# MICRO BIOLOGY

## Welcome to Microbial World:
## Nutrition & Growth

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# MICROBIAL WORLD

- **Microbiology**, study of microorganisms, or microbes, a diverse group of generally minute simple life-forms that include bacteria, archaea, algae, fungi, protozoa, and viruses. The field is concerned with the structure, function, and classification of such organisms and with ways of both exploiting and controlling their activities. The 17th-century discovery of living forms existing invisible to the naked eye was a significant milestone in the history of science, for from the 13th century onward it had been postulated that "invisible" entities were responsible for decay and disease. The word *microbe* was coined in the last quarter of the 19th century to describe these organisms, all of which were thought to be related. As microbiology eventually developed into a specialized science, it was found that microbes are a very large group of extremely diverse organisms deserve attention.

# MICROBES AND MAN

- Daily life is interwoven inextricably with microorganisms. In addition to populating both the inner and outer surfaces of the human body, microbes abound in the soil, in the seas, and in the air. Abundant, although usually unnoticed, microorganisms provide ample evidence of their presence—sometimes unfavorably, as when they cause decay of materials or spread diseases, and sometimes favorably, as when they ferment sugar to wine and beer, cause bread to rise, flavor cheeses, and produce valued products such as antibiotics and insulin. Microorganisms are of incalculable value to Earth's ecology, disintegrating animal and plant remains and converting them to simpler substances that can be recycled in other organisms.

# MAZOR MICROBIAL WORLD

- The major groups of microorganisms—namely bacteria, archaea, fungi (yeasts and molds, algae, protozoa, and viruses—are summarized below. Links to the more detailed articles on each of the major groups are provided.
- **Bacteria- Eubacteria & Archaebacteria**
- Microbiology came into being largely through studies of bacteria. The experiments of Louis Pasteur in France, Robert Koch in Germany, and others in the late 1800s established the importance of microbes to humans. As stated in the Historical background section, the research of these scientists provided proof for the germ theory of disease and the germ theory of fermentation.

# MICROBIAL WORLD

- It was in their laboratories that techniques were devised for the microscopic examination of specimens, culturing (growing) microbes in the laboratory, isolating pure cultures from mixed-culture populations, and many other laboratory manipulations. These techniques, originally used for studying bacteria, have been modified for the study of all microorganisms—hence the transition from bacteriology to microbiology.
- The organisms that constitute the microbial world are characterized as either prokaryotes or eukaryotes all bacteria are prokaryotic—that is, single-celled organisms without a membrane-bound nucleus. Their DNA (the genetic material of the cell), instead of being contained in the nucleus, exists as a long, folded thread with no specific location within the cell.

# BACTERIA

- Until the late 1970s it was generally accepted that all bacteria are closely related in evolutionary development. This concept was challenged in 1977 by Carl R. Woese and coinvestigators at the University of Illinois, whose research on ribosomal RNA from a broad spectrum of living organisms established that two groups of bacteria evolved by separate pathways from a common and ancient ancestral form. This discovery resulted in the establishment of a new terminology to identify the major distinct groups of microbes—namely, the eubacteria (the traditional or "true" bacteria), the archaea (bacteria that diverged from other bacteria at an early stage of evolution and are distinct from the eubacteria), and the eukarya (the eukaryotes). Today the eubacteria are known simply as the true bacteria (or the bacteria) and form the domain Bacteria.

# BACTERIAL WORLD

- The evolutionary relationships between various members of these three groups, however, have become uncertain, as comparisons between the DNA sequences of various microbes have revealed many puzzling similarities. As a result, the precise ancestry of today's microbes is very difficult to resolve. Even traits thought to be characteristic of distinct taxonomic groups have unexpectedly been observed in other microbes. For example, an anaerobic ammonia-oxidizer—the "missing link" in the global nitrogen cycle—was isolated for the first time in 1999. This bacterium (an aberrant member of the order Planctomycetales) was found to have internal structures similar to eukaryotes, a cell wall with archaean traits, and a form of reproduction (budding) similar to that of yeast cells.

# BACTERIA DIVERSITY

- Bacteria have a variety of shapes, including spheres, rods, and spirals. Individual cells generally range in width from 0.5 to 5 micrometers (μm; millionths of a meter). Although unicellular, bacteria often appear in pairs, chains, tetrads (groups of four), or clusters. Some have flagella, external whip like structures that propel the organism through liquid media; some have capsule, an external coating of the cell; some produce spores—reproductive bodies that function much as seeds do among plants. One of the major characteristics of bacteria is their reaction to the Gram stain. Depending upon the chemical and structural composition of the cell wall, some bacteria are gram-positive, taking on the stain's purple colour, whereas others are gram-negative.

# BACTERIA-WIDE RANGE

- The cell walls of all true bacteria contain the chemical substance peptidoglycan, whereas the cell walls of archaeans lack this substance. Many archaeans are noted for their ability to survive unusually harsh surroundings, such as high levels of salt or acid or high temperatures. These microbes, called extremophiles, live in such places as salt flats, thermal pools, and deep-sea vents. Some are capable of a unique chemical activity—the production of methane gas from carbon dioxide and hydrogen. Methane-producing archae live only in environments with no oxygen, such as swamp mud or the intestines of ruminants such as cattle and sheep. Collectively, this group of microorganisms exhibits tremendous diversity in the chemical changes that it brings to its environments

# ALGAE

- The cells of eukaryotic microbes are similar to plant and animal cells in that their DNA is enclosed within a nuclear membrane, forming the nucleus. Eukaryotic microorganisms include algae, protozoa, and fungi. Collectively algae, protozoa, and some lower fungi are frequently referred to as protists (kingdom Protista, also called Protoctista); some are unicellular and others are multicellular.
- Unlike bacteria, algae are eukaryotes and, like plants, contain the green pigment chlorophyll, carry out photosynthesis, and have rigid cell walls. They normally occur in moist soil and aquatic environments. These eukaryotes may be unicellular and microscopic in size or multicellular and up to 120 metres (nearly 400 feet) in length.

# ALGAE

- Algae as a group also exhibit a variety of shapes. Single-celled species may be spherical, rod-shaped, club-shaped, or spindle-shaped. Some are motile. Algae that are multicellular appear in a variety of forms and degrees of complexity. Some are organized as filaments of cells attached end to end; in some species these filaments intertwine into macroscopic, plantlike bodies. Algae also occur in colonies, some of which are simple aggregations of single cells, while others contain different cell types with special functions. The blue green algae shares a very significant position in the microbial world for its unique structure due to their broad range of habitats across all latitudes .The BGA being prokaryotic structure with a variety of thallus organization with 150 genera and more than 2100 species is a great significance to the biologists.

# FUNGI

- The eukaryotic organisms that, like algae, have rigid cell walls and may be either unicellular or multicellular. Some may be microscopic in size, while others form much larger structures, such as mushrooms and bracket fungi that grow in soil or on damp logs. Unlike algae, fungi do not contain chlorophyll and thus cannot carry out photosynthesis. Fungi do not ingest food but must absorb dissolved nutrients from the environment. Of the fungi classified as microorganisms, those that are multicellular and produce filamentous, microscopic structures are frequently called molds, whereas yeasts are unicellular fungi.
- In molds cells are cylindrical in shape and are attached end to end to form threadlike filaments (hyphae) that may bear spores. Individually, hyphae are microscopic in size. However, when large numbers of hyphae accumulate—for example, on a slice of bread or fruit jelly—they form a fuzzy mass called a mycelium that is visible to the naked eye.

# STUDY OF MICROBIAL DIVERSITY

- The study of the biology of microorganisms requires the use of many different procedures as well as special equipment. The biological characteristics of microorganisms can be summarized under the following categories: morphology, nutrition, physiology, reproduction and growth, metabolism, pathogenesis, antigenicity, and genetic properties.
- Morphologically it may be rod, Vibrio, Comma, Spirilum etc,
- On the basis of nutrition, it may be photoautotroph, chemoautotroph, photoheterotrophs, chemoheterotrophs etc,
- On the basis of metabolism, different types of aerobes or anaerobes,
- On the basis of temperature, pH etc, it can be categorized into different groups.

# BACTERIAL NUTRITION

- Microbes like other organisms require life supporting substances from their environment called nutrients. The nutrients are required-
- i. for the formation of structural components like cell wall, cell membrane, protoplasm etc,
- For the synthesis of bio-molecules like proteins, lipids, DNA, RNA etc,
- For catalytic activities,
- To assist the growth and reproduction.
- The nutrients may be- macronutrients and micronutrients or Trace elements like K, Na, Co, Mo, Ni, Cu, Se, Zn etc.
- The transport of the nutrients take place by passive or active process as per the cellular conditions and the energy either obtained from light energy from sun or chemical energy from organic or inorganic compounds.

# NUTRITIONAL GROUPS OF MICROBES

- On the basis of the carbon source, energy source and hydrogen source, microorganisms can be categorized into the following groups-
- Photoautotroph -Light or photon as energy source and $CO_2$ as carbon source,
- Chemoautotroph - Inorganic chemicals as energy source and convert reduced organic materials into unstable food,
- Photoheterotrophs -Use light as energy source and convert reduced organic materials into unstable food,
- Chemoheterotrophs - Use chemical as source of energy as well as absorb some organic chemicals as direct food. Besides these, Photolithographs-light as source of energy and inorganic compounds as electrons source and $CO_2$ as carbon source like Green and Purple sulpher bacteria

# NUTRITIONAL GROUPS IN MICROBES

- Chemolithotrops-They use inorganic compounds as source of electron as well as source of energy, $CO_2$ as carbon source like Sulpher oxidizing bacteria, nitrifying bacteria etc,
- Photoorganotrophs -They use light as source of energy , organic compound as carbon source as well as electrons source like Purple and non-sulpher bacteria,
- Chemoorganotrophs - They use organic compounds as the source of Carbon, energy and electron like fungi and non-photosynthetic bacteria.
- Chemoautotroph may be Sulpher bacteria, Iron bacteria, Nitrifying bacteria or Hydrogen bacteria depending upon the use of inorganic constituents as source of life supporting nutrients for the execution of life processes.

# CULTURE MEDIA

- A culture media is a growth media that consists of all sorts of nutrients for the growth of the organisms-either plant, animal or microbes. The most of the culture media generally contains all sorts of micro and macro nutrients along with hormones if it is designated for plants. The media may be solid or liquid depending upon the requirements.
- TYPES OF MEDIA:
- Media either defined or undefined on the basis of composition. In the defined media or synthetic media, the exact composition is early defined but in the undefined or complex media, the quality of the media is not defined properly; it may contain wide range of nutrients like peptone, beef extract, yeast extract etc like PDA media, Nutrient agar etc.

# MEDIA-BASED ON USE

- General media-Support the growth of many microbes
- Enriched media-Fortified by particular ingredients like blood agar
- Enrichment media-Encourage the preferential growth of desired microorganisms
- Selective media-Growth for the particular type of organisms by inhibiting the other microbes done by the addition of eosin, methylene blue etc
- Differential media-Used to differentiate the microorganisms without selecting the particular type li8ke blood agar to differentiate hemolytic and non-hemolytic bacteria.
- Selective & differential media-Select the first one group of organisms by inhibiting the other.
- Reducing media-Conditions favorable for anaerobes
- Transport media-Transport and storage of specimens

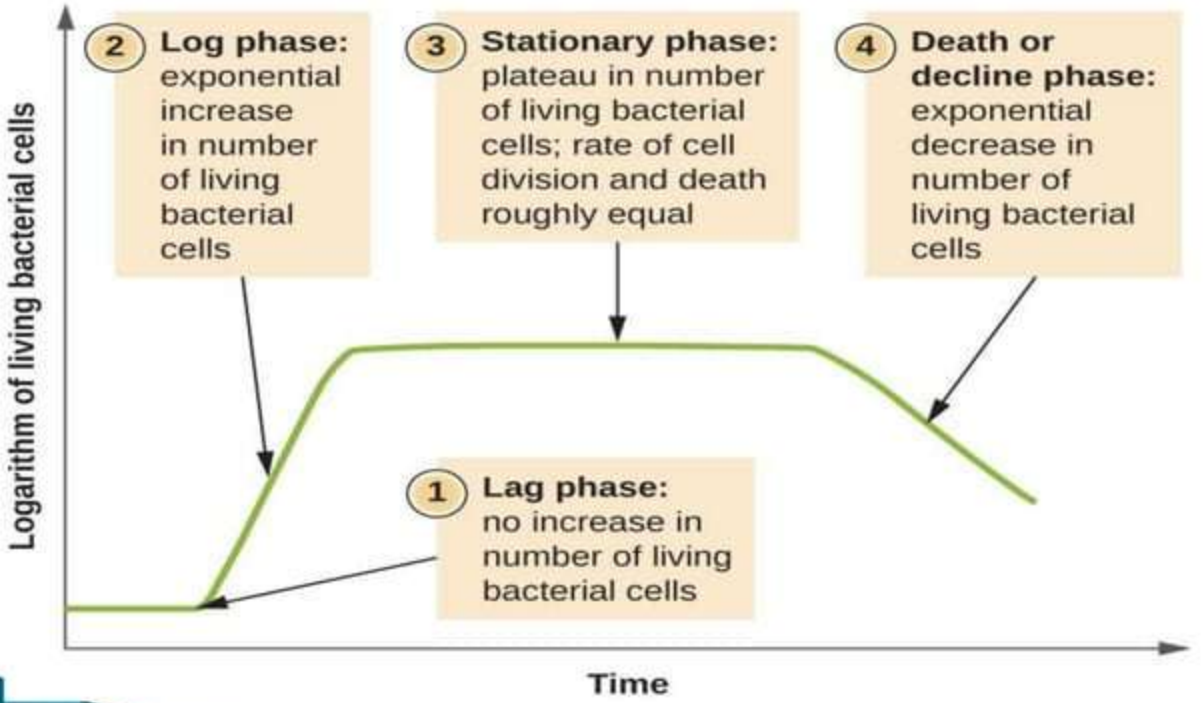
# GROWTH

- Growth is defined as the irreversible positive change of the dry weight of the body of the living organisms mostly when the anabolism dominates over catabolism. But in case of unicellular microorganisms, it refers to increase in the number of population .The number of ways by which the microorganisms increase their cell number and population by the following:
- Binary fission-It is a method of asexual reproduction where the each cell divides symmetrically into two halves to form two daughter cells of almost quantitatively and qualitatively same. The daughter cells also divides by generation time for double it by following the exponential form like 2-4-8-16 ----- Budding-An asymmetric process where a small protuberance is formed (bud) and it receives the cytoplasm and nucleus from the original and pinches off.

# GROWTH PHASE

- FRAGMENTATION- The long filament or a trichome of a multi-cellular organism breaks into fragments and each fragment gives into individual organism.
- SPORE or CONIDIA-Reproductive spore or conidia are produced from the parental organism that gives the birth of new organism.
- The growth of unicellular bacteria with relation to time is studied in a batch culture or closed system imposing the restrictions of different factors. The monoauxic growth shows the following phase as far as the nature of the growth is concerned-
- Lag phase-physiologically active state but no remarkable changes noticed

# DIFFERENT PHASES OF GROWTH



# GROWTH PHASE

- After inoculation into the sterile nutrient medium, the bacterium first undergoes a period of acclimatization. At that time, necessary enzymes and intermediate metabolites are synthesized, thereby bacterium reaches a critical stage before multiplication, multiplication takes place at this stage. The duration of lag phase depends on the type of bacteria, quality of culture medium, size of inoculums and several environmental factors such as $CO_2$, temperature, pH, etc. The average time of lag phase is 2 hours, although it varies from species to species (1-4 hours).
- **2. Log Phase or Exponential Phase:**
- In this phase, the bacteria undergo cell division and their population (number) increase exponentially at a logarithmic rate. The number of viable count, when plotted against time,

# GROWTH PHASE

- gives a straight line of inclined fashion. The average time of log phase is 8 hours, though it varies in different species.
- **3. Stationary Phase:**
- In this phase, the growth i.e., cell division, almost ceases due to exhaustion of nutrients and also the accumulation of toxic products. At this stage the cell death starts at a slow rate and is compensated by the formation of new cell through cell division. The total cell number increases at a slow rate, but the viable count remains almost constant. The duration of this phase is variable which ranges from few days to few hours. Secondary metabolites like antibiotics, toxins etc. are produced in this phase.

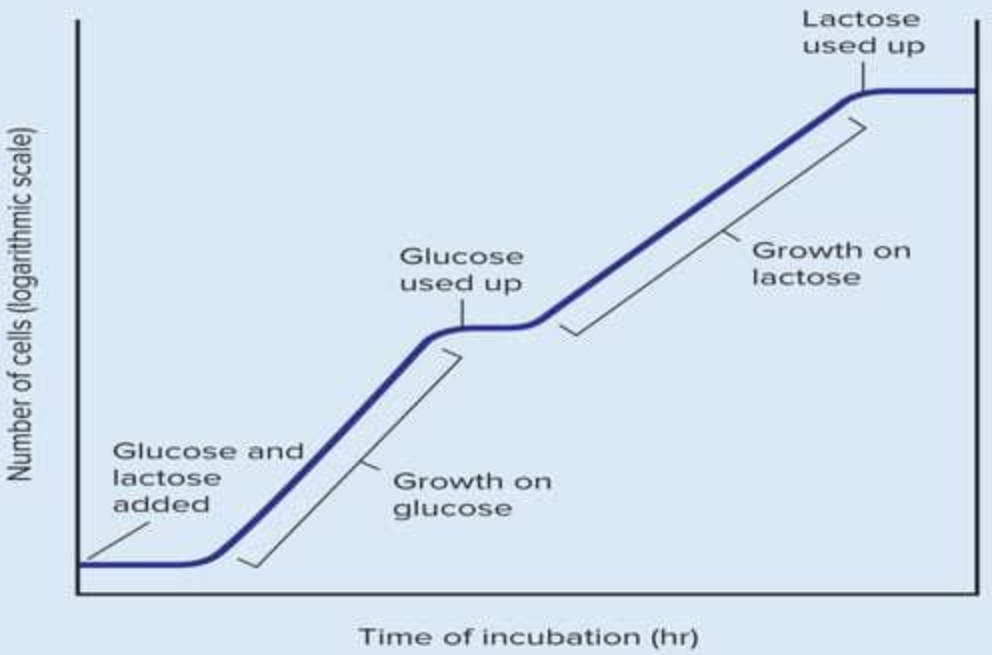
# GROWTH PHASE

- **Decline Phase:**
- In the phase of decline, the total number of cells remains constant, but the number of viable cells gradually decreases due to exhaustion of nutrients and also the accumulation of toxic products. In some cases a few- cells remain viable for long time, even after death of most of the cells. These viable cells probably grow by utilizing nutrients released from dead cells.
- The cells attain maximum size at the end of lag phase and become smaller in log phase (exponential phase). In spore forming species, the sporulation occurs at the end of log phase (exponential phase) or in the early part of stationary phase.

# DIAUXIC GROWTH

- This growth refers of an organism in presence of two substrates where one is preferentially used first and then the next substrate is used. This happens when *E.coli* grows in growth media having glucose and lactose; in this case, glucose is used first followed by lactose after a short lag phase .It is then followed by stationary phase.
- The microbial growth is measured by various methods –
- Cell number by total count or viable count using plate counts or membrane filters
- Measurement of cell mass by dry weight method, Turbidometric method or biochemical analysis of cellular components.

# DIAUXIC GROWTH CURVE



# FACTORS AFFECTING MICROBIAL GROWTH

- Growth of bacteria is affected by many factors such as nutrition concentration and other environmental factors.
- Some of the important factors affecting bacterial growth are:
- Nutrition concentration
- Temperature
- Gaseous concentration
- pH
- Ions and salt concentration
- Available water
- **Nutrient concentration:**
- If culture media is rich in growth promoting substance, growth of bacteria occurs faster. Decrease in nutrient concentration decreases the growth rate.
- Different bacteria have different nutritional requirement.

