AGM.101.FUNDAMENTALS OF MICROBIOLOGY

LECTURE NOTES

BY

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AGM 101 FUNDAMENTALS OF MICROBIOLOGY

Credit Hours : 1+1

Semester : Second

Syllabus (Theory and Practicals)

THEORY

Definition and scope of Microbiology - Spontaneous generation theory - Germ theory of diseases - Contributions of Antonie Van Leeuwenhoek, Louis Pasteur, John Tyndall, Robert Koch, Edward Jenner, Joseph Lister, Alexander Fleming and Waksman. Microscopy : Principles-Different types of microscopes: dark field, phase contrast, fluorescence, ultra-violet and electron microscope. Sterilization: Principles and equipments. Structure and organization of microbial cell- Prokaryote and eukaryote – Staining of microorganisms- principles. Morphology of bacteria, fungi, algae and viruses. Nutritional types in bacteria-nutritional requirements – growth curve-fermentation–respiration in bacteria-factors influencing the bacterial growth. Classification of bacteria (outline classification only). Genetic elements in bacteria- mutation in bacteria – conjugation – transformation – transduction. Immunology -basic principles.

PRACTICAL

Microscopes– Micrometry – Sterilization techniques and equipment – Growth media preparation – Bacteria, fungi and actinomycetes - Isolation, purification and preservation of bacteria and fungi. Staining techniques: Simple, differential and structural staining - spore staining - Measurement of bacterial growth – Identification of microorganisms: Cultural, physiological and biochemical tests for bacteria

LECTURE SCHEDULE

1. Definition and scope of microbiology-Spontaneous generation theory.
2. Contributions by Antonie Van Leeuwenhoek, Louis Pasteur, John Tyndall, Joseph Lister, Edward Jenner, Robert Koch, Alexander Fleming and Waksman.
3. Microscopy : Principles – resolution – numerical aperture, magnification-Different types of microscopes-Light microscopes- UV, dark field, phase contrast, fluorescence and electron microscope.
4. Sterilization – Principle-Physical and chemical methods.
5. Groups of microorganisms-Prokaryotes and eukaryotes.
6. Bacterial morphology- Staining of microorganisms – basic principles - arrangement of cells, structures and reproduction.
7. Morphology of algae and fungi.
8. Viruses-Bacteriophages.
9. Mid semester examination.

10. Nutritional types of bacteria-Autotrophs, heterotrophs, phototrophs and chemolithotrophs.
11. Bacterial growth-growth curve - conditions for growth; temperature requirements- aerobes and anaerobes..
12. Classification of bacteria - Bergey's manual of systematic bacteriology –outline only.
13. Microbial metabolism-fermentation-
14. Respiration - phosphorylation
15. Genetic elements in bacteria – mutation.
16. Conjugation, transformation, transduction.
17. Immunology – principles

PRACTICAL SCHEDULE

1. Microscopes – handling light microscope.
2. Micrometry-measurement of microorganisms.
3. Sterilization – equipment and apparatus used for sterilization.
4. Media preparation for bacteria, fungi and actinomycetes.
5. Isolation of microorganisms-serial dilution plate technique.
6. Purification and preservation of bacteria
7. Purification and preservation of fungi.
8. Staining techniques- simple and negative staining.
9. Differential staining - Gram-staining
10. Structural staining - spore staining.
11. Morphology of bacteria- cell arrangement.
12. Growth of bacteria- turbidometric method.
13. Physiological characters of bacteria.
14. Biochemical characters of bacteria.
15. Identification of bacteria.
16. Identification of fungi.
17. Practical examination.

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2. Madigan, M.T., Martinko, J.M. and Parker, J. 1997. Brock Biology of Microorganisms, 8th Edn. Prentice-Hall Inc. NJ.
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4. Pelczar, M.J., Chan, E.C.S.and Kreig, N.R. 1993. Microbiology. Tata McGraw Hill Publishing Co., Ltd., New Delhi.
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6. Schlegel, H.G. 1993. General Microbiology. Cambridge University Press, Cambridge.
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Lecture 1 Definition and Scope of Microbiology

Microbiology is the study of living organisms which are if microscopic in size viz. fungi, bacteria, algae, protozoa and viruses. It is concerned with their form, structure, reproduction, metabolism, taxonomy, distribution and their interaction with other groups of living organisms.

What are microorganisms?

Microorganisms are unicellular and that means, all life processes are performed by a single cell, unlike the cells of higher organisms in which the cells are arranged in tissues and organs and perform specialized functions.

Irrespective of the size of the living organisms, all living cells are fundamentally similar. When we talk about cell, we have to remember the man who aimed that name Robert Hooke (1635-1703).

Then it was two Germans Schleiden and Schwann who in 1838-1839 described cells as the basic structural and functional units of all organism and they proposed the cell theory.

The cell theory states that

1. Cells are the structural and functional unit of all living organisms.
2. Cells carry out the metabolic activities.
3. All cells arise from the pre existing cells.

Due to their size, microorganisms offer then as classical and ideal models for the study of numerous fundamental life processors. This is possible because at the cellular level many whether they be microbes or mouse or human being for *eg.* If Glucose is supplied as energy source it has to be assimilated in the cells by a series of biochemical conversion which are catalyzed by the enzymes. Glycolysis is one such biochemical pathway in which Glucose is converted to pyruvic acid by the action of different enzymes at different stages. The whole process is same in a microbial cell, mouse cell and human cell.

Microorganisms are exceptionally attractive models for studying various life processes. They can be conveniently grown in test tubes or flasks, then requiring less space and maintenance than larger plants and animals. They grow and reproduce at unusually rapid rate.

We can study the life processes white they are actively metabolizing by altering then environment we can alter then metabolic activities and even then genetic pattern can be altered, all without destroying the organisms.

Microorganisms have a wider range of physiological and biochemical potentialities then all other organisms. Some bacteria are able to utilize atmospheric nitrogen, some are able to perform photosynthesis where as certain other organisms need carbon or nitrogen.

The importance of microorganism and the scope of studying about then can be easily understood by speech of Selman A Waksmann the Nobel laureate, which he delivered in 1942 in the American society of Microbiology.

"There is no field in human endeavor, whether it be industry or agriculture, or in the preparation of food or in connection with problem of shelter or clothing or in the conservation of human or animal health and the combating of disease where the microbe dies not play an important and often dominant role"

The Scope of Microbiology:

It may be clearly understood from the words of Waksmann the scope that is offered by the studying microorganisms.

To elaborate it little further the study of microorganism *i.e* microbiology we can see their roles played by them in fields of Agriculture, Medicine, Biotechnology, and Industries.

Agriculture: Microorganisms play a major role in the field of Agriculture.

The help in soil formation, soil aggregation. Organic matter decomposition, nitrogen fixation, phosphorous mobilization, nutrient cycling-Agricultural microbiology.

Medicine: Antibiotics, Vaccines, Medical Microbiology.

Biotechnology: Insulin, Hormones, Monoclonal antibodies, disease resistance.

Industries: Beverages, Amino acids, Enzymes, Food processing, mining, Energy, Pollution control.

Lecture 2 History of Microbiology

ANCIENT MICROBIOLOGICAL HISTORY

Ancient man recognized many of the factors involved in disease. Early civilizations on Crete, India, Pakistan and Scotland **invented toilets** and **sewers**; lavatories, dating around 2800 BC, have been found on the Orkney islands and in homes in Pakistan about the same time. One archaeologist has stated that "***The high quality of the sanitary arrangements [in ~2500 BC] could well be envied in many parts of the world today***". In Rome, 315 AD, the public lavatories were places where people routinely socialized and conducted business. Ten to twenty people could be seated around a room, with their wastes being washed away by flowing water; it must have been difficult to "***stand on your dignity***" under such circumstances. The Chinese used TOILET PAPER as early as AD 589. In Europe moss, hay and straw were used for the same purpose. I can personally attest to the use as late as 1962 of "slick magazines" as toilet paper in certain European camp grounds.

The first cities to use water pipes (of clay) were in the Indus Valley of Pakistan around 2700 BC. Metal water pipes were used in Egypt (2450 BC) and the palace of Knossos on Crete around 2000 BC had clay pipes. Rome built elaborate aqueducts and public fountains throughout its empire to insure a clean supply of water for its citizens. Rome had a "WATER COMMISSIONER" who was responsible for seeing that the water supply was kept adequate and clean; the punishment for pollution of the water supply was **DEATH**. Lead was commonly used for Roman pipes and the subsequent fall of the Roman empire has been related by some to the effects of lead on the Roman brain.

Most ancient peoples recognized that some diseases were **communicable** and isolated individuals thought to carry "infections". An example of this is the universal **shunning of lepers**, which occurs even today. When the Black Death struck Europe, entire villages were abandoned as people fled in an effort to escape the highly infectious plague. Similarly, in the Middle Ages the rich of Europe fled to their country homes when small pox struck in an effort to escape its terrible consequences. The fact that people who recovered from a particular disease were immune to that disease was probably recognized many different times in many places. Often

these survivors were expected to nurse the ill. Greek and Roman physicians routinely prescribed diet and exercise as a treatment for ills.

Sadly, we know that this knowledge did not help most of our ancestors and that the human life span was, until the last 200 years, more often than not cut short due to infectious disease. Even today approximately **15,000,000 CHILDREN DIE PER YEAR**, mainly from infectious diseases that are **preventable** with basic sanitation, immunization and simple medical treatments. One might honestly question just how far we have come in our treatment of disease. An excellent synopsis of the history of Microbiology (also of chemistry and general biology) can be seen by visiting this site.

Ancient people had certainly seen masses of microbes, such as mold and bacterial colonies, on spoiled food, but it is doubtful if anyone considered that they were VIEWING living organisms. Small boys and maybe a few love-sick adults staring into a clear pond, must have seen tiny specks moving rapidly about and some may have considered them living creatures, but to express this to their friends would be equivalent to us telling our friends that we'd seen a flying saucer.

The first person to report seeing microbes under the microscope was an Englishman, **Robert Hooke**. Working with a crude compound microscope he saw the cellular structure of plants around 1665. He also saw fungi which he drew. However, because his lens were of poor quality he was apparently unable to "see" bacteria. Using the dissecting microscope in laboratory exercises # 1 and 2 you see fungi at a magnification similar to that seen by Hooke, but without the distortion of poor lenses.

THE FIRST MICROBIOLOGIST

Figure 1. Anton van Leeuwenhoek. A classical example of serendipity. By wanting better magnifying lens with which to judge the quality of the cloth he was buying Leeuwenhoek discovered bacteria.

Anton van Leeuwenhoek was a man born before his time. Although not the FIRST TO DISCOVER THE MICROSCOPE or to use magnifying lens, he was the first to see and describe bacteria. We know that he was a "cloth merchant" living in Delft Holland. and that he used magnifying lens to view the quality of the weave of the merchandise he purchased. He traveled to England in 1668 to view English cloth and there he saw drawings of magnifications of cloth much greater than any of the current lens available in Holland would do. He returned to Holland and took up lens grinding. Being meticulous, he developed his lens grinding to an art and in the process tested them by seeing how much detail he could observe with a given lens. One can guess that he chanced to look at a sample of pond water or other source rich in microbes and was amazed to see distinct, uniquely shaped organisms going, **apparently purposefully**, about their lives in a tiny microcosm. He made numerous microscopes from silver and gold and viewed everything he could including the scum on his teeth and his semen. His best lens could magnify ~**300-500 fold** which allowed him to see microscopic algae and protozoa and larger bacteria. He clearly had excellent eyesight because he accurately drew pictures of microbes that were at the limit of the magnification of his lens. He used only SINGLE LENS and not the compound lens of the true microscopes we employ today; which makes his observations all the more amazing. He wrote of his observations to the Royal Society of London in 1676 and included numerous drawings. He astonished everyone by claiming that many of the tiny things he saw with his lens were ALIVE because he saw them swimming purposefully about. This caused no end of shock and wonderment and numerous people hurried to Delft to see if this Dutchman was "in his cups" or if he was really onto something new and wonderful. A few minutes with one of his numerous

microscopes was all it took to convert his visitors to enthusiastic believers in the existence of these tiny ***beasties*** living all around them. His discovery was the equivalent of our finding life on Mars today. For more information visit this site.

Robert Hooke was the first person to propose the CELLULAR forms of life. Visit this site and view Hooke's microscope. Another great site for learning about all kinds of microscopes is here.

- In the first two laboratory exercises you will be learning to use the microscope. When you use the 40X high dry lens to view the various pond water samples you will be seeing the microbial world much as Leeuwenhoek did (40 x 10 = 400X). Imagine the reactions of Leeuwenhoek's friends and family when he told them of the wonders he had seen. Would you have the strength of character to withstand the derisive comments and to continue your investigations had you been him? Scientists who present new perspectives and data that fly in the face of conventional wisdom often face ridicule and derision from their fellow scientists and the community at large. Ask your associates/friends what they think of "global warming" and the "hole in the ozone" and the scientists that propose these theories.

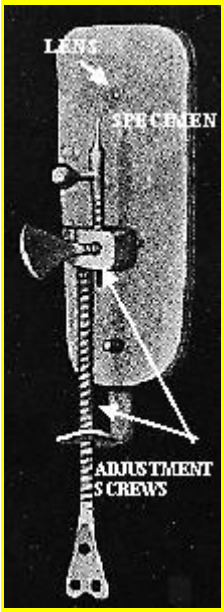


Figure 2. A copy of Leeuwenhoek's microscope. Since his scopes were made of gold and silver his family sold them after he died. This copy was made from descriptions of his microscopes. The specimens were placed on the point and adjusted so they lay in front of the tiny lens. The specimen was placed so that a beam of sunlight passed through it and the viewer looked through the tiny lens at the illuminated material. Compare this with your laboratory scope.

Many scientists and *trendy* high society people visited him to view his "little **animalcules**". including the Czar of Russia and other European royalty. He treated everyone the same and refused to even let them touch his scopes, rather he would prepare a sample and allow the visitor to come in and look briefly through the lens. He was a superior observer and an excellent scientist except for the **CRUCIAL FLAW** of not allowing others to copy his techniques and

VERIFY his results. Because of this and the failure of people to relate these tiny microbes with disease, it was another 200 years before the science of Microbiology really took off.

THE CLEAN NUT AND THE REVENGE OF THE BACTERIA

In the 1800s people (mainly the poor) began to use hospitals. Hospitals also became centers of physician training. In 1841 (30 years before the GERM THEORY of disease was established) young doctor **IGNAZ SEMMELWEIS** was hired to run a maternity ward in a Vienna hospital. There were two birthing wards in his preview, one run by midwives and the other by doctors. Semmelweiss noticed that the death rate among mothers in the doctor's ward ran as high as 18% from the blood infection (of a #streptococcus or STREP) known as CHILD BED FEVER or **PUERPERAL SEPSIS**, whereas in the midwife ward the death rate was much lower. When he suggested that the doctors might contribute to this he was **fired**. Subsequently rehired, he saw a friend die from PUERPERAL SEPSIS after cutting himself during an autopsy of a patient who had died of PUERPERAL SEPSIS. He reasoned that there was an **INVISIBLE AGENT** that caused both deaths and that one could transfer it from the autopsy room to the birthing rooms and thus infect the mothers during birthing. Acting on this assumption, Semmelweis instituted sanitary measures which included having the doctors wash their hands in disinfectant and change from lab coats ***dripping with pus and blood*** from the autopsy room to ***clean lab coats*** before examining patients or assisting in a birth. The death rate of the mothers dropped by 2/3 in his ward. However, the other doctors objected so strongly to his rules that Semmelweis was again **fired** and left Vienna. He took other hospital jobs where he instituted the same standards of cleanliness which resulted in the same decline in deaths and a revolt of his fellow physicians. Semmelweis ended up dying in an insane asylum from a blood infect that resembled puerperal sepsis (bacterial revenge?). COULD SOMETHING LIKE THIS HAPPEN TODAY in the treatment of disease?

SPONTANEOUS GENERATION

The mystery of life has puzzled and confounded humans since the first human began to contemplate his world. The religions of ancient societies were built around the seasons, the sun and the renewal of life as these were so clearly tied to survival; both of the human species through birth and death, and of the individual in the attainment of sustenance (#ENERGY). **SPONTANEOUS GENERATION** or the idea that life routinely arises from non-life was a **COMMON SENSE** explanation of the miracle of life. It had the advantage of simplicity, ease of understanding and didn't require any waste-of-time thinking. As science and the scientific method grew with the slow accumulation of knowledge, observant individuals began to consider the origin of life more deeply. Simple observations convinced many people that all the larger animals and plants produced life from previous life. Despite this, the mass of humans clung to the comfortable idea of SPONTANEOUS GENERATION Further, religions saw it as a convenient way to demonstrate the hand of God operating continuously in the WORLD. Some individuals, such as J.B. van Helment even described how one could make mice from grain, a jar and dirty rags by putting them together in a dark place for a few weeks and soon mice would appear in the jar. Other, more perceptive individuals, like **F. Redi** tested the common idea that maggots arise via SPONTANEOUS GENERATION on rotting meat. He placed a piece of meat in three jars, one he left open, one he corked tightly and the third he covered with a fine mesh gauze. Maggots only appeared in the open container, no matter how long he left the jars.

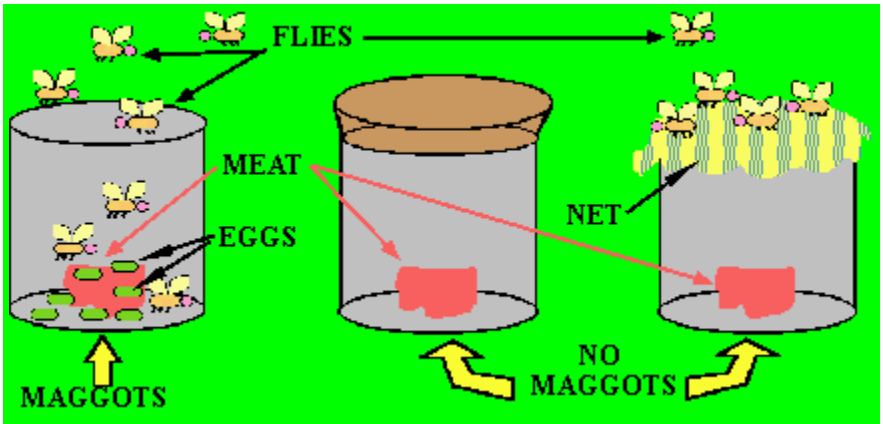


Figure 3. Redi's experiment proving maggots are not spontaneously produced in rotten meat.

Redi's experiment was important because of its eloquent simplicity. Anyone could repeat it and obtain the same clear results. Nevertheless, many people clung fiercely to the idea of SPONTANEOUS GENERATION, while others designed experiments to test it. In every case the

results of the majority of these experiments indicated that SPONTANEOUS GENERATION did not occur. The intellectual ferment this controversy stirred up gradually evolved into the #SCIENTIFIC METHOD as the various antagonists questioned each other's assumptions and, more importantly, their **experimental design**. These arguments forced the designing of **BETTER EXPERIMENTS** (with good #CONTROLS) and eventually persuaded all but the most recalcitrant believers to discard SPONTANEOUS GENERATION as an explanation for all higher life forms. Then, in the 1800's the refinement of the microscope, through which people could see tiny life forms that they assumed were SIMPLE, gave SPONTANEOUS GENERATION proponents new life.

Again, flawed COMMON SENSE led reasonable people astray. As the existence of microscopic life was accepted, the assumption was that such life must be **SIMPLE** compared to higher, more **COMPLEX** life. The reasoning that followed this erroneous assumption was that since the microbes were ***small*** they must be ***simple*** & it followed that they were formed by SPONTANEOUS GENERATION, hence God was still at work creating micro-life. As we shall see in later discussions, small is not simple! The battle over SPONTANEOUS GENERATION raged anew both from the pulpit and the lab. A number of scientists performed elementary experiments in which they treated soups and broth's, which left unheated would team with microbes after a few days, with heat to destroy any life present in them and asked the question: "***Would new life arise in these sterile soups***"? Spallanzani boiled "soup" in glass containers and melted the glass closed. The observation that nothing subsequently grew in this "heated" soup suggested that SPONTANEOUS GENERATION didn't work. His detractors, rightly criticized his experiments, proposing that since air is necessary for life and since he had sealed the flask to air, obviously NO LIFE could develop. Others boiled soups and microbes grew, thus apparently supporting SPONTANEOUS GENERATION. But again the preponderance of data suggested that SPONTANEOUS GENERATION did not even apply to the "***simple***" microbes.

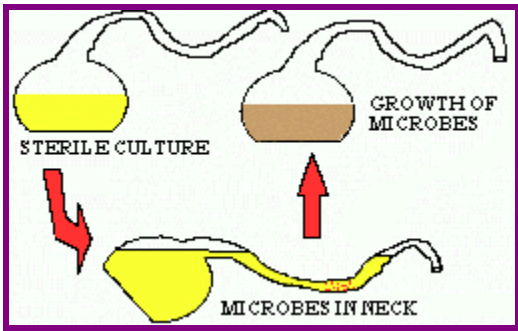


Figure 4. Pasteur's swan-neck flask experiment. Pasteur's **SWAN NECK** flasks put the nails in the coffin of spontaneous generation. The experiment was simple, & easily repeatable by anyone with modest means & the result unequivocal. However, it is important to note that few scientific experiments are this straight-forward.

In 1859 one of the fathers of modern microbiology, **L. Pasteur** (picture and short biography) decided to settle the question of SPONTANEOUS GENERATION once and for all. A genius at devising definitive experiments, Pasteur first drew the necks of glass flasks out so that they remained open to the air, but were bent so that air could only enter by a ***curved path.*** He then added broth and boiled it to destroy contaminating microbes. These flasks were then incubated and observed for months. He reasoned that the microbes in the air that could contaminate the sterile broth would be trapped on the sides of the thin glass necks ***before they reached the sterile broth***. If SPONTANEOUS GENERATION didn't occur no growth should take place. This is exactly what happened, the flasks remained sterile indefinitely, until Pasteur tipped the sterile broth up into the curved neck where he predicted the airborne organisms would have settled. After doing this the broths ***ALWAYS GREW MICROBES***. These experiments ended the SPONTANEOUS GENERATION controversy because these experiment was so elegant and simple, and the results so clear, that anyone could repeat them.

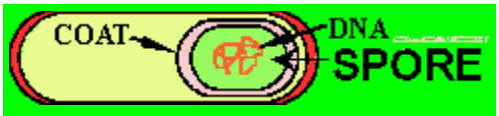


Figure 5. Spore structure.

Later, an earlier problem, in which occasional heated-broths did not remain sterile, was explained with the discovery of the heat resistant bacterial #SPORES , some of which could can survive several hours of boiling without being killed.

Pasteur discovered many of the basic principles of microbiology and, along with R. Koch, laid the foundation for the science of microbiology. In 1857 Napoleon III was having trouble with his sailors mutinying because their wine was spoiling after only a few weeks at sea. Naturally Napoleon was distraught because his hopes for world conquest were being scuttled (pardon the

pun) over a little spoiled wine, so he begged Pasteur for help. Pasteur, armed with his trusty microscope, accepted the challenge and soon recognized that by looking at the spoiled wines he could distinguish between the contaminants that caused the spoilage and even predict the taste of the wine solely from his microscopic observations. He then reasoned that if one were to heat the wine to a point where its flavor was unaffected, but the harmful microbes were killed it wouldn't spoil. As we are aware this process, today known as **PASTEURIZATION**, worked exactly the way he predicted and is the foundation of the modern treatment of bottled liquids to prevent their #spoilage. It is important to realize that pasteurization is NOT the same as sterilization. Pasteurization only kills organisms that may spoil the product, but it allows many microbes to survive, whereas **STERILIZATION** kills all the living organisms in the treated material.

Pasteur also realized that the **yeast** that was present in all the #wine produced the alcohol in wine. When he announced this, a number of famous scientists were enraged, because the current theory of wine production was that wine formation was the result of **SPONTANEOUS** chemical changes that occurred in the grape juice. Pasteur was attacked furiously at scientific meetings, to the point where certain scientists did humorous skits about Pasteur and his tiny little yeast "stills" turning out alcohol. Pasteur had the last laugh however as people all over the world soon realized that if he was right they could control the quality of wine by controlling the yeast that made it. In a short period many others verified his observations and the opposition sank without a sound.

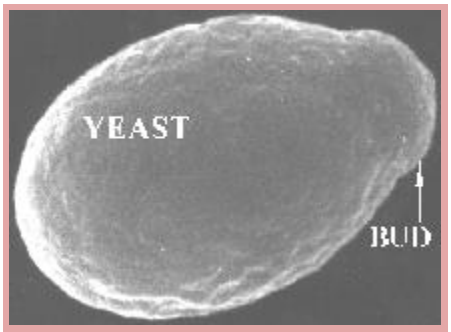


Figure 6. Budding yeast cell. Scientists who disagreed with Pasteur about the origin of ethanol in wine drew stills inside of the tiny yeasts to spoof his

idea. Pasteur also founded #MODERN IMMUNOLOGY when he realized that chickens became immune to a bacterial disease if injected with a "**weakened**" (avirulent) form of the pathogenic bacterium. While investigating chicken cholera, he injected some chickens with an old culture of the bacterial pathogen. When the chickens didn't die he realized that the culture was no longer pathogenic, perhaps because it had died, so he repeated the experiment with a fresh culture. Again he injected a number of chickens with lethal doses of a virulent culture, but only ***some*** chickens died. In questioning his technician about the source of the chickens, he learned that those chickens that ***didn't*** die when they were injected with the new, live, virulent culture were ones that had been previously injected with the "***OLD, SICK***" culture. For more information on Pasteur visit this site.

In one of those flashes of genius that can change the course of history Pasteur realized that the **OLD CULTURES** had **IMMUNIZED** the chickens. He reasoned that under adverse conditions a virulent microbe may lose its ability to produce a disease but still retain its ability to immunize the exposed host. He went on to produce vaccines against a number of scourges including anthrax and rabies using this approach. Still today, most vaccines are made with a modification of this general technique. Only with the rise of molecular biology are we developing newer methods of vaccine production and #immunization. Lately it has been discovered that Pasteur didn't quite tell the whole truth about some of his data as he was under terrific pressure to show successful results. Do you think this is a problem in science today?

A case of the "Microbes Revenge": During his investigation on bacteria two of Pasteur's daughters and his father died of #Typhoid fever.

ROBERT KOCH

In the late 1870s a country physician, **R. Koch** became interested in anthrax, a common disease of both the farmers and their animals in his rural practice. Using a microscope purchased with his meager funds, Koch saw a large bacterium in the blood of anthrax victims. He reasoned that it might be the agent of the disease, but he knew that as a ***hick country doctor*** he would have difficulty getting such a controversial proposal accepted. Using a closet at home as his lab and developing basic microbiological techniques as he proceeded, Koch painstakingly teased out the

anthrax bacterium and purified it. He then inoculated the purified bacteria into healthy animals and produced the classical clinical disease. When he examined the blood of the inoculated animals he was able to re-isolate the same bacterium. He repeated the isolation, infection and disease cycle until he was certain he had found the agent of anthrax. Because it was such an important commercial disease and because his techniques could be easily duplicated, others quickly verified his findings and Koch became famous. He soon had his own institute (like Pasteur) and other discoveries soon followed. Koch attracted other bright scientists and together they (along with Pasteur's group) developed the basic techniques of microbiology labs we still use today. These include the sterile culture techniques, pure culture techniques, the use of petri plates, inoculation needles, solid medium, the use of agar and gelatin to produce a solid surface, the Gram stain and other staining procedures. In addition Koch discovered the etiological agents of #cholera, and #tuberculosis. His studies, in combination of those of Pasteur's, established the **GERM THEORY** of disease. His procedure for defining the agent of any disease, called **KOCH'S POSTULATES**, consists of the following 4 steps.

 FIRST, **isolate** the suspected agent from a disease victim.

 SECOND, grow the agent in **pure culture**.

 THIRD, infect a healthy host and show that the organism produces the **CLASSICAL CLINICAL DISEASE**.

 FOURTH, **ISOLATE** the "**same**" organism from the new victim.

In general we still follow these dictates today (Visit this site). However, there are cases where it is not possible to do this. For example, many viral diseases of man (e.g. #HIV) do not infect other species, even our close primates, so it is not possible to carry out steps 3 and 4. In other case such as #syphilis, leprosy and the agent of #genital warts it has been difficult if not impossible to grow the organism as an isolated ***in vitro*** (= in the test tube) culture, which severely limits our ability to study the organism. For more on Koch visit this site.

MICROBIAL SERENDIPITY: Until recently, few women have played a significant role in microbiology, however this young science owes the development of a **crucial technique** to a woman. Fanny Angelina Eilshemius was born in 1850 in New York of a wealthy Dutch immigrant family. As a young girl, Angelina toured Europe where she met a young German doctor, Walther Hesse, whom she married in 1874. Angelina Hesse settled down to being the wife of a busy country physician. W. Hesse, became interested in the new science of Microbiology and joined Koch's lab in 1881. Dr. Hesse studied many aspects of bacterial public health and bacterial metabolism . His wife, nicknamed Lina, assisted him, much like my wife does me, as a laboratory technician. She was a talented artist who drew illustrations for his publications. One hot summer when Walther was attempting to do counts on bacterial air contaminates he was having trouble with his **GELATIN** (what we call **Jell-O**) plates melting in the heat and being digested by many of the bacteria he tried to grow on them. In frustration, he asked his wife "***Why do your jellies and puddings stay solid in the warm weather?***". She explained to him that she used **AGAR-AGAR**, a complex polysaccharide extracted from seaweed, to keep them solid in hot weather. AGAR-AGAR had been used as a ***gelling agent*** in ASIA for centuries. She had learned of it as a youngster in New York from a Dutch neighbor who had immigrated from Java. Presumably Dr. & Mrs. Hesse discussed the possibility of using agar-agar to prepare microbial media and Dr. Hesse subsequently found that it worked beautifully. The following characteristics of AGAR-AGAR make it almost perfect for the growth of microbes on solid medium:

- (a) non-toxic to most microbes.
- (b) only melts at 100^{o}C, but solidifies at about 45^{o}C (a temperature most bacteria can survive).
- (c) nontoxic to other forms of life.
- (d) stable to sterilization temperatures.
- (e) physiologically inert as very few bacteria have the #enzymes for digesting it.

This kitchen ingredient revolutionized the science of microbiology as it made what had been an arduous task of separating and growing microbes on solid surfaces a routine procedure. Dr. Hesse went on to make other important bacteriological discoveries and advances, such as bringing the pasteurization of milk to Germany; which prevented the death of children from TB-

and milk contaminated with intestinal pathogens. Angelina died in 1934, ***when I was one year old***, so you see how NEW the science of microbiology is? Ref.: ASM News 58:425 (1992).

E. JENNER

Smallpox was one of the greatest scourges of mankind. For thousands of years it swept through human populations, killing up to 40% of its victims and leaving many of the survivors horribly scarred for life; their faces covered with deep red pits. Because of the propensity of the male to appreciate females with healthy complexions, many a beautiful young woman became a ***pox-scarred spinster*** because no man would marry them. The ancient Chinese recognized that those who recovered from a case of "the pox" were **IMMUNE** to smallpox. Some unknown person in CHINA, perhaps noting that even people who only developed a few scabs were as immune as those whose bodies were covered with scars, took material from a dried scab and scratched it into an uninfected person in an attempt to **IMMUNIZE** them. It worked and the process was repeated by others, with the technique eventually reaching India. From India it traveled by various routes to Europe in the 17th century where it came into common usage. The only problem was that the scab from a victim contains fully #virulent virus capable of producing the clinical disease. Thus, while one person might have only a mild case and become immune, they shed the virulent virus and were capable of starting their own #epidemic. Whereas the next person inoculated with the same scab-material could just as easily develop a **FULL BLOWN CASE** and die in agony.

This was the situation when Edward Jenner entered the picture. Through a series of serendipitous events, Jenner was led to the discovery of immunization and to the eventual elimination of the scourge of smallpox from the earth. As a young man he had lived in the country and had been told by a milkmaid that "***she never had to worry about catching smallpox because she had had "cowpox***", a mild chronic disease of cows that milk-maids usually contracted as a rash on their hands. Later, after Jenner became a physician and took up a country practice, he remembered the milkmaid's story. He began asking questions and was told by local men that "***if you want to marry a woman who will never be scarred by the 'POX', marry a milkmaid***". By 1796 he became convinced that the story was true so he inoculated an 8-yr. old boy with cowpox and 8

weeks later inoculated the same boy with the pus from a smallpox lesion. The boy showed no effects and Jenner repeated the experiment. As word of his results spread, others began to test it and by 1803 it was an established medical procedure in England. Shortly thereafter Ben Franklin encouraged American doctors to adapt this technique in view of the dangers inherent in the older technique. For more on Jenner visit this site.

THE MAGIC BULLET

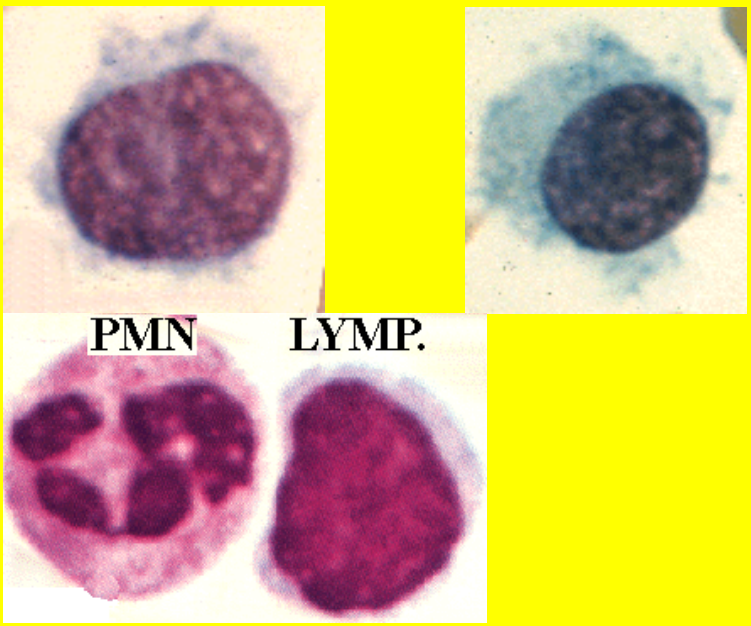


Figure 7. Differential staining of blood cells. These figures show the type of differential staining that gave Ehrlich his idea for a "Magic Bullet". Different parts of the cells in these three pictures stain differently with a stain composed of the same mixture of dyes. Look in your Atlas to see if you can identify these cells.

Paul Ehrlich worked in Koch's lab where he learned to study bacteria. While considering the phenomenon of #differential staining of different bacteria and of different components of eukaryotic cells, he speculated that if a dye chemical could bind to one cell and not another or to one substance within a cell and not others, perhaps you could find chemicals that would selectively kill certain pathogens without harming the surrounding host cells; this would act like a MAGIC BULLET selectively killing the villain and sparing the innocent victim. He embarked on a search for a magic bullet to cure syphilis, which in the late 19th century was a scourge as terrible as AIDS is today. In the final stages of syphilis, a sexually transmitted disease or #STD, its victim suffered horribly and eventually died insane as the brain was destroyed by the infection. Over many years he tested 100s of chemicals and finally in 1910 he found one, he named SALVARSAN or compound 606, that killed the syphilis organisms

without killing the host (usually). This discovery laid the ground for the discovery of #antibiotics and other chemotherapeutic agents.

SUMMARY

The major points to take away from this Chapter are:

- The knowledge that humans have recognized the transmissibility of disease for centuries, but without the technology to "SEE" the disease agents we were helpless before the onslaught of pathogens.
- A relationship of the names of the individuals covered with that of their discoveries and an idea of the time frame.

History URLs:

1. http://www.stlcc.cc.mo.us/fp/users/kkiser/History.page.htm; This is an excellent beginning of a history review. If the author continues to add to the information it has the potential to become a major microbiological reference site.

Contributions by Microbiologists (in brief)

1. **Spontaneous generation**
    a. Key to developing the germ theory of disease was a refutation of the concept of *spontaneous generation*.
    b. *Spontaneous generation* is the idea that, in modern times, living things can arise from non-living things (a violation of basic cell theory).
    c. Of course, what was really being observed was the appearance of visible organisms or populations of organisms which were initially microscopic contaminants.
    d. "As long as (individuals) believed that microorganisms could arise from nonliving substances, scientists saw no purpose in considering how diseases were transmitted or how they could be controlled."

2. **van Leeuwenhoek, Anton (1670s)**
    a. **First microbiologist:**
        i. First observation of individual, live microorganisms (used simple microscope).
        ii. "It was Anton van Leeuwenhoek, a Dutch cloth merchant and amateur lens grinder, who first made and used lenses to observe living microorganisms. The lenses Leeuwenhoek made were of excellent quality; some gave magnifications up to 300x and were remarkably free of distortion. Making these lenses and looking through them were the passions of his life." (p. 8, Black, 1996)
3. **Pasteur, Louis (1860s)**
    a. **Anti-spontaneous generation experiments:**
        i. *Pasteur* definitively demonstrated that microorganisms are present in air but not created by air.
        ii. This was critical for refutation of the concept of spontaneous generation and the for development of germ theory of disease.
    b. **No contamination when air is withheld:**
        i. Sterilized broth by boiling.
        ii. Exposure of sterilized broth to air resulted in contamination of broth by microorganisms (i.e., the broth became turbid).
        iii. Protection from *air*, by sealing, prevented contamination.
    c. **Contamination due to air-borne particles:**
        i. Curved neck flasks allowed contact with air but inhibited movement of non-gaseous particles.
        ii. Contamination was prevented (microdes stuck to neck of flask, did not reach broth)---air alone was not sufficient to induce contamination, must be something *carried* by air.
    d. **Vaccine discoverer:**
        i. *Pasteur* played key roles in the discovery and development of vaccines such as the rabies vaccine.
        ii. "In gratitude for Pasteur's development of vaccines, people from around the world contributed to the construction of the Pasteur Institute in Paris, France." (p. 10, Prescott *et al.*, 1996)

e. Microorganisms responsible for fermentation:

i. Fermentations are such things as the formation of alcohol or acetic acid in grape juice to form wine or vinegar.

ii. Demonstrated that fermentations occur as a consequence of the actions of micoorganisms.

4. Aseptic technique

a. Specific *aseptic techniques* are employed to avoid microbial contamination (e.g., Pasteur's experiments).

b. "Modern aseptic techniques are among the first and most important things that a beginning microbiologist learns." (p. 8, Tortora et al., 1995)

5. Pasteurization

a. Method of prevention of spoilage of liquid foodstuffs (milk, wine, beer) which utilizes heating.

b. Trade-off between applying enough heat (high enough temperature for long enough) to kill most microorganisms present (and delay or prevent spoilage) and applying so much heat that foodstuff is negatively altered.

6. Semmelweis, Ignaz (1840s)

a. Hand washing/childbirth fever:

i. Demonstrated that hand washing prevented the spread of childbirth fever.

ii. At the time (early 19th century) doctors would deliver babies without first washing their hands and, worse, would do so after performing autopsies on patients who had died from childbirth fever. This not only assured transmission, but biased that transmission so that the most virulent forms of the organism (i.e., those that killed women while they were still in the hospital) would be transmitted.

7. Lister, Joseph (1860s)

a. Chemical inhibition of infection:

i. Connected and applied Semmelweis' and Pasteur's work to develop and popularize the chemical inhibition of infection during surgery.

ii. Washed surgical wounds with phenol (a.k.a., carbolic acid)

iii. *Lister* is considered to be the father of antiseptic surgery.

8. **Koch, Robert (1870s)**
    a. Developed Koch's Postulates which are a sequence of experimental steps for directly relating a specific microbe to a specific disease.

Koch Postulates

- The specific organism should be shown to be present in all cases of animals suffering from a specific disease but shold not be found in healthy animals.
- The specific microorganism should be isolated from the diseased animal and grown in pure culture on artificial laboratory media.
- This freshly isolated microorganism, when inoculated into a healthy laboratory animal, should cause the same disease seen in the original animal.
- The microorganism should be reisolated in pure culture from the experimental infection.

    b. *Koch* discovered:
        i. Bacillus anthracis (causing anthrax)
        ii. Mycobacterium tuberculosis (causing TB)
        iii. Vibrio cholera (causing cholera)
    c. Technique developer:
        i. In addition to Koch's postulates, *Koch* played an important role in the developement of the use of agar as solid medium.
        ii. *Koch* also invented nutrient broth and nutrient agar.
9. **Iwanowski, Dmitri (1890s)**
    a. *Dmitri Iwanowski* discovered the first virus, tobacco mozaic virus.

Lecture: 3 Microscopy

As microorganisms are too small to be seen with naked eyes, the developments in the field of microbiology were not spectacular. It is only because of the invention of microscope the entire new world of invisible incredible organisms was opened to humans from then on, the field of microbiology revealed many amazing factors. Its all because of the invention of microscope and techniques developed in the field of microscopy.

A Dutch spectacle maker, Zaccharius Janssen (1590) found that a combination of two hand lenses can enlarge the image. In 1660 Robert Hooke developed compound microscope with which he described the cork cells.

Anton Van Leeuwenhoek (1632-1723), Delft, Holland, was almost the first person to individual microorganisms. Leeuwenhoek's microscope consisted of single convex lens mounted on stand of brass or silver. He used one microscope for one specimen so in his life time he constructed 419 microscopes.

He observed many fluid samples through his microscope and found many little things moving in the samples which he called "**animalcules**". He made drawings of his observations and sent then to Royal Society of London. Because of his observations and descriptions the existence of microbes was revealed to the scientific community.

[Anton Van Leeuwenhoek, 1683]

"I have had several gentlemen in my house, who were keen on seeing the little eels in Vinegar; but some of them here so disgusted at the spectacle that they vowed they would never use vinegar again. But what if one should tell such people in future that there are more animals living in the scum on the teeth in a man's mouth, them there are men in the world.]

Microscopy:

It is the technology of making very small things visible, and microscopes are the tools.

The early microscopes were "light microscopes because they used light, either from sunlight or from an artificial source. Today we have mainly two types of microscopes

i) Light microscopes
ii) Electron microscopes

i) Light microscopes:

This is most widely used type of microscopy. It refers to the use of any kind of microscopes that uses visible light to make specimens observable. Here the microscopic field is brightly illuminated and the microorganism mounted on a glass slide appears dark because they absorb resulting in greater contrast and colour differentiation. Light microscopes generally enlarge upto 1000x and beyond this limit it will cause a fuzzy image.

The modern light microscope is a descendent hot of Leeuwenhoek's but of Hook's compound microscope.

ii) Compound microscope:

Since Leeuwenhoek's time the light microscope has undergone various improvements and its current forms reached shortly before the turn of the twentieth century. This microscope is the compound microscope that is; it has more than one lens.

The compound microscope of today usually consists of a strong metal stand with a broad base. The base contains a strong light source – either an electric lamp or a mirror. Above the light source is a system of Iris diaphragms which regulate the passage of light and eliminate undesirable peripheral light rays from the source. Attached to the stand above the diaphragm is an adjustable condenser, which concentrates the light rays on the object. Above the condenser is the working stage or platform of the microscope with an opening at the center to admit light from the condenser. The glass slides with specimen should be kept in the center of the platform .A body tube is attached to the stand. At the lower end of the body tube objective lenses are mounted thorough a rotating nose piece. The top end of the body tube is housed with the eye piece (ocular lens). The objective lenses are usually of 10x, 45x and 100x magnification power and the ocular lens normally 10x. The eye piece end may either be monocular or binocular. The focusing mechanism consists of a coarse adjustment knob, which changes the distance between the objective lens and the specimen fairly rapidly, and the fine adjustment knob which changes the distance very slowly. The coarse adjustment knob is used to locate the specimen and fine adjustment knob is used to bring the specimen into sharp focus.

The total magnification of a light microscope is calculated by multiplying the magnifying power of the objective and ocular lenses. Thus an ordinary student microscope with 10x ocular and 10x, 45x and 100x objective lenses will produce useful magnification of 100,450 and 1000 times of the size of the specimen.

Working of compound microscope:

Light enters the microscope from a source in the base (mirror or electric lamp) and passes through a blue filter, which filters out the long wavelength of light, leaving shorter wavelengths and improving resolution. It then goes to through the condenser, which converges the light beams so that they pass through the specimen. The iris diaphragm controls the amount of light that passes through specimen and into objective lens. The higher the magnification, the greater the amount of light needed to see the specimen clearly. The objective lens magnifies the image before it passes through the body tube to the ocular lens in the eye piece. The ocular lens further magnifies the image. The mechanical stage allows the precise control of moving the slide, which is especially useful in the study of microorganisms.

Most microscopes are designed so that when the microscopist increases or decreases the magnification by changing from one objective to another, the specimen will remain very nearly in focus. Such microscopes are said to be par focal.

To have a better understanding of microscopes the following criteria are to be understood.

Resolving Power (RP): of a lens is a numerical measure of the resolution that can be obtained with that lens. It can also be defined as the ability of a microscope to distinguish two adjustment points distinct and separate for eg. Two points or lines with less than 200nm space between them are not visible to the unaided to eyes. There magnification without the ability to distinguish minute structural details is not beneficial in a microscope. That is the smaller the distance between the objects that can be distinguished, the greater the resolving power of the lens. The resolving power of a microscope depends on two factors, the wavelength of light and the numerical aperture (NA) of the lens system. (Wavelength one of the most important property of light is its wavelength or the length of the light ray. It is equal to the distance between two adjacent crests or two adjacent troughs of a wave and it is represented by the Greek letter lambda (λ).

The wavelength used for observation is crucially related to the resolution that can be obtained. The wavelength of the visible light used in light microscopy is 400 – 750nm. Resolution is inversely proportional to wavelength.

The resolving power of a lens can be worked out if the Numerical aperture (NA) of a lens is known.

Numerical Aperture (NA):

It is a mathematical expression relating to the extent that light is concentrated by the condenser and collected by the objective. The measure of the aperture of the objective is the angle 0 subtended by the optical axis and the outermost rays covered by it. Angle 0 is the half-aperture angle. The magnitude of this angle is expressed as a sine value. Sine value of this half aperture angle multiplied by the refractive index 'n' of the medium filling the space between the objective lens and the slide gives the numerical aperture. If the medium is just air the n value is 1.0 and if its is oil (to increase the resolutions all microscopes have one immersion lens and the oil is called immersion oil) then the refractive index is 1.56.

The formula for calculating R.P is

$$RP = \frac{\lambda}{2\ NA}$$

where NA = Numerical aperture = Sine value x Refractive index.

Low Power (10x)	High Power (45x)	Oil immersion (100x)
$\frac{64}{2} = 32^o$	$\frac{96}{2} = 48^o$	$\frac{116}{2} = 58^o$
RI = 1	RI = 1	RI = 1.50

The numerical aperture for the any objective is less than 1.0 for oil immersion objective the value ranges from 1.2 to 1.4. Thus the resolving power of the light microscope is limited by wavelength of the visible light and the numerical aperture. The diameter of the smallest object on the least distance between two objects which may be resolved by a lens system i.e. the resolving power of a microscope (R.P) may be calculated as follows

$$RP = \frac{\text{Wavelength}}{2\ NA}$$

$$\text{Eg. } = \frac{600nm}{2 \times 1} = 300nm.$$

$$\frac{600nm}{2 \times 1.4} = 213nm = 0.21mm$$

This value is called the limit of resolution that is, the object that try to see through microscope should approximate have a diameter of this size to be seen very clearly.

Spherical and chromatic aberrations:

Inequalities of refraction by the peripheral portion of the objective lens result in spherical aberrations resulting in fuzzy images. Chromatic aberration (colour rings) is due to the prism like effect of the outermost portions of the lens. In modern microscopes, spherical aberration is overcome by the ingenious design and curvature of the lens and partly by the iris diaphragm. Lens system corrected for chromatic abrration in the red and blue ranges are corrected is called achromatic lenses and those corrected in red, blue and other ranges apochromatic lenses.

Dark field microscopy:

In this microscope objects are seen brightly illuminated against a dark background. The condenser used in an ordinary light microscope causes light to be concentrated and transmitted directly through the specimen. This gives a bright illumination. The dark field microscopy requires a special type of condenser (cardoid or paroboloid condenser) that transmits a hallow cone of light from a source of illumination. It is particularly useful for identifying thin microbes such as Treponema pallidum (syphilis organism).

Phase contrast Microscopy:

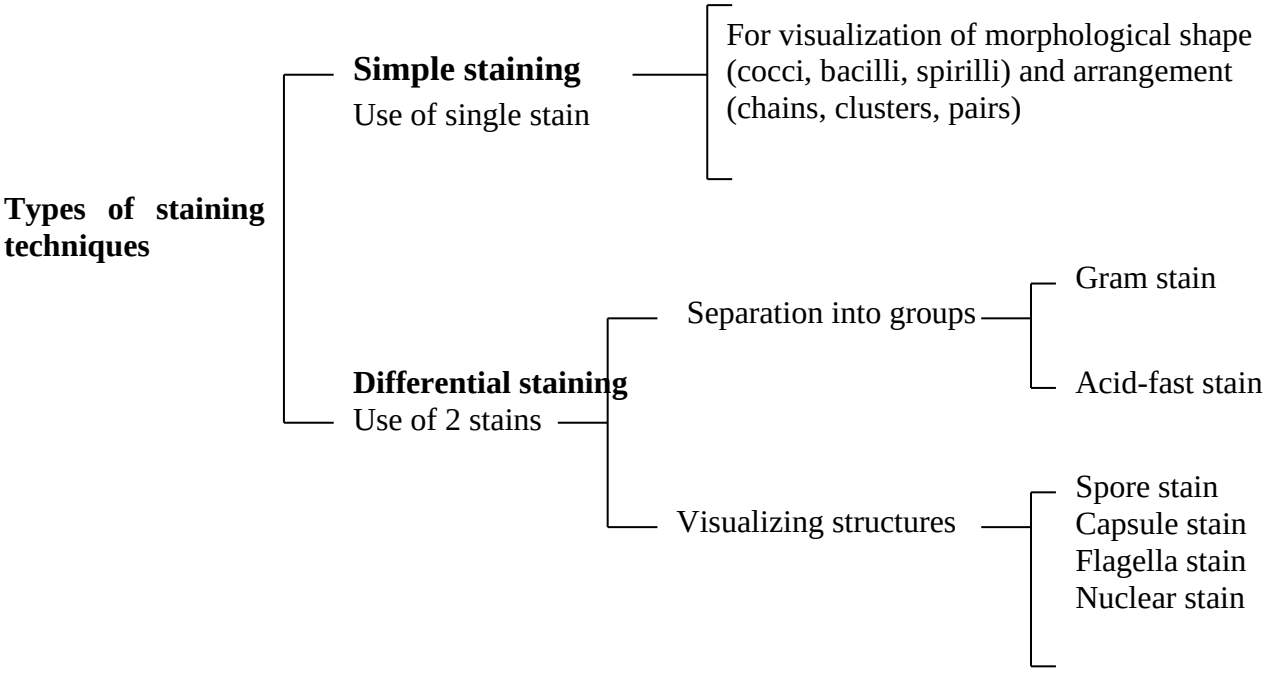
It is a form of light microscopy in which light rays are advanced or retarded ¼ of a wavelength by means of a special condenser and objective attachments. It is useful for examining cell ultrastructure. It is particularly useful for examining intracellular structures that appear transparent by other forms of light microscopy.

Lecture: 4 Staining techniques

Visualisation of microbes in the living state is most difficult, not only because they are minute, but also they are transparent and practically colorless when suspended in an aqueous medium. To study their properties and to divide microbes into specific groups for diagnostic purposes, biological stains and staining procedures in conjunction with light microscopy have become major tools in microbiology.

A stain may be defined as an organic compound containing a benzene ring plus a chromophore and auxochrome group. Acidic (negative charge) and basic dyes (positive charge) are used in staining procedures to impart colour to the bacteria. Basic dyes are more commonly used for bacterial staining as bacterial surface is negatively charged. Acidic dyes are used to stain the positive constituents (proteins) of the cell.

Numerous staining techniques are available for visualization, differentiation and separation of bacteria in terms of morphological characteristics and cellular structures. A summary of commonly used procedures and their purposes is outline below.



Preparation of slides (Methods)

1. Wet mount:

In this method the organisms are examined under their normal living conditions. A drop of fluid containing the microbial cells is placed on clean glass slide and it is covered with a cover slip. Then it is viewed in a microscope.

Another technique is the “hanging drop technique” in which the fluid drop having the microbial cells, is placed on a cover slip and it is encircled with petroleum jelly. The cover slip is then inverted and placed on a clean glass slide, which is viewed under a microscope.

These two methods are advantageous to observe the morphology of spiral bacteria as these cells get distorted when they are stained. If one wants to know the mobility of an organism then it should be viewed only by these methods.

In cases where one wants to study about the changes occurring during cell division, or spore formation and germination, the organisms should be observed only under living state.

In all these cases wet mount or hanging drop technique are useful to study about the organisms under living conditions.

But these methods may not be useful when it is required to study about the organisms structural details or cell inclusions. In such cases staining procedures can be adopted.

The preparation of slide for staining is the very important. The steps to be followed are

(1) Preparation of smear

In this a drop of solution to be examined is placed over a clean grease free glass slide. It is spread in the slide to about $1Cm^2$ with a glass rod as a thin film or smear. Too thick or too thin smear will not be useful.

(2) Fixation:

The slide with a smear is then air dried for some time and then heat fixed by showing the slide over flame. By this the cells are fixed on the slide.

(3) Application of stain:

Depending upon the nature of study and type of organisms.

Staining methods:

Stained preparations of microbial cells are most frequently used to study about the morphological characteristics of bacteria. The staining has certain advantages.

1. The cells are made clear as they are coloured.
2. Differences between cells of different species and within the species can be easily elucidated by adapting suitable staining methods.

There are various staining methods and stains are available which make us to study about the bacteria in greater detail.

In all the staining methods microbial cells have to be processed.

A basic dye has positive charge and generally stains the acidic cell components.

A neutral dye is a mixture of acidic dye and basic dye. Eg. Eosin and methylene blue.

The process of staining involve ion exchange reactions between the stain and active site at the surface of or within the cell certain cell proteins or nucleic acids involve in the formation of salt with positively charged ions such as Na^+ or K^+.

(Bacterial cell $^-$) (Na^+)

In a basic dye like methylene blue, the coloured ion is positively charged and it is represented as MB and the dye is actually methylene blue chloride then it is represented as

$MB^+ CL^-$

Then the staining reaction or ion exchange reaction can be represented as an equation. (Bacterial cell$^-$) (Na^+) (MB^+) (Cl^-) (Bacterial cell$^-$) (MB^+) + (Na^+ Cl^-)

What is a stain ?

A stain or dye is a molecule that can bind to a cellular structure and give it colour. These stains make the microorganisms stand out against their backgrounds. They are also helpful to examine the structural and chemical differences in cellular structures and chemical differences in cellular structures.

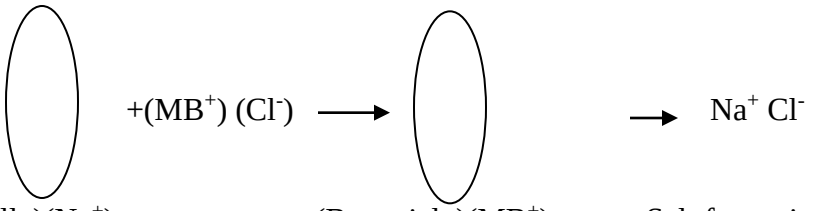
Microbiological stains:

A large number of coloured organic compounds are available for staining microorganisms. These compounds are chemically complex molecules. On the basis of their chemical nature they can be grouped as triphenylmethane dyes, oxazine dyes and thiazine dyes.

They can be also classified more practically as acidic, basic or neutral dyes.

An acidic dye is an ammonic dye which has the negative charge. They stain generally basic cell compounds.

Thus we may view the peripheral areas of the cell as coloured.



Different staining methods:

There are various staining methods, which help as to examine the bacterial cells and their compounds clearly and help as to differentiate between organisms.

1. Simple staining: In this method a single stain or dye is used, to stain the smear. The fixed smear is flooded with a dye solution for a specified period of time, after which the solution is washed with water and blotted dry. The cells uniformly stained and viewed clearly in a microscope.

Differential staining:

Staining methods which make the differences between bacterial cells or parts of cells visible is called differential staining. This method is more elaborate than the simple staining in the sense that more than one dye solution is used.

Gram staining:

The most important and widely used staining technique is gram staining. This technique was developed by Hans Christian Gram in 1884.

In this procedure the stain solutions are added in the following order.

1. Crystal violet
2. Iodine solution
3. Alcohol
4. Saffranin.

By this method Bacteria are classified into two groups viz, Gram positive and Gram negative. The Gram positive bacteria will look deep violet as they retain the crystal violet.

The Gram negative bacteria appear red as they lose crystal violet and retain saffranin.

Why this difference?

The plausible explanation for this phenomenon lies in the structure and composition of cell wall.

Gram – ve bacteria are thinner than those of gram + ve bacteria.

Gram – ve bacteria contain a higher percentage of lipid than do the Gram + ve bacteria.

In gram – ve bacteria the lipids are extracted by alcohol and increase the porosity or permeability of the cell wall. Thus the crystal violet is washed off and thus the crystal violet is washed off and decolourized. These cells will subsequently take the saffranin. Whereas in the Gram + ve bacteria because of their power lipid content become dehydrated during the treatment with alcohol. So the pore size decreases, permeability is reduced and crystal violet can not be extracted. Therefore these cells remain purple violet.

Another explanation is that the Gram + ve bacteria has higher peptidoglycan layer and trap the crystal violet as the ethanol treatment causes a diminution in the pore size.

Gram – ve bacteria is less extensively crosslinked and the pore size is sufficiently large enough even after ethanol treatment to allow the crystal violet be extracted.

This fact is proved by treating the Gram + ve bacteria with lysozyme to remove the cell wall and then they are stained with crystal violet and then given with alcohol treatment the cells lose crystal violet.

Sometimes the certain bacteria are gram variable. In such cases antibiotic treatment or KOH test would be helpful. Gram + ve bacteria are more susceptible to penicillin treatment and gram – ve to streptomycin.

In KOH treatment test a clump of cell are taken and placed in a glass slide and 2 drops 3% KOH solution is added to the cells and stirred with a glass rod for 30 seconds when the glass rod is lifted above the slide a string of DNA will come along with the glass rod if the cells are gram – ve if the cells are gram positive there will not be a string formation.

Special staining methods:

1. Negative staining or capsule staining:

Certain bacteria have slimy layer around them, which acts a host defense burrier. These capsules resist stain. So in negative staining the cells with capsule appears clearly again dark background of dye solution. The stains used are Indian ink or nigrosin.

2. Flagella staining:

Flagella are appendages of some bacterial cells and used for locomotion. These are too this to be seen with the light microscope. It is a painstaking and time-consuming process in which the surfaces of the cells are carefully coated with a dye or neutral like silver. It is done when it is exactly required to study about the presence and arrangement of flagella.

3. Endospore staining:

A few types of bacteria produce resistant cells called endospores. Endospore walls are resistant to penetration of ordinary dyes. Schaffer-Fulton spore stain maker the spore to be seen clearly. Heat fixed smear are covered with malachite green and gently heated until they steam. After 5 minutes of steaming the endospore walls become permeable to the dye. The slide is then rinsed with water. The endospores only retain the colour.

Lecture: 6 Structure of a Prokaryotic cell

Structurally a bacterial cell consists of

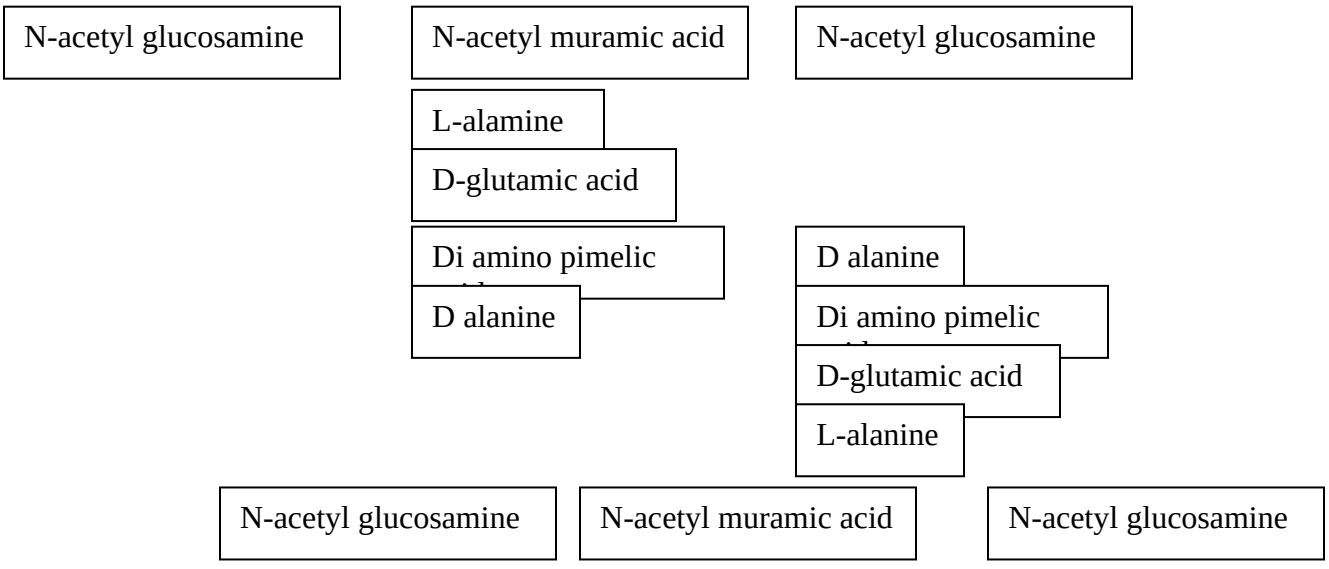
1. A cell membrane usually surrounded by a cell wall
2. An internal cytoplasm with ribosome's a nuclear region and in some cases granules and vesicles.
3. A variety of external structures such as capsules, flagella and pili

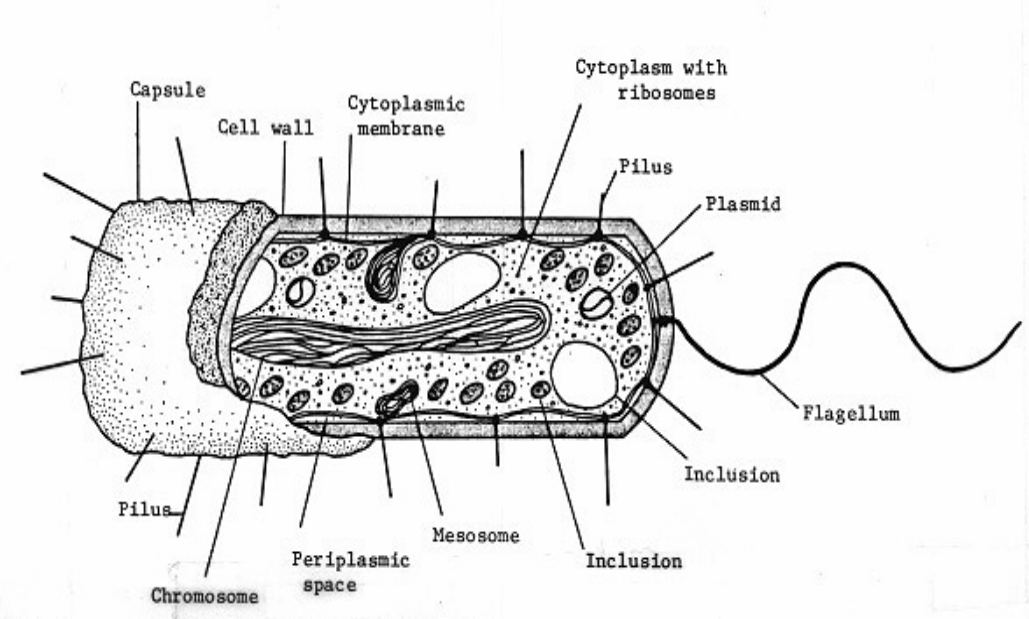
Cell Wall:

A semi rigid wall lies outside the cell membrane is called cell wall. It confers the cell, the shape by its rigid nature and prevents the cell from bursting out when fluids flown into the cell through osmosis. It is regulating entry of minerals into the cell.

Chemical nature of cell wall:

The single, most important component of cell wall is the Peptidoglycan it is a polymer comprised of molecules of N-acetyl glucosamine alternately linked with N-acetylmuramic acid. These two molecules are cross linked with tetrapeptides i.e. a chain of 4 amino acids in gram positive bacteria the tetrapeptide contain L. alanine, D-glutamic acid, lysine, D-alanine. In gram negative organisms wistead of lysine, diaminopimelic acid is present. Cell walls of gram + ve organism have an additional molecule, teichoic acid which consists of glycerol phosphate and sugar alcohol ribitiol. It sometimes extends outside the cell wall and its exact function is get unknown, and it is assumed that it furnishes attachment site for bacteriophages.





Outer membrane:

It is found only in G-ve bacteria. It is the outermost layer of cell wall and it is attached to the peptidoglycan by a continuous layer of small lipoprotein molecules. These lipoprotein are embedded in the outer membrane and covalently bonded to the peptidoglycan. The outer membrane acts as a coarse sieve and has little control over movement of substances into cell. Proteins called Porins form channels through the outer membrane. The outer membrane contains important constituent called lipopolysaccharides (LPS) and acts as an endotoxin. It is an integral part of the cell wall and it is not released until the cell walls of dead bacteria are broken down. It consists of polysaccharides and lipids A. the lipid A is responsible for the toxic properties.

Periplasmic space:

The gap between the cell membrane and cell wall is the periplasmic space. It represents a very active area of cell metabolism. This space contains many digestive enzymes and transports metabolites into bacterial cytoplasm. The periplasm consists of peptidoglycan protein constituents and metabolites found in the periplasmic space. This is rarely observed in Gram + ve bacteria.

The cell wall of Gram + ve bacteria has a thick layer of peptidoglycan 20 to 80nm.

The cell wall of Gram – ve bacteria is thinner, only 10 to 20 % of it is peptidoglycan rest with polysaccharides, proteins and lipids.

Cell wall Characteristics of G + ve & G – ve

Sl.No.	Characters	Gram Positive	Gram Negative
1.	Peptidoglycan	Thick layer	Thin layer
2.	Teichoic acid	Often present	Absent
3.	Lipids	Very little	Hippo polysaccharide
4.	Outer membrane	Absent	Present
5.	Cell shape	Always rigid	Rigid or flexible
6.	Results of cell wall degradation	Protoplast	Spheroplast
7.	Sensitivity to dyes and antibiotics	More sensitive	Moderately sensitive

The cell membrane:

It is otherwise called the plasma membrane. It forms a boundary between cell and its environment. Bacterial cell membranes are similar to that of the membranes of all other cells. They consist mainly of phospholipids and proteins. The phospholipids form a bilayer or two adjacent layers. In each layer the phosphate ends of the lipids molecule toward the membrane surface and fatty acid end extends inwards. The drained ends of the phosphate are hydrophilic and fatty acids hydrophobic. Protein molecules are interspersed along lipids

Cell membranes are dynamic constantly changing entities materials constantly go through and out of the membrane. the main function of the membrane is to regulate the movement of materials into and out. It synthesizes cell wall components, assists DNA replication, secrete protein carry out respiration contain bases of appendages called flagella.

Internal structures:

Bacterial cells contain ribosome's vacuoles, nuclear region, chromatophores in this the cytoplasm.

Cytoplasm:

The prokaryotic cells contain a semifluid substance inside the cell membrane, called cytoplasm. Cytoplasm is about $^{4}/_{5}$ th water and $^{1}/_{5}$ th other substances.

This substances include enzymes and other proteins, carbohydrates lipids and a variety of inorganic ions, many biochemical reactions, both anabolic and catabolic occur in the cytoplasm. There is no cytoplasmic streaming in the prokaryotes as that found in eukaryotic cells.

Ribosomes:

They consist of ribonucleic acid and protein. They are abundant in the cytoplasm and grouped in chains called polyribosomes. They are nearly spherical and serve as sites for protein synthesis. The sedimentation rate of ribosomes is 70s (Svedberg units).

Nucleic Region:

One of the key features of prokaryotic cells is the absence of definite membrane bound nucleus, instead they have a nuclear region or nucleoid. They consist mainly DNA but have some RNA and protein associated with it. The DNA is arranged in one large circular chromosome. Some bacteria also contain circular DNA molecules of DNA called plasmids. Genetic information in plasmids supplements information in the chromosomes.

Internal membrane systems:

Photosynthetic bacteria and cyanobacteria contain internal membrane systems and are known as chromatophores

Volutin granules:

They are also known as metachromatic granules and are composed of polymetaphosphate. It serves as a reserve source of phosphate. As it was first found in *Spirillum volutans* hence the name.

Poly β-hydroxy butyrate:

This serves as a reserve carbon and energy source.

Polysaccharide granules:

Contains glycogen.

Mesosomes:

Bacteria have specialized invaginations of the cytoplasmic membrane in the form of localized infoldings which increase surface area of membrane. these are particularly well developed structures in bacilli.

Structures outside cell wall

Flagella:

They are hair like structures extending through bacterial cell wall and are used for mobility.

Types of flagella

Atrichous : If any bacterium lacks flagella (eg) cocci

Monotrichous: If a single polar flagellum is present it is called monotrichous eg. *Psedomonous aeruginosa*

Amphitrichous: When the bacterium possesses flagella at both ends. Eg. *Aquaspirillum serpens*.

Lophotrichous: When the bacterium possesses a tuft of flagella at one or both ends eg. *Pseudomonas fluorescens*

Peritrichous: When the bacterium possesses flagella all around the cell eg. *Salmonella typhi*

Bacterial flagella are helical appendages that protrude through the cell wall and responsible for swarming mobility. They are thinner than flagella or cilia of eukaryotes. The diameter of bacterial flagella is protein subunits called flagellin.

Bacteria propel themselves by rotating thin helical flagella. It is known that the flagella motor is driven by the proton motive force. I.e. the force derived from the electrical potential and hydrogen ion gradient across the cytoplasmic membrane.

Each flagellum is attached to a cell membrane by a basal region consisting of a protein other than flagellin. The basal region has a hook like structure and a basal body. The basal body consists of a central rod or shaft surrounded by a set of rings. Gram – ve bacteria have a pair of rings embedded in the cell membrane and another pair of rings associated with the peptidoglycan and lipopolysaccharide layer of cell wall. Gram positive bacteria have one ring embedded in the cell ring and another in the cell wall.

Chemotaxis: Some times bacteria move toward or away from substances in their environment by a process called chemotaxis concentration of most substances in the environment varies along a gradient i.e. from high to low concentration. If the bacteria run towards the chemical substance it is positive chemotaxis. If it goes away from the substance it is negative chemotaxis.

Phototaxis:

Some bacteria move toward or away from light. This is called phototaxis. Especially photosynthetic bacteria move towards light.

Axial filament:

Spirochetes have axial filaments or endoflagella, instead of flagella that extend beyond cell wall. Each filament is attached at one of its ends to an end of the cytoplasmic cylinder that form the body of a spirochete.

Pili (Pilus – singular) or Fimbriae: are hollow, filamentous appendages that are thinner, shorter and larger in number compared to the flagella. They are genetically coded in the plasmid DNA called 'F' plasmid or fertility plasmid. They can also integrate with chromosomal DNA. They do not help in motility. Though these are restricted to gram –ve bacteria they are noticed in *Corynebacterium renale* , a gram +ve bacterium.

The length of the pili varies from 0.2mm – 20mm and their width from 30A to 140A . They can be seen only in electron microscope. They are made up of a protein called "pilin". Generally the term pili is used to designate sex pili and are involved in a genetic transformation process called "conjugation" whereas the other groups are called as 'fimbriae". Several functions are associated with fimbriae.

Capsules: Certain bacterial cells are covered by a viscous or gelatinous substance. Capsules can be seen by microscopes with specific staining techniques. If they are too thin they are called as "microcapsule" and when many cells are embedded in a matrix it is known as "slime". They are made up of polysaccharides. In some cases they are made up of poly peptides. Eg. Capsule of Bacillus anthracis is made up of polymer of D- glutamic acid..

Cytoplasmic Membrane: Beneath the cell wall lies the cytoplasmic membrane and that is bounding layer of the inner contents. It is the osmotic and permeability barrier. It is approximately 7.5 nm thickness and composed of phospholipids and proteins.

It is selective layer and so it allows the entry and exit of small molecules such as nutrients and waste materials. It also possesses enzymes involved in respiration and synthesis of capsular and cell wall materials. More importantly it is the site of ATP synthesis and control of flagellar motility. So damage to this membrane will result in the death of the cell.

Mesosomes: Bacteria have specialized invaginations of the cytoplasmic membrane in the form of localized infoldings which increases the surface area of the membrane. These are involved in the export of extra cellular enzymes.

Cytoplasm: By treatment with lysozyme periplast i.e bacterial cell material bounded by the cytoplasmic membrane is obtained in Gram + bacteria and in the case of gram –ve bacteria the outer layer of the outer membrane is not dissolved by lysozyme and it is called as spheroplast.

The cytoplasm is granular in appearance and rich in ribosomes , the structures on which ribosomes are synthesized. Nuclei are not present in bacteria instead chromatin rich areas called nucleoid are present. Unlike animal and plant cells the endoplasmic reticulum, plastids and

mitochondria are absent. The respiratory enzymes and photosynthetic pigments are not localized but distributed in the cytoplasmic membrane and ribosomes. The nuclei are absent and the DNA rich chromatin areas are called nucleoids.

Lecture 7 VIRUSES

Viruses are infectious agents with both living and nonliving characteristics.

1. Living characteristics of viruses

a. They reproduce at a fantastic rate, but only in living host cells.

b. They can mutate.

2. Nonliving characteristics of viruses

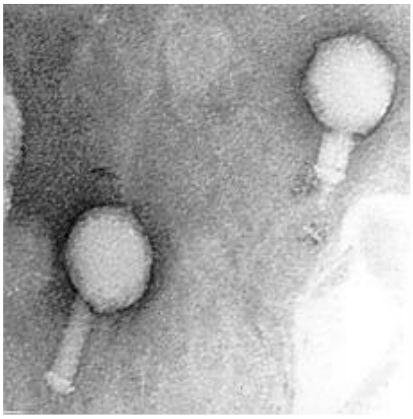
a. They are acellular, that is, they contain no cytoplasm or cellular organelles.

b. They carry out no metabolism on their own and must replicate using the host cell's metabolic machinery. In other words, viruses don't grow and divide. Instead, new viral components are synthesized and assembled within the infected host cell.

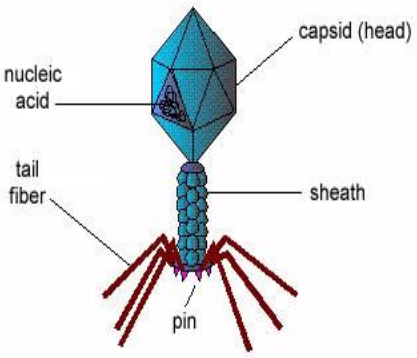
c. They possess DNA or RNA but never both.

Bacteriophages

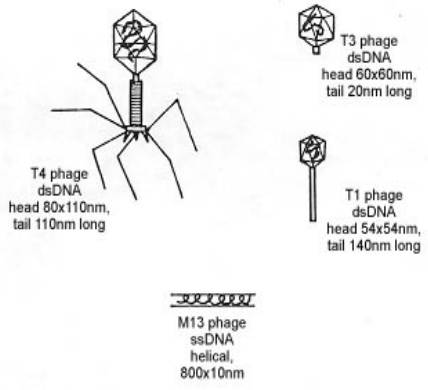
Bacteriophages are viruses that only infect bacteria



SEM of T4 bacteriophagephage



Structure



Types of phages

There are two primary types of bacteriophages: **lytic bacteriophages** and **temperate bacteriophages**.

1. Bacteriophages that replicate through the lytic life cycle are called **lytic bacteriophages**. They lyse the host bacterium as a normal part of their life cycle.

2. Bacteriophages capable of a **lysogenic life cycle** are termed **temperate phages**. When a temperate phage infects a bacterium, it can either replicate by means of the lytic life cycle and cause lysis of the host bacterium, or, it can incorporate its DNA into the bacterium's DNA and become a noninfectious prophage.

The lytic life cycle bacteriophages

There are five major steps in the life cycle.

a. Adsorption

Attachment sites on the phage adsorb to receptor sites on the host bacterium (see Fig. 1). Most bacteriophages adsorb to the bacterial cell wall, although some are able to adsorb to flagella or pili. Specific strains of bacteriophages can only adsorb to specific strain of host bacteria. This is known as **viral specificity** *(def)*.

b. Penetration

In the case of phages that adsorb to the bacterial cell wall, a phage enzyme "drills" a hole in the bacterial wall and the **phage injects its genome into the bacterial cytoplasm** (see Fig. 2). Some phages accomplish this by contracting a sheath which drives a hollow tube into the bacterium. This begins the eclipse period. The genome of phages which adsorb to flagella or pili enter through these hollow organelles. In either case, only the phage genome enters the bacterium so there is no uncoating stage.

c. Replication

Enzymes coded by the phage genome shut down the bacterium's macromolecular (protein, RNA, DNA) synthesis. **The phage replicates its genome and uses the bacterium's metabolic machinery to synthesize phage enzymes and phage structural components** (see Fig. 3 and Fig. 4).

d. Maturation

The phage parts assemble around the genomes (see Fig. 5).

e. Release

Usually, a phage-coded **lysozyme breaks down the bacterial peptidoglycan causing osmotic lysis** and release of the intact bacteriophages (see Fig. 6).

f. Reinfection

From 50 to 200 phages may be produced per infected bacterium.

The Lysogenic Life Cycle of Temperate Bacteriophages

Bacteriophages capable of a lysogenic life cycle are termed **temperate phages**. When a temperate phage infects a bacterium, it can either **replicate by means of the lytic life cycle** and cause lysis of the host bacterium, **or**, it can **incorporate its DNA into the bacterium's DNA** and become a noninfectious **prophage.**

The different steps include

1. Adsorption
2. Penetration
3. Formation of prophage

The cycle begins by the phage adsorbing to the host bacterium or **lysogen** (Fig 1) and injecting its genome as in the lytic life cycle (see Fig. 2).**The phage DNA inserts or integrates into the host bacterium's DNA** (see Fig. 3). At this stage the virus is called a **prophage**.

Expression of the phage genes controlling phage replication is blocked by a repressor protein, and the phage DNA replicates as a part of the bacterium's DNA so that every daughter bacterium now contains the prophage (see Fig. 4).

The number of viruses infecting the bacterium as well as the physiological state of the bacterium appear to determine whether the temperate phage enters the lytic cycle or becomes a prophage.

In about one out of every million to one out of every billion bacteria containing a prophage, **spontaneous induction** occurs (Fig.5). The **phage genes are activated and new phages are produced by the lytic life cycle** (Fig. 3-6 of lytic cycle)

PROPHAGE:A bacteriophage that has integrated its DNA into the nucleoid of a host bacterium.
LYSOGEN:A bacterium containing a prophage
LYSOGENIC LIFE CYCLE: The life cycle of temperate bacteriophages in which the virus can either replicate via the lytic life cycle or become a latent prophage within the infected host bacterium and not replicate.
TEMPERATE BACTERIOPHAGE:A bacteriophage having a lysogenic life cycle and capable of inserting its DNA into that of the host bacterium. Since it may or may not lyse the host bacterium it is considered more temperate in its action.

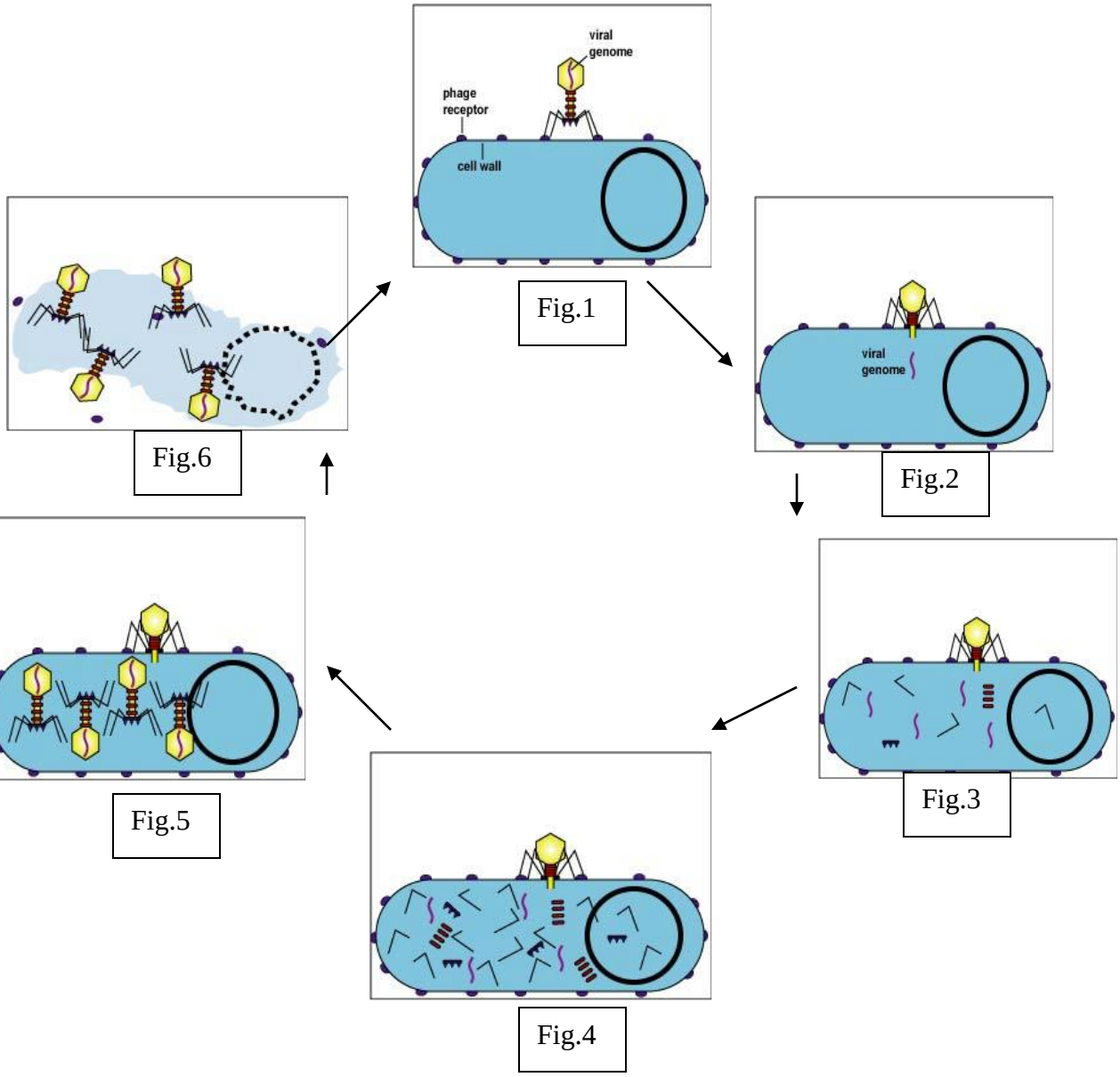
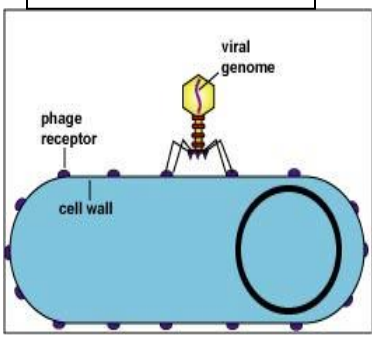


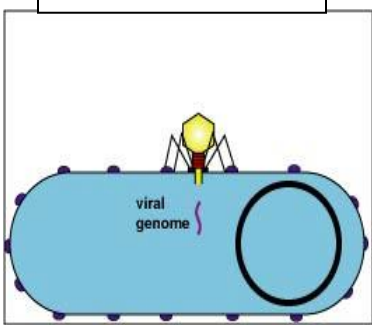
Fig 1. Adsorption of phage to the bacterial cell receptor sites
Fig. 2 Penetration of DNA into the bacterial cell
Fig. 3 Replication of phage DNA
Fig. 4 Synthesis of phage structural components
Fig. 5 Assembling of phage components
Fig. 6 Release of phages

LYTIC LIFE CYCLE

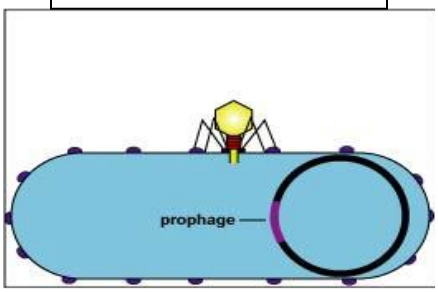
Adsorption (Fig 1)



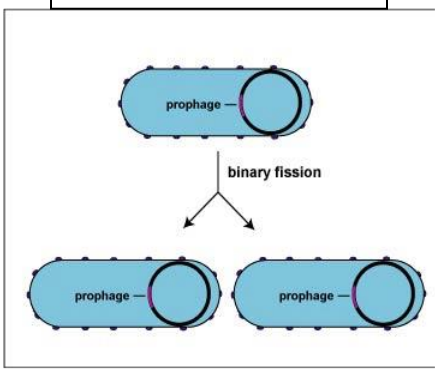
Penetration (Fig.2)

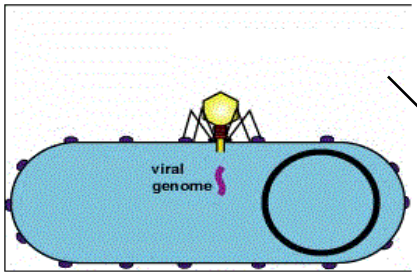


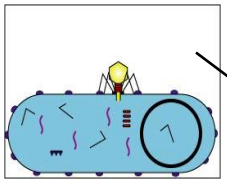
Prophage formation (Fig.3)

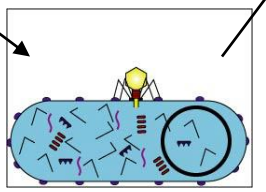


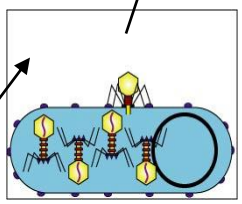
Prophage in bacteria (Fig.4)

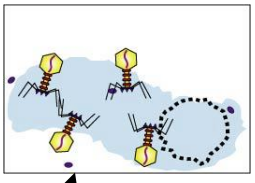












LYSOGENIC LIFE CYCLE

Lecture 8 Bacterial Growth Curve

Microbial growth

(a) "Because individual cells grow larger only to divide into new individuals, **microbial growth** is defined not in terms of cell size but as the increase in the number of cells, which occurs by cell division."

(b) This emphasis has practical application since it is typically far easier to measure increases in cell number than it is to measure increases in cell size

(c) Furthermore, unless cell division is synchronized, cells will typically vary in size across an even homogeneous population, thus making measurement of cell size almost irrelevant as a means of measuring microbial growth

(2) Binary fission

(a) The majority of bacteria reproduce by a mechanism termed binary fission

(b) Binary fission is much simpler than the mechanisms of cell division seen in eukaryotic cells

Some of the basic shapes of bacteria are **cocci** (round shaped), **bacilli** (rod shaped), **vibrio** (curved rods) **spirillum** (spiral shaped), **spirochaete** (corkscrew shaped), star shaped and square shaped. Predominant shapes are given in the figures. The arrangement of bacteria is also important. Cocci can either occur as **diploccocci** (cocci in pairs)**, tetrad** (4 cocci together), **staphylococci** (chain of cocci), **streptococci (cluster of cocci)** and **sarcinae (cluster of 8 cocci)** . Similarly, there are **streptobacilli** and **diplobacilli**.

(6) Standard bacterial growth curve

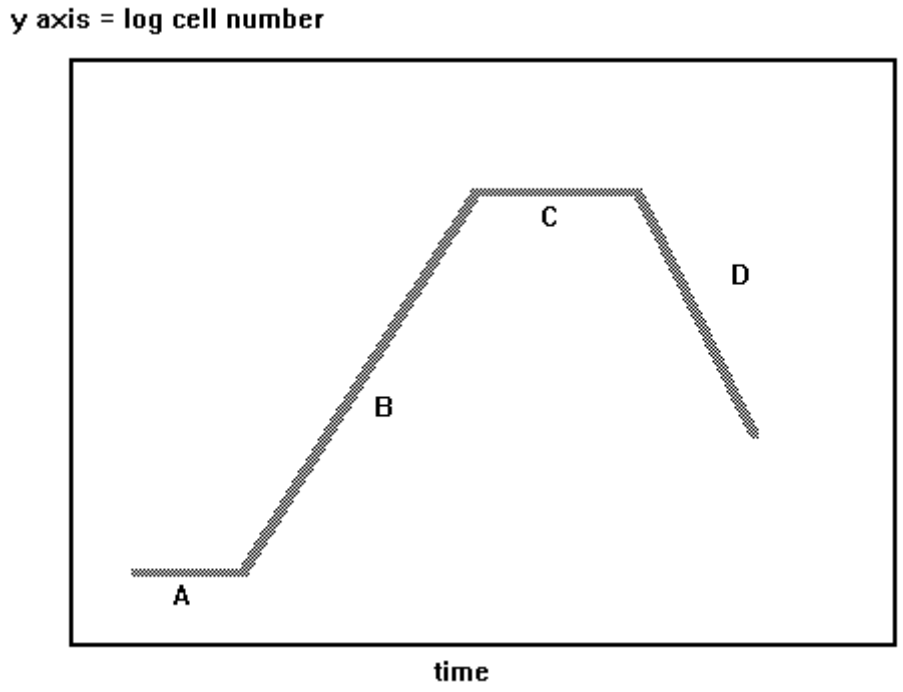
(a) Bacteria added to fresh media typically go through four more-or-less distinct phases of growth

(i) Lag phase (A)

(ii) Log (logarithmic or exponential) phase (B)

(iii) Stationary phase (C)

(iv) Decline (death) phase (D)



(7) Lag phase

(a) Transfers of bacteria from one medium to another, where there exist chemical differences between the two media, typically results in a lag in cell division

(b) This lag in division is associated with a physiological adaptation to the new environment, by the cells, prior to their resumption of division

(c) That is, cells may increase in size during this time, but simply do not undergo binary fission

(8) Log phase (logarithmic phase, exponential phase)

(a) Lag phase is followed by log phase during which binary fission occurs

(b) This phase of growth is called logarithmic or exponential because the rate of increase in cell number is a multiplicative function of cell number

(c) This can be seen in a graph of cell number versus time where cell numbers increase at ever increasing rates with time or generation; that is, the rate of increase is a function of absolute cell number such that the more cells present, the faster the population of cells increases in size (at least, during log phase)

(e) When graphed on semi-log graph paper (Figure 6.3, i.e., log cell number versus time), log-phase growth produces a straight line

(9) Continuous culture (serial transfer)

(a) A means of keeping cultures in log phase can be accomplished either by employing a chemostat or via serial transfer

(b) A chemostat involves adding fresh medium to a culture, mixing, and then allowing an equal volume of culture to drain from the vessel; this is typically done continuously (i.e., a steady stream of fresh medium is added)

(c) Serial transfer means taking a volume of culture and diluting that volume into fresh media

(10) Generation time

(a) Generation time it takes a bacterial population to double in size (number) during log-phase growth

(b) Note that the time it takes for the population to double in size does not change with cell number (so long as cells remain in log phase)

(c) That is, with exponential growth, the absolute increase in cell number increases as cell number increases while the relative increase remains invariant

(d) Typically, generation times range from 20 minutes to 20 hours depending on the bacterial species/strain and the conditions during which log-phase growth is occurring

(11) Stationary phase

(a) Stationary phase is a steady-state equilibrium where the rate of cell growth (division) is exactly balanced by the rate of cell death (i.e., increase in cell number due to cell divisions exactly balanced by a decrease in cell number due to death)

(b) Cell death (or, at least, lack of cell growth) occurs because of a loss of limiting nutrients (due to their incorporation into cells during log-phase growth) or a build-up of toxins (due to their release during log-phase growth, e.g., fermentative products)

(c) Note that the simplest conditions that will result in a stationary phase is when both the rate of cell increase and the rate of cell death together equal zero (i.e., cells neither die nor are born)

(12) Decline phase (death phase)

(a) Stationary phase, in a standard bacterial growth curve, is followed by a die-off of cells

(b) Cell death in bacteria cultures basically means that the cells are unable to resume division following their transfer to new environments

(c) Typically this die-off occurs exponentially, i.e., such that cell number graphed against time, using a semi-log scale for cell number, results in a straight line (i.e., see Figure 6.3)

(d) This death occurs because vegetative cells can survive exposure to harsh conditions (few nutrients or too-many toxins) for only so long

Environmental Factors Affecting Bacterial Growth

(A) physical

(B) nutritional factors.

(A) Physical Factors Affecting Growth:

i) pH - ***measurement of the acidity or alkalinity of the medium***

Microorganisms have an optimum pH for growth and generally most bacteria grow best at neutral pH. However, some grow best at acidic pH and are called ***acidophiles*** e.g.Lactobacillus, whereas, other grow best at high pHÕs and are called ***alkaliphiles*** e.g.Vibrio cholerae

ii) temperature - most species can grow over a 30°C temperature range, with the maximum and minimum temps varying greatly for different species, e.g because sea water remains liquid below

32°F, bacteria living in the sea can tolerate temperatures below freezing, likewise, some bacteria can tolerate and grow in temperatures around 75°C (a very hot bath for us is about 60-65°C).

a) **psychrophiles** - cold-loving bacteria that grow best at 15-20°C. These bacteria generally live in cold water and soil - none can survive in the human body. These are the bacteria that cause food to spoil in the fridge.

b) **mesophiles** - majority of bugs, grow best between 25-40°C. Human pathogens are included in this group.

c) **thermophiles** - heat-loving bacteria, grow best at temperatures 50-60°C. Some can survive temps as high as 110°C in boiling hot springs, or, in deep-sea vents.

The temperature effect is generally on enzyme stability i.e. high temperatures tend to denature proteins. However, thermophiles are becoming attractive to industry because chemical reactions tend to be more efficient at higher temps. This means less money need be spent to obtain the final product.

(B) Nutrient factors Affecting Bacterial Growth

Generally the concentration of solutes (i.e. chemical growth components) is higher within the microbial cell than in the extracellular environment. The major barrier governing this differential passage of chemical components is the ***cell membrane***.

Membrane function is:
i) keep essential nutrients and macromolecules inside the cell.
ii) pump certain nutrients inside the cell **against** a concentration gradient.
iii) permit free flow of nutrients across the membrane.
iv) exclude some solutes within the environment from entry into the cell.

Nutritional Types for Bacteria

Nutritional classification of microorganisms based on carbon and energy sources

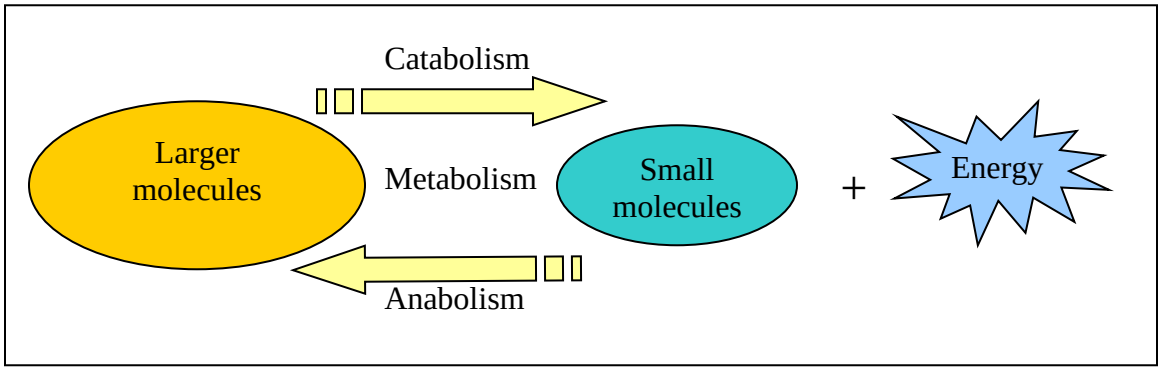
Nutritional type	Energy source	Carbon source	Examples
Chemoautotroph/ Chemolithotroph	Chemicals	Carbondioxide	Hydrogen, iron and nitrifying bacteria
Chemoheterotroph	Chemicals	Organic compounds	Animals (including humans) Protozoa, most bacteria and fungi
Photoautotroph	Light	Carbondioxide	Green plants, algae, purple and green bacteria
Photoheterotroph	Light	Organic compounds	Some cyanobacteria and purple and green bacteria

Lecture 9 Microbial metabolism

Metabolism is the sum of all the chemical processes carried out by living organisms. It includes

Anabolism : reactions that require energy to synthesise complex molecules from simpler ones. The molecules synthesised in this way are used for growth, reproduction and repair.

Catabolism : reactions that release energy by breaking complex molecules into simpler ones. Large molecules are always high in energy compared to smaller ones.



In general, there are two types of microbial metabolism

1. Anaerobic metabolism
    a. **Glycolysis** (oxidation of glucose to pyruvic acid)
    b. **Fermentation** (conversion of pyruvic acid to ethanol, lactic acid and other organic compounds)

2. Aerobic metabolism
    a. **Respiration** (oxidation of pyruvic acid to carbon dioxide and water)

I Anaerobic metabolism

A. Glycolysis

It is the metabolic pathway used by most autotrophic and heterotrophic organisms, few aerobes and majority of anaerobes. In this process, glucose is oxidised to pyruvic acid and it do not require oxygen. However, it can occur both in the presence and absence of oxygen.

There are 4 important steps involved in glycolytic pathway

1. Phosphorylation of glucose
2. Breaking of six carbon molecule (glucose) to three carbon molecule

3. Transfer of 2 electrons to coenzyme NAD
4. The capture of energy in ATP

1. Phosphorylation

Phosphorylation is the addition of phosphate group to a molecule from ATP (Adenosine Tri Phosphate). It increases the energy level of the molecule and often phosphate group serves as energy carriers in biochemical reactions. In glycolysis, 2 phosphate groups are added from 2 molecules of ATP (i.e., glucose → glucose- 5 phosphate → fructose 1,6-diphosphate). The addition of phosphates increases the energy level of glucose. Glucose then participates in the following reactions.

2. Breaking of six carbon molecule (glucose) to 2 three carbon molecules

After phosphorylation, Fructose 1,6 diphosphate breaks into 2 three-carbon molecules (i.e., formation of dihydroxyacetone phosphate and glyceraldehyde-3-phosphate).

3.Transfer of 2 electrons to coenzyme NAD

With the addition of phosphate, each molecule of Glyceraldehyde-3-phosphate is then oxidised to 1,3 diphosphoglyceric acid. It releases 2 electrons that are transferred to NAD (NAD → NADH).

4. Capture of energy in ATP

Energy is captured at substrate level (**Substrate Level Phosphorylation**) in 2 separate reactions later during

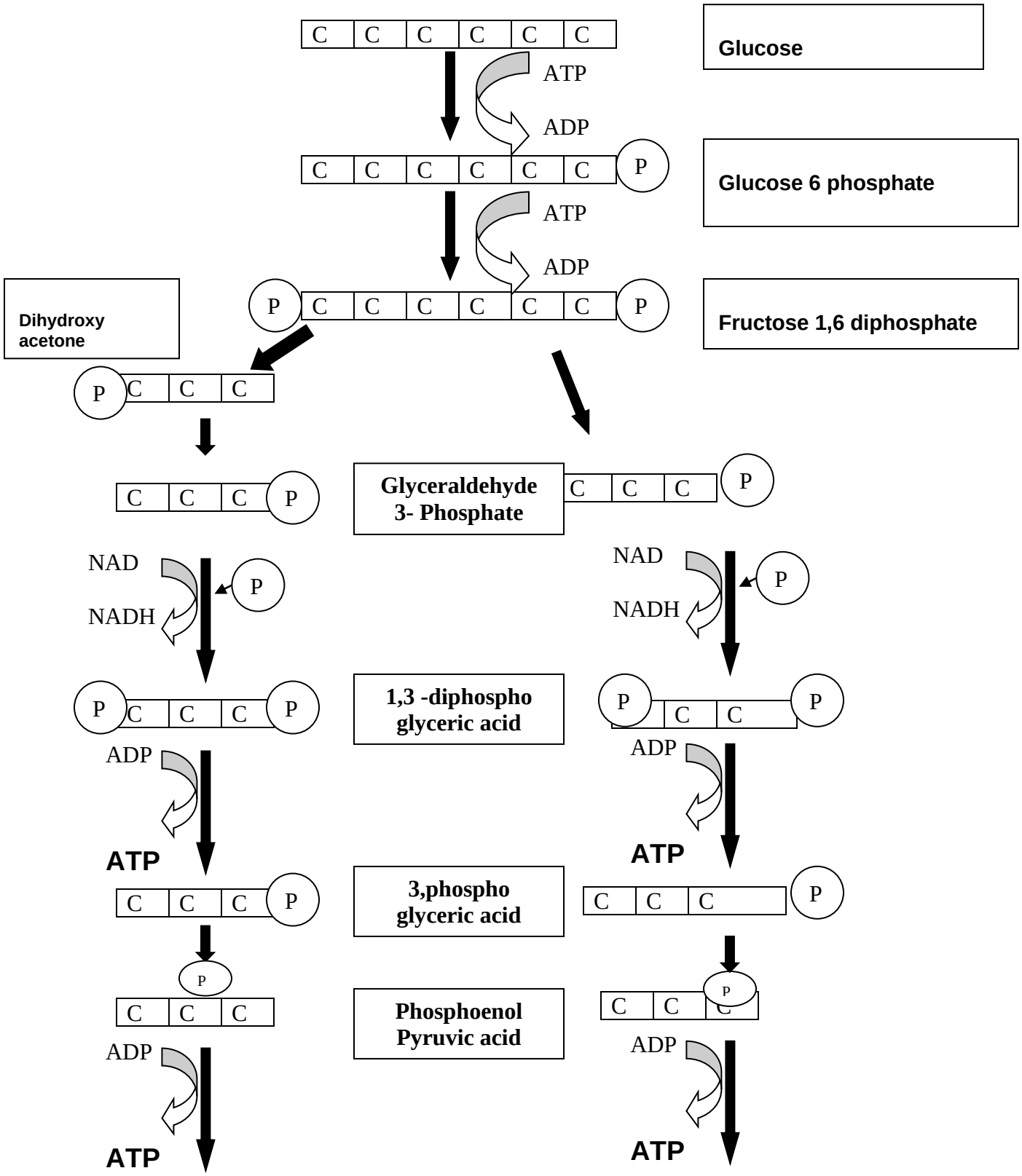
a) conversion of 1,3-diphosphoglyceric acid to 3-phosphoglyceric acid and
b) conversion of phosphoenol pyruvate to pyruvic acid

In substrate level phosphorylation, ATP is formed from ADP by transfer of a high-energy phosphate group from an intermediate.

ADP + Pi (phosphate) + Energy ⟶ ATP (Adenosine Tri Phosphate)

- Glycolysis provides cells with a relatively less amount of energy
- Glycolysis results in the formation of totally 4ATPs and 2 NADH from one glucose molecule
- Energy is captured in 2 ATPs during metabolism of one 3 carbon molecule. Hence from one glucose molecule totally 4 ATPs are formed. However, two ATPs are used initially for the start up process. Hence, the net ATP production is 2 ATPs
- Apart from glucose other sugars are also metabolised by microbes. These microbes contain certain specific enzymes that convert a sugar into an intermediate of glycolytic pathway.

Glycolysis / Glycolytic pathway
(Glucose to pyruvic acid)



Pyruvic acid ($CH_3COCOOH$) **Pyruvic acid ($CH_3COCOOH$)**

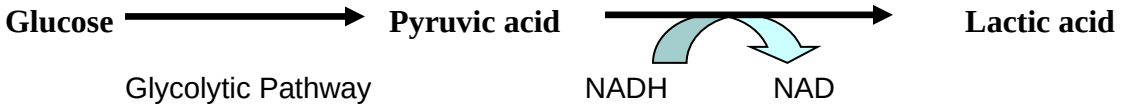
B. Fermentation

The metabolism of pyruvic acid (formed by glycolysis) in the absence of oxygen is termed as fermentation. During this process, electrons are passed on from NAD to other molecules by two different pathways.

a. Homolactic acid fermentation
b. Alcoholic fermentation

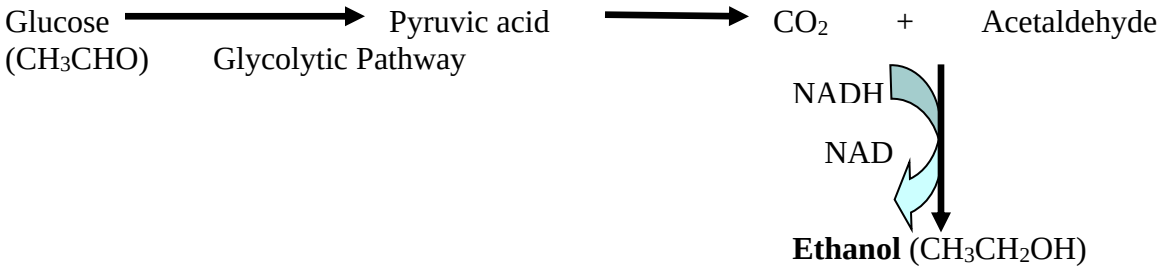
a. Homolactic acid fermentation

Only one acid (homo) i.e., lactic acid is formed from pyruvic acid by using 2 electrons from NAD. This process does not produce gas. It occurs in lactobacilli, streptococci and in mammalian muscle cells. This pathway is lactobacilli are used to make cheeses.



b. Alcoholic fermentation

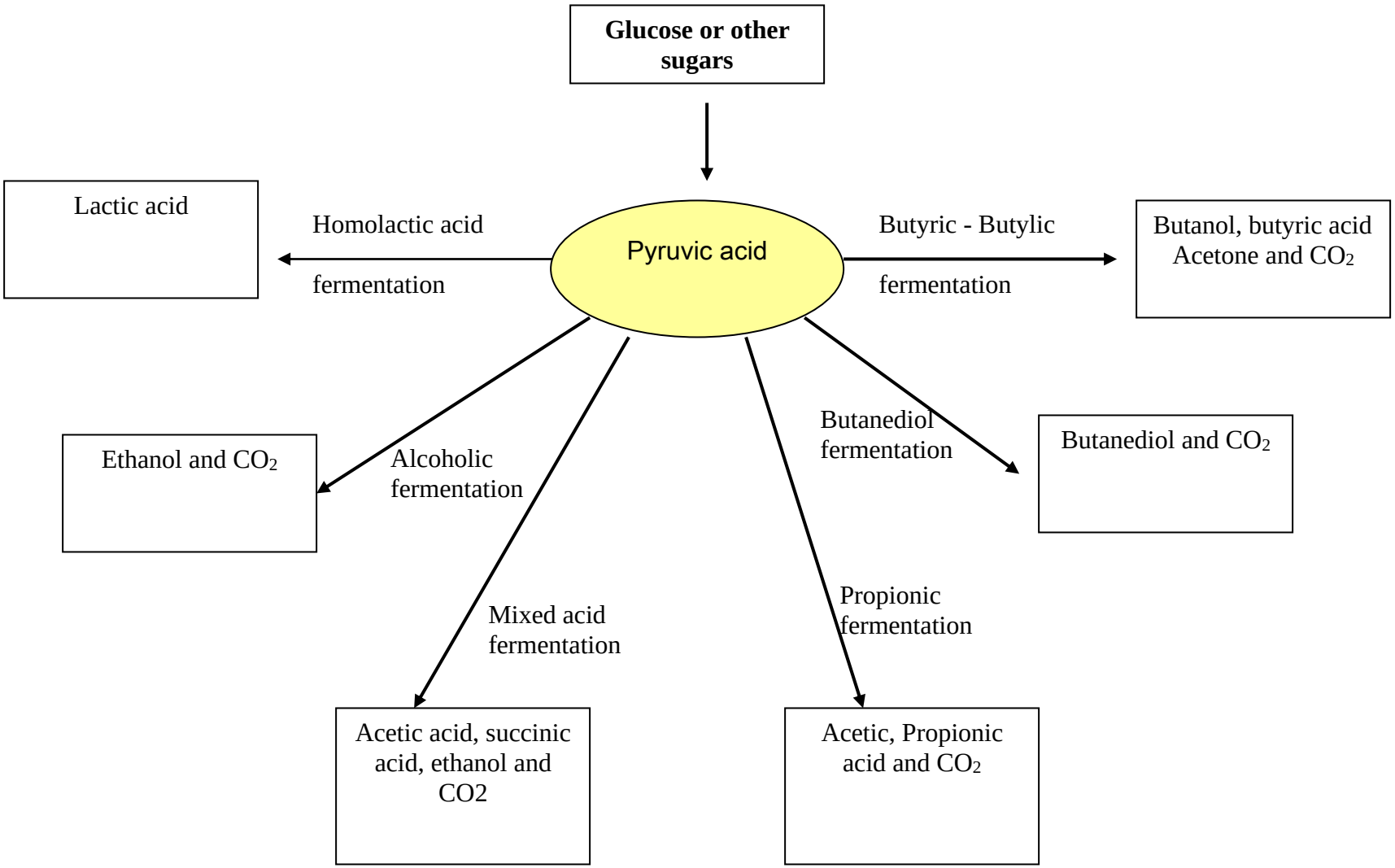
In alcoholic fermentation, carbondioxide is released from pyruvic acid to form acetaldehyde. Later this acetaldehyde is quickly reduced to ethanol by electrons from NADH. This fermentation is common in yeast, however less in bacteria. This process is widely used in making bread and wine.



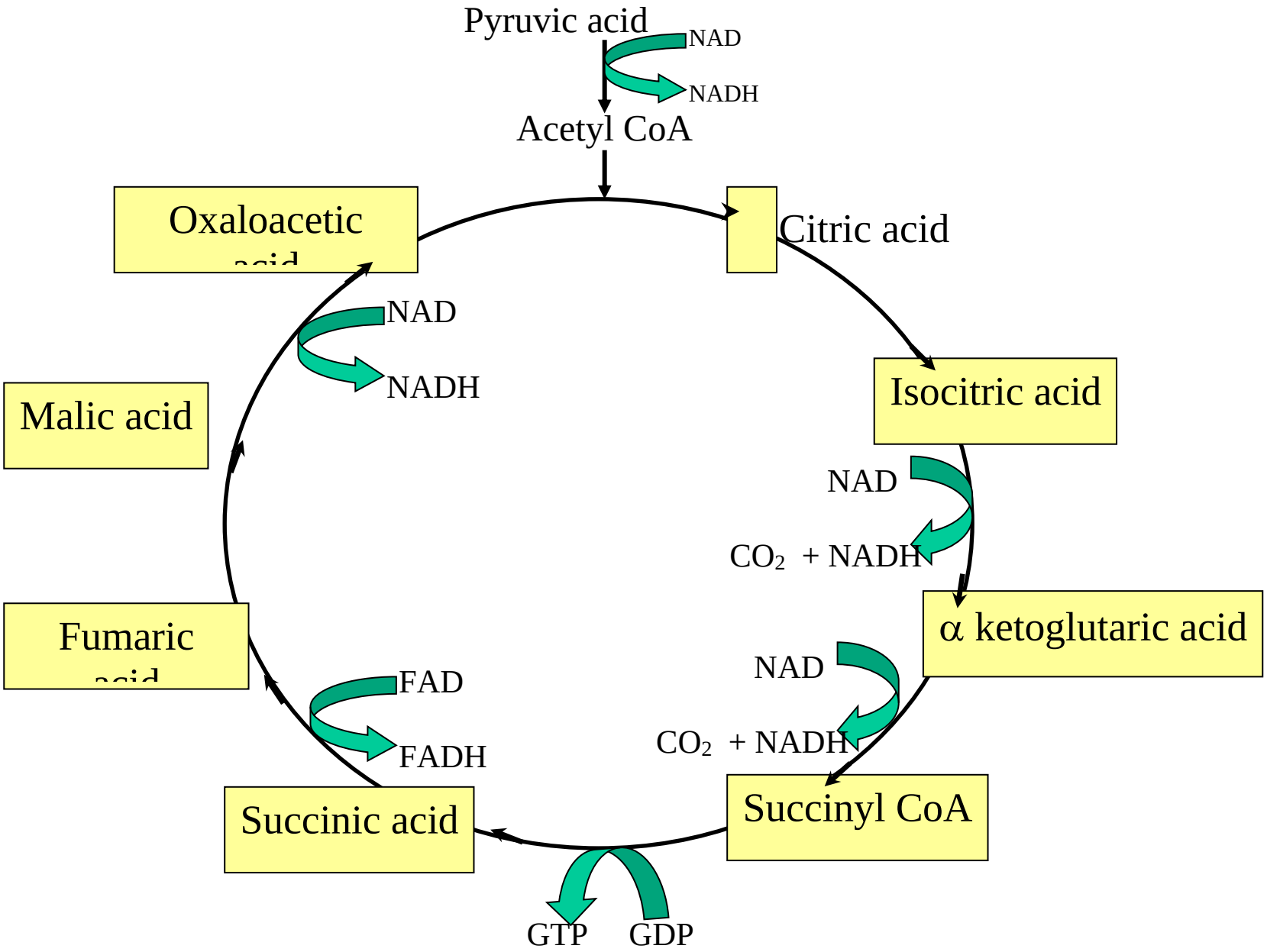
c. Other fermentations

- Butanediol fermentation
- Butyric – Butylic fermentation
- Acetic, propionic acid fermentation

Different fermentation pathways



KREBS / TCA/ CALVIN CYCLE



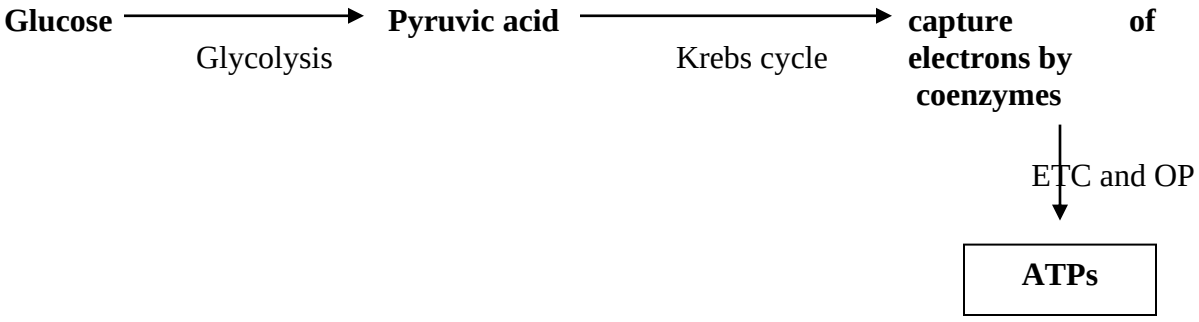
II Aerobic Metabolism

Respiration

Aerobic organisms to obtain more energy from pyruvic acid formed during glycolysis use this metabolic pathway (36 ATPs). In this process, pyruvic acid is completely oxidised to CO_2 and water. Aerobic respiration is carried out by

1. Kreb's cycle (also called as Calvin cycle/ Tricarboxylic acid, TCA cycle)
   followed by
2. Electron transport chain (ETC) and Oxidative Phosphorylation (OP)

Steps in aerobic respiration



1. Kreb's / Calvin / TCA cycle

Kreb's cycle is a sequence of reactions in which

- acetyl groups are oxidised to CO_2.
- Hydrogen atoms are removed and their electrons are transported to coenzymes. Hydrogen ultimately combines with oxygen to form H_2O.
- Each reaction is controlled by a specific enzyme
- The reactions form a cycle as the first reactant (oxaloacetic acid) is regenerated at the end of the cycle

(DRAW THE TCA CYCLE HERE)

Main events in TCA cycle

- Two molecules of CO_2 are released from complete oxidation
- Four pairs of electrons are transferred to coenzymes (3 pairs to NAD and one pair to FAD)
- Some energy is captured in GTP (Guanosine triphosphate)
- Since each molecule of glucose produces two molecules of acetyl CoA, the amount of products formed are doubled.

2. Electron transport and Oxidative Phosphorylation

Electron transport is the process leading to the transfer of electrons from substrate to oxygen through a series of oxidation-reduction reactions. Ultimately, energy is captured in high-energy bond as Pi to form ATP (ADP + Pi → ATP). This process of conversion of ADP to ATP by Pi obtained is called **oxidative phosphorylation**.

Compounds like NAD, FAD and cytochromes carry out this reaction. These carrier compounds form an **electron transport or respiratory chain**. Each compound in this chain gets gains electrons from previous compound and passes it on to next compound. At the end, this electron reduces O_2 to H_2O and released energy.

ATP generation by aerobic respiration

From one molecule of glucose,

10 pairs of electrons are trapped in NAD (2 pairs from glycolysis, 2 pairs from pyruvic acid to acetyl CoA conversion, 6 pairs from kreb's cycle)

2 pairs from FAD (Kreb's cycle) and

2 pairs from GDP (Kreb's cycle)

Source	Electron pairs	ATPs	Total ATPs
Glycolysis	-	2	2
NAD	10	10x3	30
FAD	2	2x2	4
GDP	2	2x1	2
Total			**38**

(One pair of electron from NAD can produce 3 ATPs, FAD can produce 2 ATPs and GDP can produce 1 ATP)

Lecture 10 GENETIC RECOMBINATION IN BACTERIA

Genetic recombination in bacteria

Genetic recombination is the transfer of DNA from one organism to another. The transferred donor DNA may then be integrated into the recipient's nucleoid by various mechanisms.

Mechanisms of genetic recombination:

- **Transformation**
- **Transduction and**
- **Conjugation**

Transformation

Bacterial transformation was first studied by Frederick Griffith while he was studying the pneumococcal infections in mice. Subsequently Avery, added more information of transformation in bacterial cells

Transformation is defined genetic recombination in which a **DNA fragment from a dead, degraded bacterium enters a competent recipient bacterium** and is exchanged for a piece of DNA of the recipient.

DNA fragments (usually about 20 genes long) from a **dead degraded bacterium** bind to DNA binding proteins on the surface of a competent recipient bacterium. Nuclease enzymes then cut the bound DNA into fragments. One strand is destroyed and the other penetrates the recipient bacterium. This DNA fragment from the donor is then exchanged for a piece of the recipient's DNA by means of Rec A proteins

Refer Fig.1 for more details

Transduction

Genetic recombination in which a **DNA fragment is transferred from one bacterium to another by a bacteriophage**

1. **Generalized transduction**

   Genetic recombination in which a DNA fragment is transferred from one bacterium to another by a lytic bacteriophage that is now carrying donor bacterial DNA due to an error in maturation during the lytic life cycle.

During the replication of a **lytic phage**, the capsid sometimes assembles around a small **fragment of bacterial DNA**. When this phage infects another bacterium, it injects the fragment of donor bacterial DNA into the recipient where it can be exchanged for a piece of the recipient's DNA. Plasmids (Small molecules of circular, extrachromosomal DNA found in some bacteria), such as the penicillinase plasmid of *Staphylococcus aureus*, may also be carried in a similar manner.

Refer Fig. 2 for more details

2. Specialized transduction

Genetic recombination in which a DNA fragment is transferred from one bacterium to another by a temperate bacteriophage that is now carrying donor bacterial DNA due to an error in spontaneous induction during the lysogenic life cycle.

This may occur occasionally during the lysogenic life cycle of a **temperate bacteriophage**. During spontaneous induction, **a small piece of bacterial DNA may sometimes be exchanged for a piece of phage genome** (that remains in the nucleoid). This piece of bacterial DNA replicates as a part of the phage genome and is put into **each phage capsid**. The phages are released, adsorb to recipient bacteria, and inject the donor bacterium DNA/phage DNA complex into the recipient bacterium where it inserts into its nucleoid.

Refer Fig.3 for more details

Conjugation

Genetic recombination in which there is a **transfer of DNA from a living donor bacterium** to a recipient bacterium. Often involves a sex pilus.

In Gram-negative bacteria, a **sex pilus (**A tubular protein structure extending from a bacterial surface used for attachment to environmental surfaces or cells. A sex pilus is involved in bacterial conjugation) produced by the donor bacterium binds to the recipient. The sex pilus then retracts, bringing the two bacteria in contact. In Gram-positive bacteria sticky surface molecules are produced that bring the two bacteria into contact. DNA is then transferred from the donor to the recipient.

There are three types of conjugation

1. **F^+ conjugation** *(def)*. This results in the transfer of an **F^+ plasmid** (coding only for a sex pilus) but not chromosomal DNA from a male donor bacterium to a female recipient bacterium. One plasmid strand enters the recipient bacterium while one strand remains in the donor. Each strand then makes a complementary copy. The recipient then becomes an F^+ male and can make a sex pilus. Other plasmids present in the cytoplasm of the bacterium, such as those coding for antibiotic resistance, may also be transferred during this process.

Refer Fig. 4

2.Hfr (high frequency recombinant) conjugation *(def)*. An F^+ plasmid inserts or integrates into the nucleoid (bacterial DNA) to form an Hfr male. The nucleoid then breaks in the middle of the inserted F^+ plasmid and one DNA strand begins to enter the recipient bacterium. The bacterial connection usually breaks before the transfer of the entire chromosome is completed so the remainder of the F^+ plasmid seldom enters the recipient. As a result, there is a transfer of some chromosomal DNA, that may be exchanged for a piece of the recipient's DNA, but not maleness.

Refer Fig.5

3. **Transfer of F' plasmids**

   In HFr bacterial cell, sometimes the F plasmid can get separated from bacterial DNA. In some cases this occurs improperly and a fragment of bacterial DNA is carried out along with F-plasmid, creating what is called as F' plasmid (F prime plasmid). Cells containing F' plasmids are termed as F' strains. When F' cells conjugate with F^- cells the whole plasmid (including the genes from bacterial DNA) is transferred. Hence, the recepient cells have two of some chromosomal genes – one on the bacterial DNA and one associated with plasmid

   Refer Fig. 6

4. **Resistance plasmid conjugation** *(def)*. This results in the transfer of a **resistance plasmid (R-plasmid)** from a donor bacterium to a recipient. R plasmid is one having genes coding for multiple antibiotic resistance and often a sex pilus.

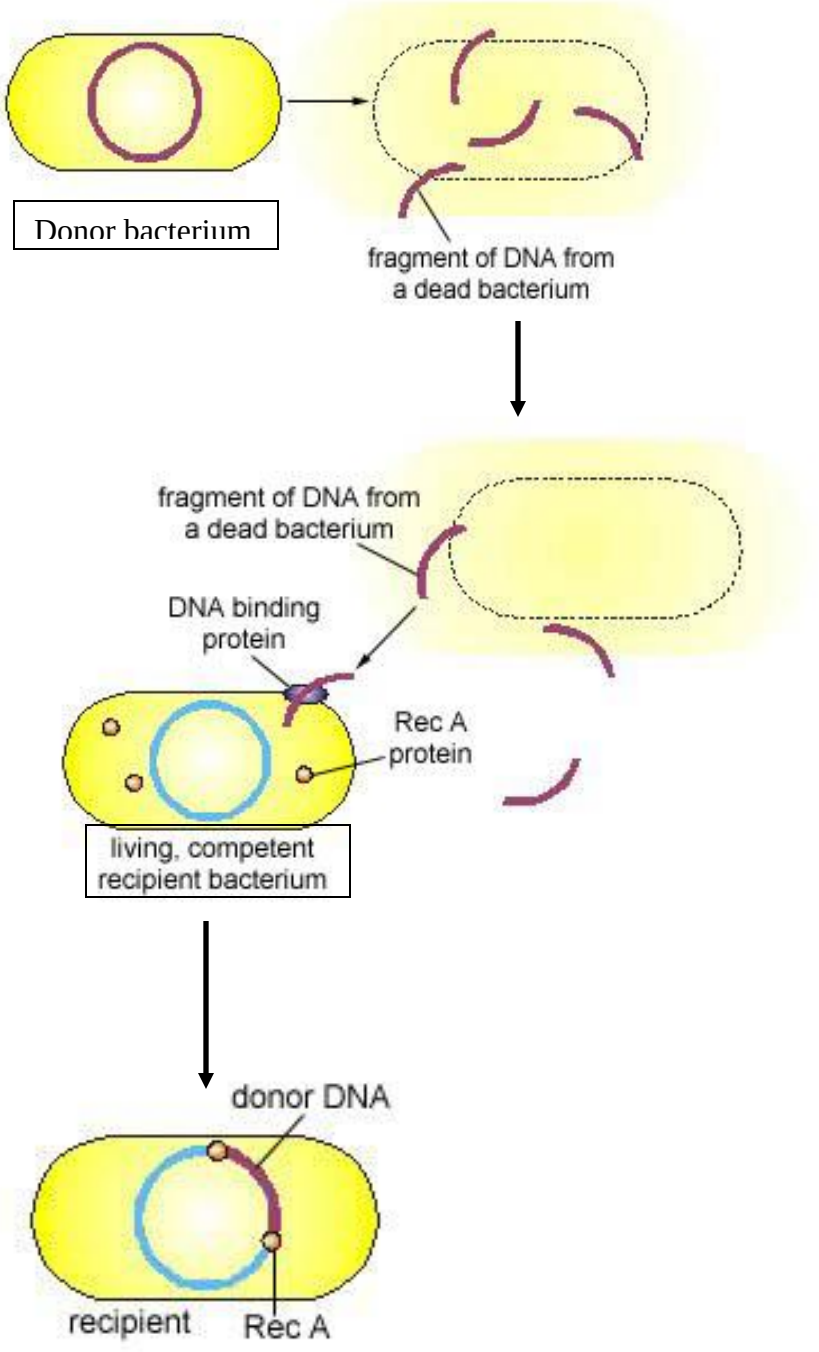
   One plasmid strand enters the recipient bacterium while one strand remains in the donor. Each strand then makes a complementary copy. The R-plasmid has genes coding for **multiple antibiotic resistance** and **sex pilus formation**. **The recipient becomes multiple antibiotic resistant and male**, and is now able to transfer R-plasmids to other bacteria.

   Refer Fig.7

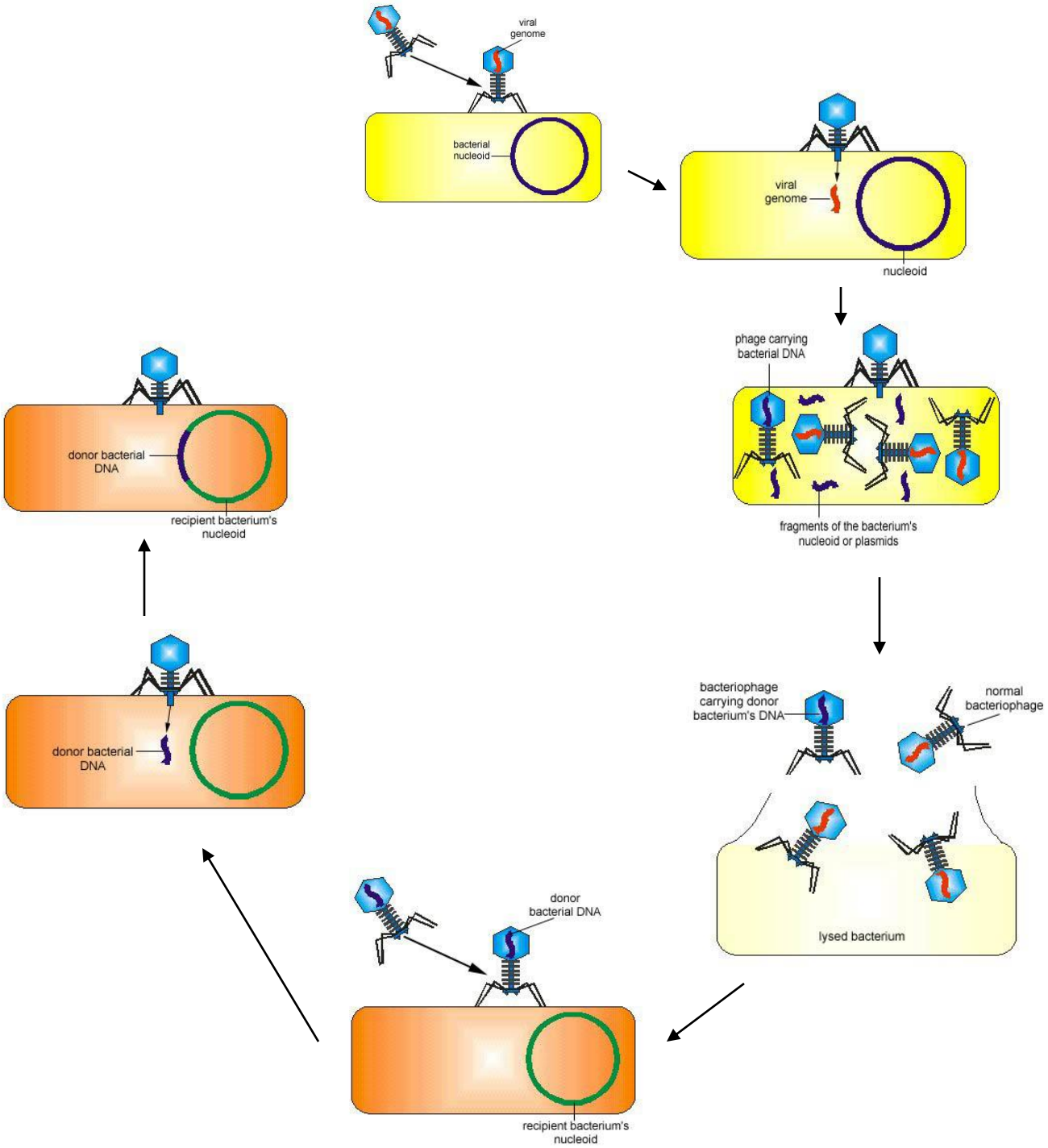
   This is a big problem in treating opportunistic gram-negative infections (urinary tract infections, wound infections, pneumonia, septicemia) by such organisms as *E. coli, Proteus, Klebsiella, Enterobacter, Serratia*, and *Pseudomonas,* as well as with intestinal infections by organisms like *Salmonella* and *Shigella* (see below).

   Genes in bacteria can also be altered artificially through recombinant DNA technology. In recombinant DNA technology, discussed later in this unit, endonuclease and ligase enzymes are routinely employed. Restriction endonuclease enzymes are naturally occurring enzymes in bacteria that help protect bacteria from viral attack by cutting up the foreign viral DNA while not harming the bacterium's own DNA. **Restriction endonuclease enzymes** recognize specific palandromic deoxyribonucleotide base sequences (base sequences that read the same forward and backward on the complementary DNA strands), and then split each DNA strand at a specific site within that sequence. For example, *Escherichia coli* makes a restriction endonuclease called *Eco* R1 that recognizes the deoxyribonucleotide base sequence G-A-A-T-T-C and cuts the DNA strand between the G and the A. Since the complementary strand has the sequence CTTAAG, it is also cut between the G and the A. This leaves short, complementary, single-stranded **sticky ends** capable of hydrogen bonding with the complementary sticky ends of DNA fragments cut by the same enzyme.

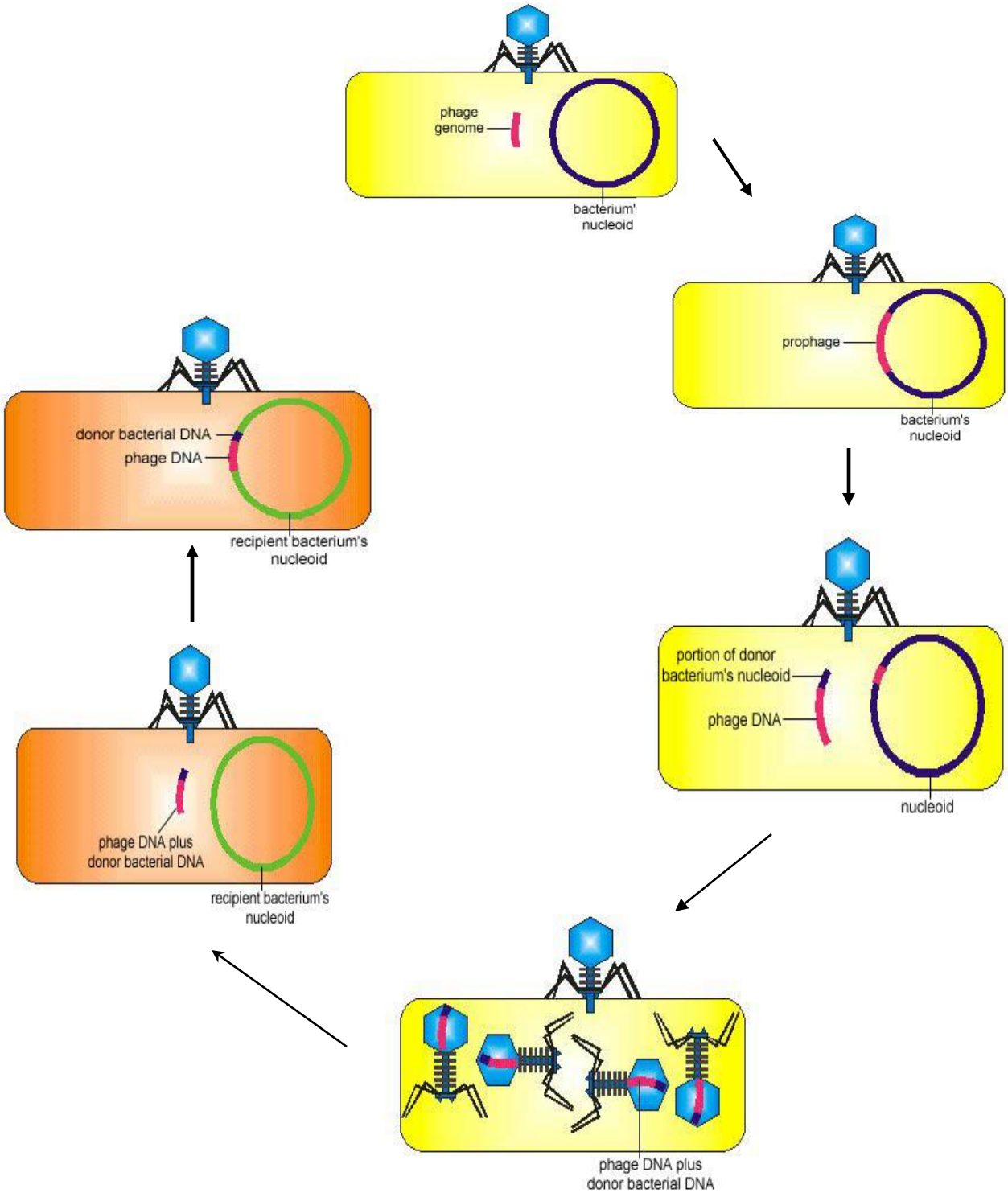
Transformation



Generalised Transduction

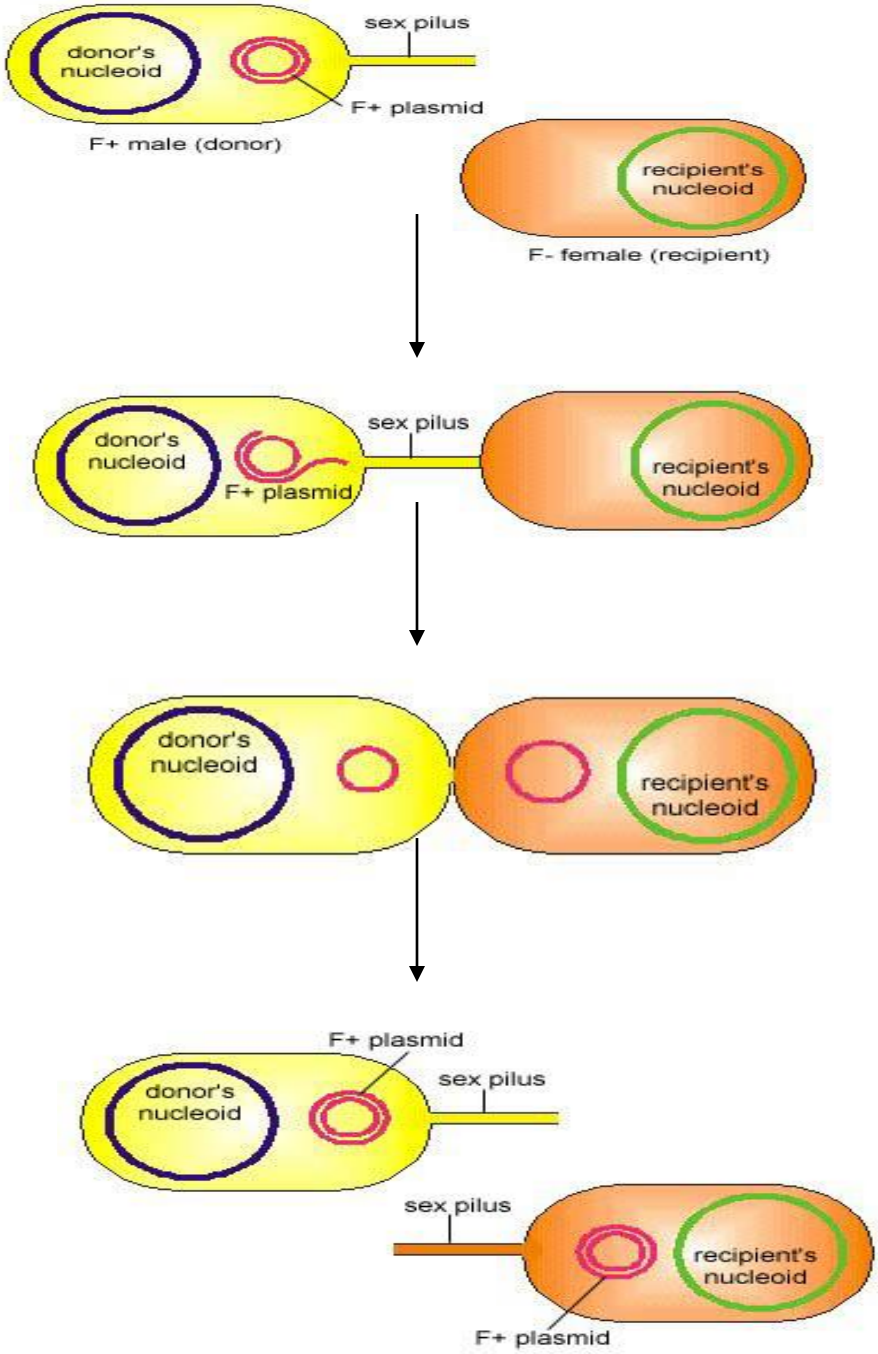


Specialised Transdution

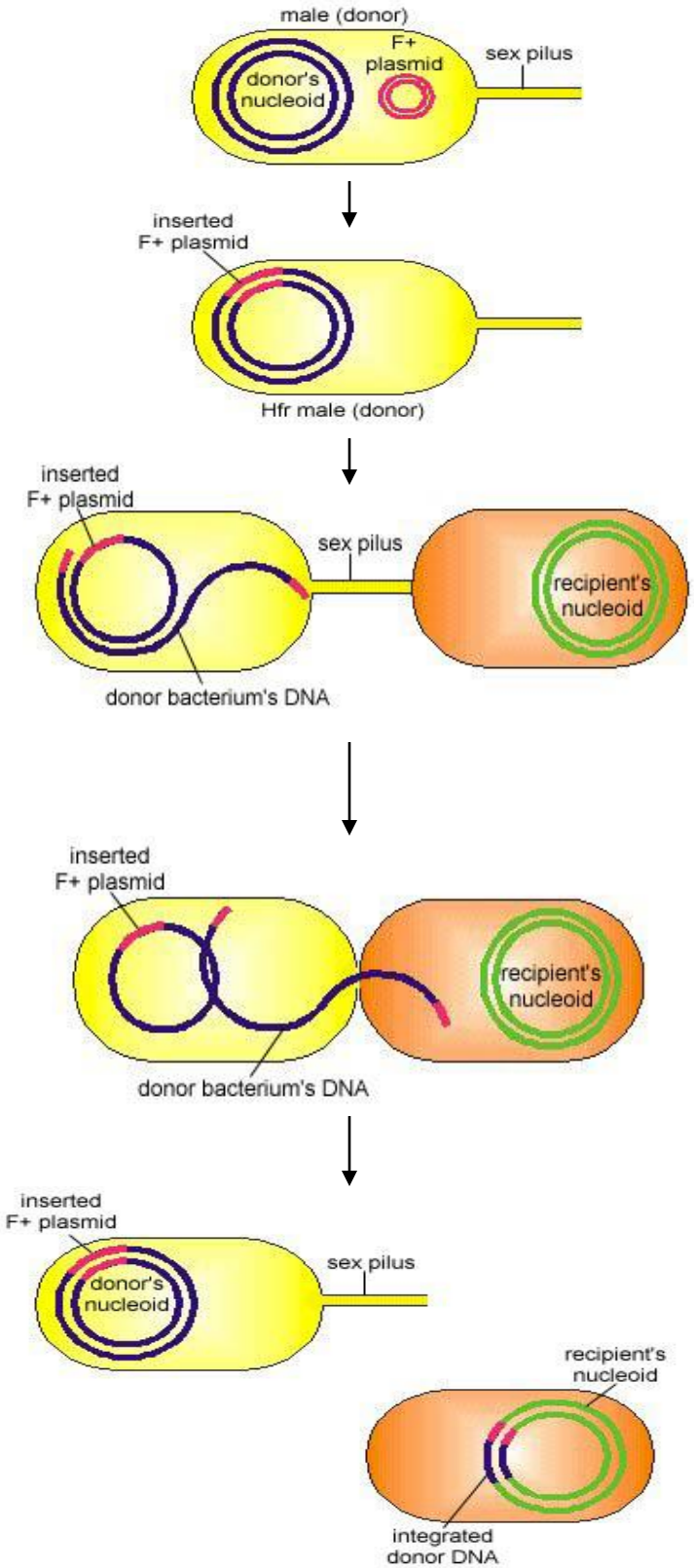


Conjugation

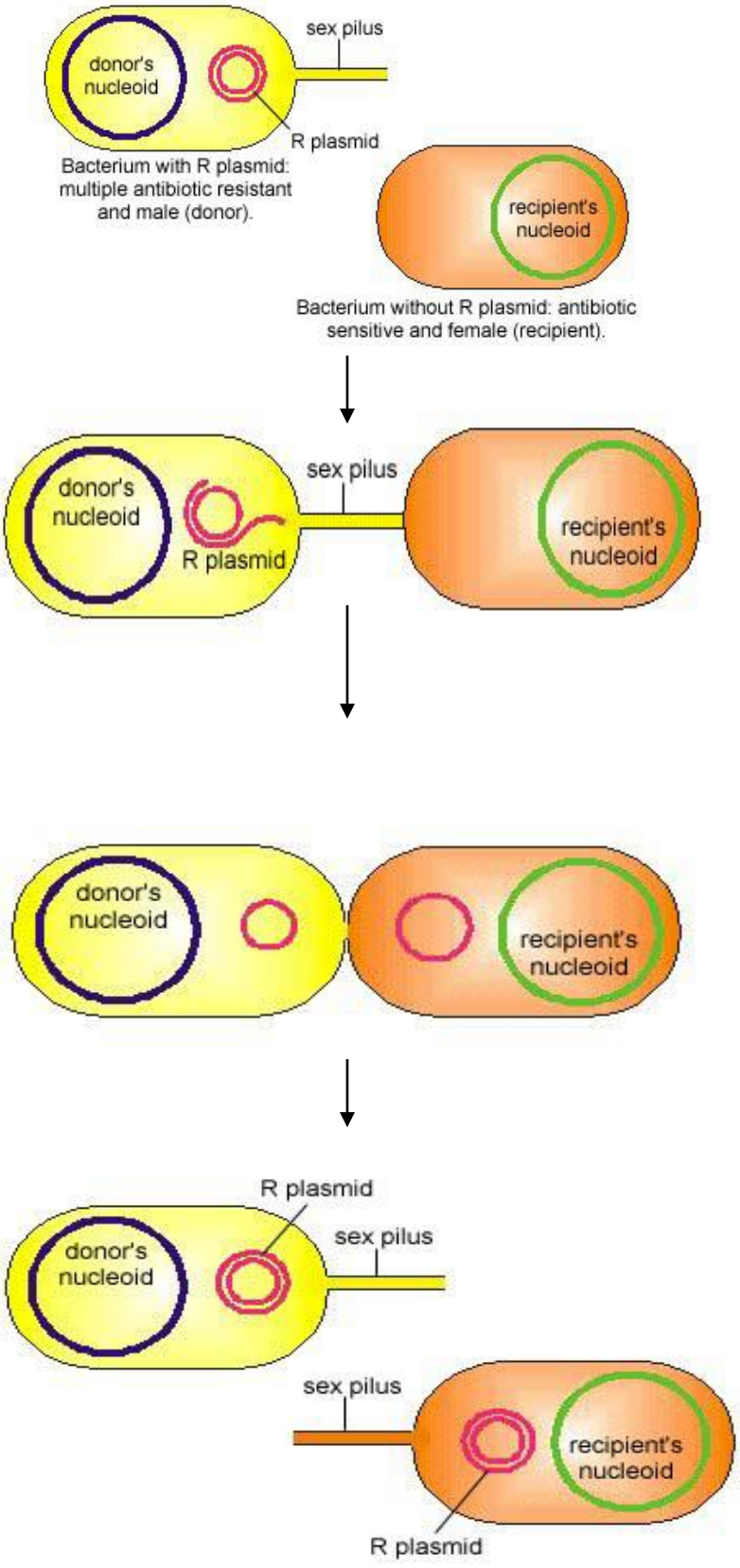
F+ conjugation



HFr Conjugation



R-Plasmid Conjugation



Lecture 11 Immunology

Immunity is the ability of an organism to recognise and defend itself against infectious agents. **Immunology** is the study of specific immunity and how the immune system responds to specific infectious agents. The immune system consists of various cells, especially lymphocytes and organs such as the thymus gland.

