hydrolyzed by acids or by enzymes** such as pepsin, trypsin, and papain. The products are free amino acids, peptides (polymers of amino acids) and proteoses (large size peptides). **All forms of peptone are not coagulated by heat.**

Peptone provides **nitrogen** for growing microor-ganisms. Plant proteins such as **soya peptone** also provide **carbohydrates,** and most peptones contain **nucleic acid fractions, minerals and vitamins.**

\*Peptone powder should be light in color, dry, and have a neutral pH. The concentration and form of peptone used depend on the uses of individual culture media, for example peptones with a high tryptophan content are used in indole testing media, proteose peptone is used in media for bacterial toxin production, tryptose in enriched media, and tryptone which is particularly rich in amino acids is added to several media including blood culture media.

## Meat extracts

Beef extract such as *Lab Lemco* provides organisms with a further supply of amino acids, and also with essential growth vitamins and mineral salts including phosphates and sulphates. It is an ingredient of many culture media including nutrient agar and nutrient broth. Trypsin digested meat extracts are also used

## Yeast extract

This is contained in many culture media as a bacterial growth stimulant, for example in xylose lysine deoxycholate (XLD) medium, modified New York City (MNYC) medium, and thiosulphate citrate bile salt sucrose (TCBS) medium.

## Mineral salts

For cell growth, sulphates are required as sources of sulphur and phosphates as sources of phosphorous. Culture media should also contain traces of magnesium, potassium, iron, calcium and other elements which are required for bacterial enzyme activity. Sodium chloride is also an essential ingredient of most culture media.

## Carbohydrates

Simple or complex sugars are added to many culture media to provide bacteria with sources of carbon and energy.

Carbohydrates are also added to media to assist in the differentiation of bacteria, for example lactose is added to MacConkey agar and deoxycholate

citrate agar to differentiate enterobacteria, and sucrose to TCBS agar to differentiate *Vibrio* species. Fermentation of the sugar with acid production is detected by a change in colour of the indicator. Fermentation is often accompanied by the production of gas (carbon dioxide and hydrogen).

## Agar

This is an inert polysaccharide extract obtained from a variety of red-purple seaweeds (rhodophyceae) which form the agarophyte group of marine algae. It consists of two main polysaccharides, agarose (70-75%) and agarpectin (20-25%).

Agar is used to solidify culture media because of its high gelling strength and its setting temperature of 32-39°C and melting temperature of **90-95°C**. Most agars used in bacteriological work produce a firm gel at a concentration of **1.5% w/v**. The low gelling temperature allows heat-sensitive nutrients such as whole blood to be added safely at **45-50 °C**.

At a concentration of **0.4-0.5% w/v, agar is added to transport media** such as Amies medium to give a **semisolid gel**.

Besides being used to solidify culture media, agar also provides microorganisms with calcium and other organic ions.

Water

This is essential for the growth of all microorganisms. It must be free from any chemicals which inhibit bacterial growth. **Deionized or distilled** water must be used in the preparation of culture media if the local water supply has a high mineral content.

## TYPES AND SELECTION OF CULTURE MEDIA

The main types of culture media are:

Basic

Enriched and enrichment

Selective

Differential

Transport

Basic media

These are simple media such as nutrient agar and nutrient broth that will support the growth of microorganisms that do not have special nutritional requirements. ,

They are often used in the preparation of enriched media, to maintain stock cultures of control strains of bacteria, and for subculturing pathogens from differential or selective media prior to performing biochemical and serological identification tests.

### Enriched media

These are media that are enriched with whole blood, lysed blood, serum, extra peptones, special extracts, or vitamins to support the growth of pathogens that require additional nutrients or growth stimulants.

Enriched media are required for the culture of *Haemophilus influenzae*, pathogenic *Neisseria*, and several *Streptococcus species*. Blood agar and tryptone soya media are used to produce a better and more rapid growth of a wide range of pathogens.

Note: The term enrichment is used to describe a fluid medium that increases the numbers of a pathogen by containing enrichments, and, or substances that discourage the multiplication of unwanted bacteria. For example, selenite F broth is used as an enrichment medium for salmonellae in faeces or urine prior to subculturing on xylose lysine deoxycholate (XLD) agar or other enteric selective medium.

## Selective media

These are media which contain substances that prevent or slow down the growth of microorganisms other than the pathogens for which the media are intended. For example, XLD agar selects for *salmonellae* and *shigellae* by containing bile salts that inhibit the growth of many faecal commensals.

In recent years, antimicrobials have become increasingly used as selective agents in culture media. Examples of antimicrobial selective media include modified New York City (MNYQ) medium for isolating *Neisseria gonorrhoeae* from urogenital specimens, and Butzler medium for isolating *Campylobacter* species from faeces.

Selective media are available for isolating most of the important pathogens.

## Differential (indicator) media

These are media to which indicators, dyes, or other substances are added to differentiate microorganisms, for example TCBS agar contains the indicator **bromothymol**. blue which differentiates sucrose fermenting from non-sucrose fermenting

*Vibrio* species.