# TEMPERATURE

- **Temperature:**
- Temperature affects the growth of bacteria by various ways.
- The lowest temperature that allows the growth is called minimum temperature and the highest temperature that allows growth is called maximum temperature.
- There is no growth below minimum and above maximum temperature.
- Below minimum temperature cell membrane solidifies and become stiff to transport nutrients in to the cell, hence no growth occurs.
- Above maximum temperature, cellular proteins and enzymes denatures, so the bacterial growth ceases.

# TEMPERATURE

- On the basis of temperature preference, bacteria can be classified as-
- Psychrophiles - 0-15°C found in Arctic and Antarctica habitats. e.g. *Polaromonas vacuolata*
- Mesophiles - 20 °C -30 °C and maximum at 45 °C , Most human pathogens belong this category,
- Thermophiles - 53 °C -65 °C with max. 80 °C , found in hot springs, most of them are prokaryotic like *Cyanidium caldarium, Mucor pussilis*
- *Hyper thermophiles- Grow at very high temperature with maximum 100 °C , Pyrococcus abyssi & Pyrodictium occultum*

# pH

- pH is the indicator of the concentration of Hydrogen ion in the media and is the negative logarithm of hydrogen ion concentration. It ranges from 0-14, value less than 7 indicates acidity and above 7 indicates basic nature of the media. With few exceptions, most of the bacteria prefer to grow in neutral pH( 6.5-7.5). Depending upon the requirements of hydrogen ion concentration, it can be grouped as-
- Acidophilus - Grow in very acidic conditions like *Sulphodes acidiocaldaius*
- Basophiles – Grow in between 8.5-11.5 with optimum value 10, *Bacillus alkyophilus*
- Neutrophiles – in between 5.5-8.0 , like *Staphylococcus aureus.*

# OXYGEN CONCENTRATION

- On the basis of oxygen availability during respiration. Microorganisms can be categorized into 5 categories-
- Obligate aerobes – Oxygen is obligatory for all metabolic activities, Ex. *Micrococcus luteus*
- Microaerophiles – 2-10% oxygen conc. Is required but more than is detrimental, Ex. *Campylobacter s*p.
- Facultative anaerobes – Grow both in the presence and absence of oxygen to become aerobes or anaerobes like E.coli.
- Aero tolerant anaerobes – Although they do not use oxygen but can grow in the presence of oxygen *like Lactobacillus plantarum,*
- Obligate anaerobes- Grow in the strict absence of oxygen like *Clostridium, Methanococcus* etc.

# OTHER FACTORS

- **Ions and salt:** All bacteria requires metal ions such as K+, Ca ++, Mg++, Fe++, Zn++, Cu++,Mn++ etc to synthesize enzymes and proteins. Most bacteria do not require NaCl in media however they can tolerate very low concentration of salt. There is some halophilic bacteria such as *Archeobacteria* that require high concentration of salt in media.
- **Available water:**
- Water is the most essential factor for bacterial growth.
- Available water in the culture media determines the rate of metabolic and physiological activities of bacteria.
- Sugar, salts and other substances are dissolved in water and are made available for bacteria.

# CONCLUSION

- The bacteria can grow in the diverse condition as stated earlier but the growth can be controlled by applying different methods for the sake of the diverse purposes. Sterilization. Disinfection, Sanitization and antiseptic are the some of the methods used in this regard. Different physical agents like heat treatment by moist heat( Boiling, Tyndalization, Pasteurization, Autoclaving), dry heat ( Incineration, hot air sterilization, heating in oil), Radiation ( Non- ionising, ionizing, Microwave), Filtration; Chemical mewt5hods like use of alcohol, phenols, halogens, heavy metals, aldehydes, peroxides, Gases etc are used for the control of the growth of the microorganisms as well as manipulated for extraction of the different products required for the welfare of human beings.

# THANK YOU FOR YOUR PATIENCE TO STAY WITH