Types of immunity

1. Innate immunity
2. Acquired immunity
3. Active immunity
4. Passive immunity

Innate immunity/ Genetic immunity

This immunity is genetically determined. E.g., Species immunity. All human beings have immunity to infectious agents from pet animals. Similarly the animals have immunity to human diseases. *Bacillus anthracis* causes diseases in all mammals and some birds but not other animals.

Acquired immunity

This is immunity acquired in some manner other than heredity. It can be acquired either naturally or artificially. Naturally acquired immunity is most often obtained through having specific diseases. During the course of disease, the host produces antibodies and creates specific defense against that disease. In contrast, artificially acquired immunity is obtained by receiving the antigen by injection of vaccine or antigen.

Active immunity

Active immunity is created when the person's own immune system produces antibodies or other defenses against an infectious agent. This can last for a lifetime or weeks or months.

a. Naturally acquired active immunity is produced when the person is exposed to an infectious agent
b. Artificially acquired active immunity is produced when a person is injected with the vaccine of live, weakened organisms or toxins.

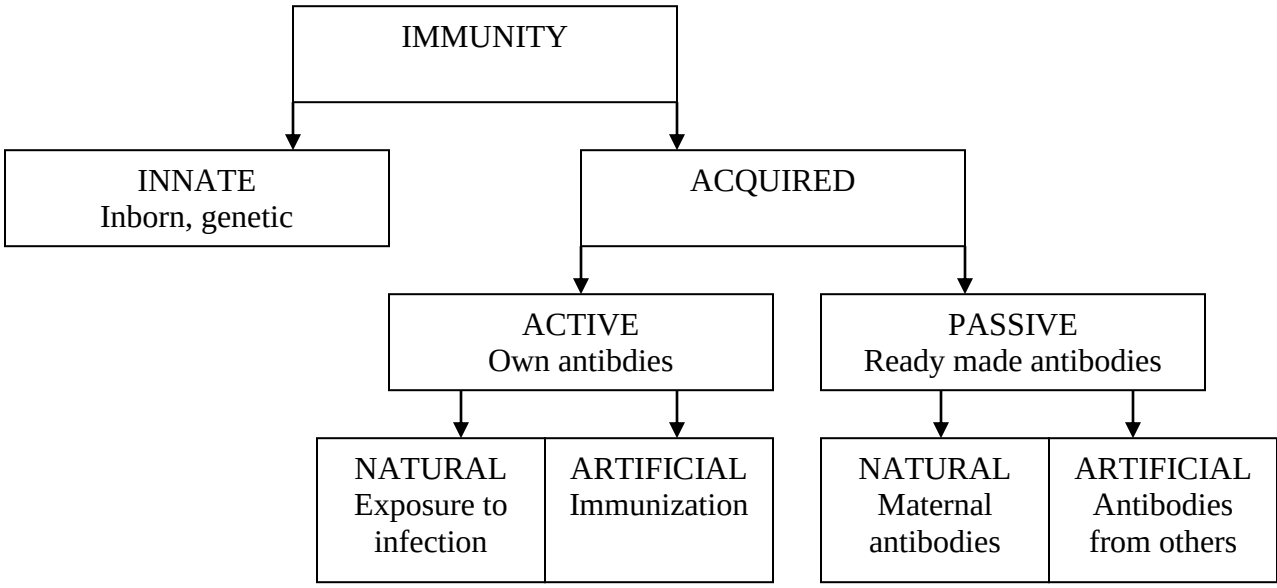
In both natural and artificial types, it's the person's immune system responding to the specific disease.

Passive immunity

Passive immunity is created when ready-made antibodies are introduced into the body. This immunity is passive because the host's own immune system does not produce antibodies.

Naturally acquired passive immunity is produced when antibodies made by mother's immune system pass on to the babies. New mothers are encouraged to breast feed as the colostrum (protein rich milk secreted by mammary glands) contains lot of antibodies.

Artificially acquired passive immunity is produced when antibodies produced by other host are introduced into new host. For e.g,. a person bitten by snake is given antivenin injection. Antivenins are antibodies produced in another host like horses and rabbits.



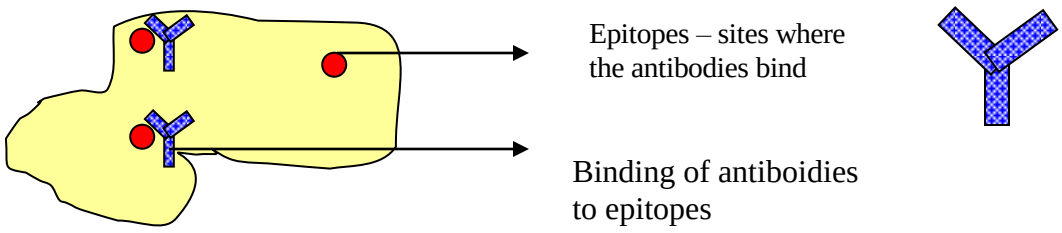
Antigen and Antibodies

Antigen is a foreign substance into the body towards which the body mounts an immune response. It is also called as **immunogen**. Most antigens are large complex protein molecules with complex structures. Some antigens are polysaccharides and few are glycoproteins. The proteins usually have more antigenic strength compared to polysaccharides. Also these complex protein molecules contain sites called **epitopes**, where the antibodies bind. In some instances, a small molecule called **hapten** can also act as an antigen if it binds to a large protein molecule.

e.g., antigens are found on surface or viruses, bacteria and other microorganisms. Bacteria have antigens on capsules, cell wall and flagella. Antigens on the surfaces of red blood cells determine the blood group and other cells determine whether the tissue transplanted from one person will be rejected.

Antigen (a protein molecule) with epitopes

Antibodies



Antibodies (or **Immunoglobulins, Ig**) are Y shaped protein molecules produced in response to an antigen. These are highly specific. Each kind of antibody binds to a specific epitope. This binding may or may not contribute to inactivation of the antigen.

Y shaped protein molecules are composed of four polypeptide chains, two identical **light chains** and two identical **heavy chains**. Disulfide bonds hold these chains together. These disulfide bonds have *constant regions* and *variable regions*.

The constant regions determine the class of that immunoglobulin

The variable regions bind the molecule to a specific antigen (They have definite shape and charge)

There are five classes of immunoglobulins identified in humans and higher vertebrates. They are **IgG, IgA, IgM, IgE and IgD**. All these have a antigen binding site and a tissue binding site.

IgG is the main class of antibodies found in blood and accounts for 20%. IgG is produced in larger quantities during secondary response. The antigen binding sites are attached to antigen and the tissue binding sites are attached to phagocytic cells. IgG is the only immunoglobulin that can cross the placenta and provide antibodies to the foetus.

IgA occurs in small amounts in blood and more in body secretions like tears, milk, saliva and mucus. The main function of IgA is to bind antigens before they enter tissues. IgA does not cross the placenta but is plenty in colostrum.

IgM is the first antibody secreted into blood and is produced by both B cells and plasma cells. As IgM binds to antigen, it causes microbes to clump together.

IgE is also called reagin. Its antigen-binding site is attached to allergens like pollen, drugs and certain food. The tissue-binding site is attached to plasma membrane of basophils. Asthma and hay fever are common allergic symptoms to pollen. IgE are elevated in patients with allergies are found mainly in body fluids and skin.

IgD is found mainly ob B cell membranes and rarely secreted. Though it binds to antigens, its function is unknown.

Dual Nature of immune system

Lymphocytes give rise to two types of immune responses, **humoral immunity** and **cell- mediated immunity**.

Humoral immunity

- Carried out by antibodies circulating in the blood.
- When stimulated by antigen, B cells initiate the process and releases antibodies
- Is most effective in defending against bacteria, bacterial toxins and viruses before they enter the cells.

Cell mediated immunity

- It occurs at cellular level where antigens are embedded inside the host cells.
- T cells initiate the process of releasing antibodies
- Is most effective in clearing virus infected cells, cancer and foreign tissues

How antibodies are produced ?

- Initiation of antibody production occurs in host' B cells (B lymphocytes) or T cells (T lymphocytes) that re derived by differentiation of stem cells.
- The B cells grow and mature. A number of B cells are formed which are specific for a particular antigen.
- When an antigen enters the host, the B cells specific for that antigen gets activated and form B clones.
- These clones further divide to form lot of plasma cells
- Plasma cells synthesise the antibodies specific for that antigen and are released into blood stream
- In blood stream, the antibodies complexes with antigens (microbes, allergens, etc) and causes neutralisation, opsonisation and ultimately cell lysis.

Kinds of antigen antibody reaction

1. Neutralisation: Antibodies for bacterial toxins, viruses. Since these are very small they are inactivated just by forming antigen-antibody complex. It stops toxin from further damage to the host.

IgG is main neutraliser for bacterial toxins and IgM, IgG and IgA are effective against viruses.

2. Opsonisation : Antibodies neutralise the toxins and coat microbes so that they can be phagocytized (engulfed and digested)

3.Cell lysis : destruction of cells by antibodies e.g., IgM

4. Agglutination: The bacterial cells are relatively large and the antigen antibody complexes will also be large. Such reactions are called agglutination (sticking to microbes)

Monoclonal Antibodies

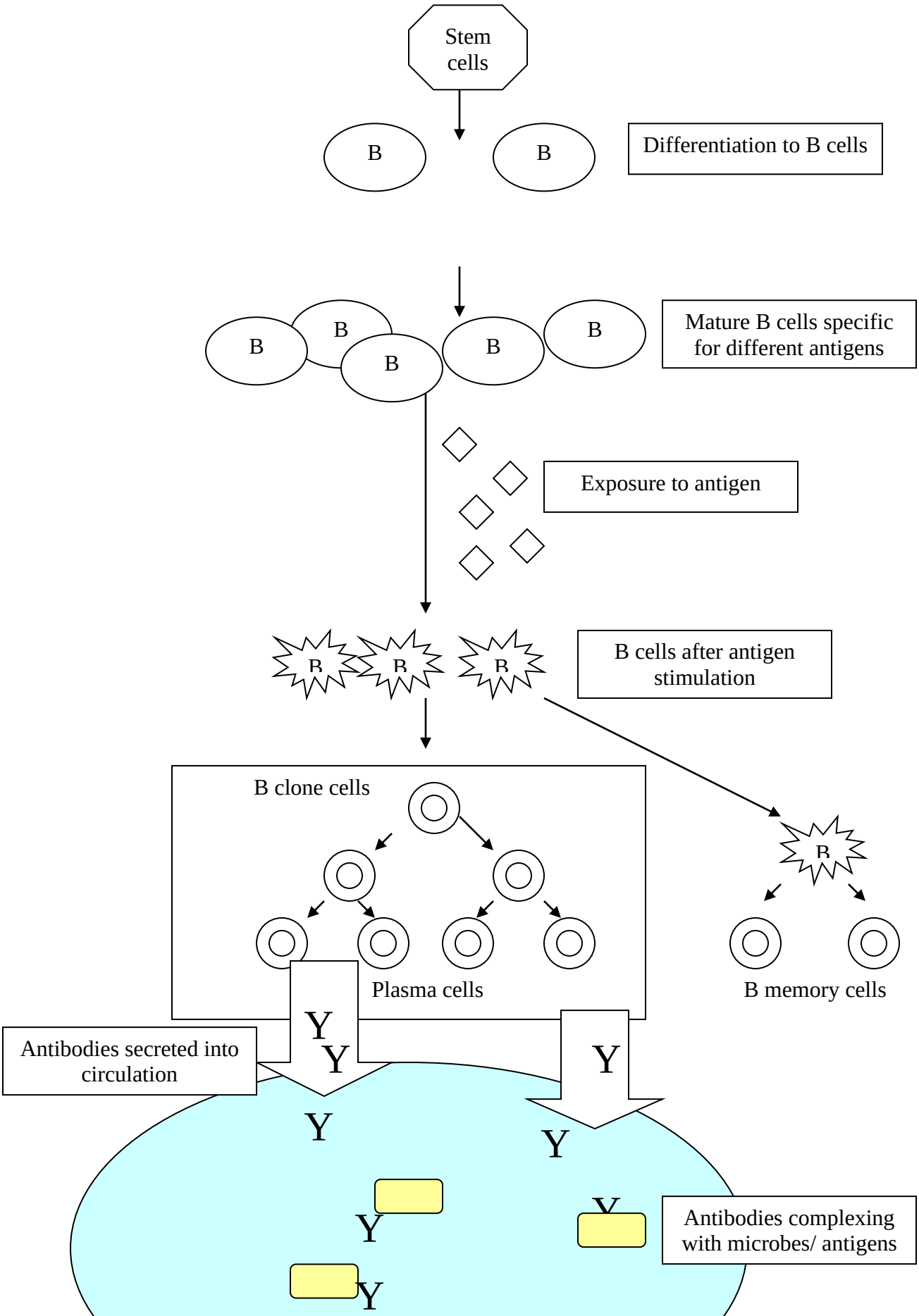
A single pure antibody produced in the laboratory by a clone of cultured hybridoma cells.

Coenzymes

Coenzyme is a non-protein organic molecule bound to or loosely associated with an enzyme.
NAD – Nicotinamide Adenine Dinucleotide
FAD – Flavin Adenine dinucleotide

Oxidation/ Reduction

Reduced compound - Gain electrons – gain energy
Oxidised compound - Lose electron – lose energy


Stem cells
B
B
Differentiation to B cells
B
B
B
B
B
Mature B cells specific for different antigens
Exposure to antigen
B
B
B
B cells after antigen stimulation
B clone cells
B
Plasma cells
B memory cells
Y
Y
Y
Y
Y
Y
Antibodies secreted into circulation
Antibodies complexing with microbes/ antigens

Previous Question papers

June 2004 (AGM 101)

An example of unicellular fungus …….

The main constituent of cell wall of bacteria

The antibiotic streptomycin was discovered by …..

In bacterial cell, the flagella are arranged throughout the body then it is called as …… flagellation

Virus is an ……parasite

Pure culture technique was developed by ……..

The highest resolving power is …… microscope

True or false

The bacillus subtilis is an example for gram positive

The gelling agent used first in solid media is gelatin

Illumination with transmitted light is achieved in microscope by objective

Louis Pasteur discovered vaccine for the prevention of small pox

The theory of spontaneous generation was disproved by spallanzani

The liquid medium is sterilized in hot air oven at a temperature of 160C for 2 hrs

Define

Sterilization, Saprophytes, Pili, antibiotic, endospore, numerical aperture

Choose the correct answer

The taxonomy that classify a bacterium giving equal weightage of several characteristics for each strain

Genetic relatedness, intuitive method, numerical , none of these

Which of the following is an example for cyanobacteria

Rhodospirillum, Rhizobium, Acetobacter, Nostoc

The wavelength of light in germicidal UV lamp is

4000A, 2650A, 3850A, 5650A

The bacteria can be purified by ……..….method

Single hyphal tip method, streak plate method, single spore isolation, none of these

The term animalcule was coined by

Louis Pasteur, Leeuwenhock, Needham, Edward Jenner

The bacterial DNA is transferred from one cell to an other with the help of virus is known as ……..

Conjugation, Transduction, mutation, transformation

Short notes

Germ theory of disease, transduction, contributions fron joseph lister, morphology of virus, numerical taxonomy, oligotrophic bacteria, mutation

Brief answers

Define growth curv. Mentionfour important phases of growth curve. Factors influencing growth curv

Describe the principles of phase contrast microscope. Write its advantages and limitations

Give and outline classification of bacteria

Describe the gene transfer techniques in bacteria

Describe the principles and operations of autoclave. What are the materials sterilized in it

June 2004 (AGM 201)

The vaccination to prevent small pox was introduced by ……..

An example for anaerobic spore forming bacteria ……..

Ribosomes of prokaryotic organisms are of …..

An example of mordant is …….

Cell membrane of mycoplasma contain ……

The scientist who discovered streptomycin is ……

The gene transfer through pili in bacteria is known as ……..

Intermittent sterilization is also known as ……..

The molecules produced in the body in response to antigens are ……

The fluorescent dye used in UV microscope is ……..

The vegetative body of fungus is known as ……..

The cold loving bacteria are called as ……..

Define : Endospore, Thermophile, phototroph, pasteurization, capsule, fermentation

Short notes : Transduction in bacteria, algae, nutritional types of bacteria, negative staining, mycoplasma, spontaneous generation theory.
Brief questions
Define generation time and describe the bacterial growth curve with illustrations
Give an account of principles and techniques involved in the use of hot air oven and UV lamp
Describe the different types of microscopes and their uses
Describe the principles an immunology and explain the antigen antibody reaction
Write briefly the historical development of modern microbiology

Question papers 2002
The bacteria generally reproduce by …….
The energy source for heterotrophs …….
The cell wall of prokaryotes are made of …….
The father of immunology is ……..
The antibiotic streptomycin was discovered by the scientist ……
An example for asexual method in surgery was first introduced by …….
The bacteria that survive under low temperature condition below 10 are called ….
The genetic principle is transmitted between bacteria by ……. in transduction
It is possible to crystallise …… group of MO
The cotton type of growth produced by fungi is called ……
An example for anaerobe……..
ELISA – Enzyme Linked Immunosorbent Assay
Define
Autotrophs – Respiration – Resolving power – Structural staining – Respiration – Antibody – Resolving Power – Mutation
Short notes
Sterilisation – Bacteriophage – Transformation – Phase contrast microscope – Fermentation – Types of arrangement of bacterial cells
Explain
Draw a neat diagram of a typical prokaryotic cell, label all parts and write in brief any four structures in the prokaryotic cell
Discuss briefly on the factors influencing bacterial growth
Write in detail abut the principles of immunology
Describe the transfer of genetic material through conjugation
Explain the theory of spontaneous generation

2002 – special semester
Define
Prokaryotic – conjugation – phagocytosis – tyndallization – endospore – immunoglobulins – micron – antigen – dryheat sterilisation – staining – penicillium – pure culture
Explain briefly
Antibiotics – Heterocysts – viruses – pasteurisatio – micrometry – immobilisation
Differentiate the light and dark microscopy
What is the nature of growth curve in bacteria
Mention about the reproduction in algae
Mention the economically important products of microbial origin
Write a note on koch postulates
Differentiate germicide and bactericide
Draw a typical bacterial cell and label the parts
Differentiate pro and eukaryotes
Describe the methods of preventing microbial contamination
What are culture media ? Explain different nutritional media

2001
genetic code – Pure culture – Glycolysis – Co enzymes – Akinetes- m RNA – Myxotrophs – Numerical aperture – Immunodiffusion – Mesosomes – Phototrophs – Chlamydospores

Name the contributions of Robert Koch
Mention contributors to spontaneous generation theory
Write the advantages of phase contrast microscope
Name two functions of mesosomes
Give two importance of yeasts
Give the classification based on oxygen requirement
Describe streak plate method
Explain negative staining technique
Draw the morphological features of Penicillium sp. and name them. Name two species of economic importance of the genus
What is substrate level phosphorylation. Explain it
Explain the process of bacterial transduction
Explain the antibodies. List different immunoglobulins
Define mutation, Explain different mutations in bacteria
Give the structural features of a bacterial cell membrane and explain its role in metabolism
Give outline function of fungi with few examples in each
Give the important characters of myxobacteria, sheathed bacteria and oxygenic photosynthetic bacteria
What is microbial metabolism – explain different types of fermentations

1999
Swan neck flask – pleomorphism – heterokaryosis – antibiotics – meiosis – amphitrichous – counterstain – disinfection – lithotrophs – glycolysis – zygospore – antigen
Name any two contributions made by Robert Koch
Name any two chemolithotrophs
What are the principles involved in gram staining
How the bacteria reproduce
Name two microorganisms served as food
Explain the term lysogenic bacterium
Who disproved the theory the spontaneous generation and how ?
Describe single spore isolation method
Write a short account on bacteriophage
Describe sexual reproduction in yeast
Define koch postulates and give in significance
Describe the various parts of a bacterial cell with diagram
Briefly explain the contributiosn made by Selman A waksmann
Differentiate between prokaryotes and Eukaryotes
Write a short account on the biosynthesis of proteins
Draw a growth curve and explain different stages and its significance
Give an outline classification of viruses

1995
Sterilisation – autotrophs – magnification – bacteriophages – vaccines – conjugation – RNA – mutation – anaerobic – complex medium – phagocytosis
Lytic cycle – Plasmids – Antigen antibody complex – cyanobacteria – Louis pasteur – endospores – yeasts – autoclave – ascomycetes – streptococci – flagella – phenol coefficient
Explain with the help of a neat diagram the typical growth curve of a bacterial culture
Give a brief note on the classification of fungi
Discuss the economic significance of different types of algae
Explain the principle and use of UV microscope
Explain different chemical methods for microbial control
How do you classify bacteria based on their temperature requirement ? Explain the groups
Write an essay on the role of MO in the welfare of human beings
Explain the different steps and changes during gram staining

May 1995
Sterilisation – binary fission – autotrophs – magnification – bacteriophages – vaccines – conjugation – RNA – mutation – anaerobic – complex medium – phagocytosis
Lytic cycle – plasmids – antigen-antibody complex – cyanobacteria – louis pasteur – endospores – yeasts – autoclave – ascomycetes – streptococci – flagella – phenol coefficient
Explain with a neat diagram the typical growth curve of bacterial culture

Give a brief account on the classification of fungi
Discuss the economic significance of different types of algae
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