Most, but not all differential media distinguish between bacteria by an indicator which changes colour when acid is produced following carbohydrate fermentation. Blood agar,

however, can also be described as a differential medium when it differentiates haemolytic from non-haemolytic bacteria.

As shown in the Chart on p. 43-44, many culture media are both differential and selective such as TCBS agar, MacConkey agar, XLD agar and DCA. Enriched media may also be made selective and, or, differential. For example, crystal violet blood agar is an enriched, selective, and differential medium for *Streptococcus pyogenes* (Group A *Streptococcus*).

### Transport media

These are mostly semisolid media that contain ingredients to prevent the overgrowth of commensals and ensure the survival of aerobic and anaerobic pathogens when specimens cannot be cultured soon after collection. Their use is particularly important when transporting microbiological specimens from health centres to the district microbiology laboratory.

Examples of transport media include Cary-Blair medium for preserving enteric pathogens (see p. 405) and Amies transport medium (see p. 402) for ensuring the viability of gonococci and other pathogens in specimens collected on swabs. Other transport media are listed in the Chart on p. 43-44.

### Choice of culture media

The selection of culture media to use in district microbiology laboratories will depend on:



- The major pathogens to be isolated, their growth requirements, and the features by which they are recognized.
- Whether the specimens being cultured are from sterile sites or from sites having a normal microbial flora.

Although a selective medium is usually more expensive than a non-selective one, the use of a selective medium often avoids subculturing, isolates a pathogen more quickly, and makes it easier to differentiate and interpret bacterial growth especially by laboratory staff with limited experience.

- The cost, availability, and stability of different media in tropical and developing countries.
- The training and experience of laboratory staff in preparing, using, and controlling culture media.

Note: Information regarding the preparation and control of culture media can be found in 48: 1.

## SOLID, SEMISOLID AND FLUID CULTURE MEDIA

Culture media can be used in three forms:

Solid

Semisolid

Fluid

## Solid culture media

This form of media is used mainly in petri dishes as plate cultures. It can also be used in bottles or tubes as stab (deep) or slope cultures. The inoculation of plates, slopes, and deeps

When grown on solid media, microorganisms multiply to form visible colonies. Colonial appearances and any changes in the surrounding medium help to identify bacteria and differentiate commensals from pathogens. Some cultures also have a distinctive smell, for example those of *Proteus* and *Pseudomonas aeruginosa*.

## Colonial appearances

Bacterial colonies should be examined in a good light. A low power magnifying lens is required to see morphological details.

When viewed from above, colonies may appear round, irregular, crenated, or branching. They may be transparent or opaque and their surface may be smooth or rough, dull or shiny. The colonies of capsulated species appear mucoid. Mature colonies of pneumococci have a ringed appearance.

When viewed from the side, colonies may appear flat or raised in varying degrees sometimes with bevelled edges or with a central elevation or depression.

When touched with a wire loop, some colonies are soft and easily emulsified such as *Staphylococcus aureus*, whereas

others are difficult to break up such as *Streptococcus pyogenes*.

The colour of colonies also helps to identify bacteria, especially when using differential media containing indicators.

### Medium changes

These include haemolytic reactions, pigment production, colour changes surrounding carbohydrate fermenting colonies, and blackening due to hydrogen sulphide production.

An example of a pigment-forming organism is *Pseudomonas aeruginosa* which produces a yellow-green colour in media such as blood agar and MacConkey agar.

Examples of carbohydrate fermenting bacteria that produce color changes in media include sucrose fermenting *Vibrio cholerae* that gives a yellow colour in TCBS agar, lactose fermenting *Clostridium perfringens* that produces a pink-red color in lactose egg yolk milk agar, and manitol fermenting *Staphylococcus aureus* that gives a

yellow color in mannitol salt agar.

Blackening in the medium due to hydrogen sulphide production is seen with many salmonellae cultured in Kligler iron agar.

Haemolytic reactions in blood agar are seen with beta-haemolytic streptococci and alpha-haemolytic pneumococci.

## Fluid culture media

The growth and multiplication of bacteria in a fluid medium is usually described in four stages, or phases, as follows:

- Lag phase, during which the organisms adjust to their new surroundings.
- Logarithmic phase, during which the bacteria reproduce rapidly. In multiplying, the organisms use up the food substances in the medium and introduce into it toxic products of metabolism.
- Stationary phase, during which there is no further increase in the concentration of living bacteria in the medium. There is a balance reached between the number of bacteria dying and those being produced.
- Decline phase, during which the concentration

of living organisms is reduced as the number of dying bacteria out-number the living bacteria in the medium.

The inoculation of fluid culture media is described in 35:4. Growth is shown by a turbidity in the medium. A surface growth is shown by some organisms, for example vibrios in alkaline peptone water.

Fluid media are used mainly as enrichment media, biochemical testing media, and blood culture media (see Chart at the end of this subunit)

## Semisolid culture media

This form of medium is prepared by adding a small amount of agar (0.4-0.5% w/v) to a fluid medium.

Semisolid media are used mainly as transport media and for motility testing. Examples are given in the following Chart: