Introduction

Physiology of fungi is a branch of science which deals with studying the functions of these microorganisms, forms of manifestations and their activities of feeding, growth and reproduction. There is no doubt that these processes are related to the shape and structure of the living organism. It is often this science scholar familiar with the composition and structure of these organisms. The larger role carried out by the fungi in nature is to get rid of the remains of dead plants and dead wood by their consumption and growth on it, they can produce other materials to help other living organisms to grow in a manner directly and indirectly. Since knowing the cultivation of fungi used in many beneficial purposes for humans as using yeast in the baking industry, the production of other species within the food industry and some industries such as alcohol, which is used in laboratories, industry and organic acids, antibiotics, vitamins....etc. This branch of science concerned with the study of biological processes carried out by these organisms and their activities, as well as its impact on the surrounding factors and the impact of these factors.

$$C_6H_{12}O_6 \xrightarrow{\text{Enzyme}} 2C_2H_5OH + 2\ CO_2 + \text{ENERGY}$$

This branch of science, like any other science is closely related to other sciences such as heredity, chemistry, physics, anatomy and cell biology and other sciences. Most of the basic principles that govern the physiology of fungi are almost the same principles that govern any other group and this is reflected clearly that the vitamins necessary for their growth are almost the same for the growth of human, animal and higher plants, where the function of vitamins and one in each of those. However, there is a slight difference in the ability of these test organisms on the synthesis of some vitamins without the other. In order to understand the scholar's problems other sciences such as plant diseases it is necessary to study the parasitic fungus away from the host to show nutritional needs that requires the fungus and perhaps helped to know the methods of resistance where at the present time

some obligate fungi are difficult to develop on the industrial or synthetic media at least at the present time.

In the last years, the importance of the physiological characteristics of fungi has been increased in the taxonomic studies that are depended for many years on the morphological characters mainly or may be completely.

Some of the outstanding leaders in the development of fungal physiology are worthy of special mention.

1– Louis Pasteur (1822-1895):

French Scientist was originally a chemist then became a biologist. Pasteur discovered that some microorganisms are inhibited by free oxygen (Pasteur Effect). Also he discovered that some fungi change the physiological and morphological characteristics when grown Anaerobic

2– H. Anton de Bary (1831-1888):

German scientist focused his interests on the life cycles in fungi, as well as parasitism of fungi and in his studies of nature was more physiological than divisional. In addition to that it was the first author about the physiology of fungi authored by de Bary.

3– Oscar Brefeld (1839 – 1925):

German Scientist of the most important discoveries introduced a number of methods of sterilization for either culture media or tools and devices used in the study of the physiology of fungi, but the main was achieved in mycology through his studies on the life cycles of fungi. He was the first to use the single–spore technique.

4– Buller (1874 – 1944):

Canadian Scientist from England origin. His chief interests lay in production of fruit bodies and spores and in the effect of the environment on these activities. His research has been published separately in detail in seven volumes "Researches on fungi".

5– Leonian (1888-1945):

American Scientist. His principle contributions were made in the study of fungus nutrition with emphasis on the factors which are required by fungi for growth and reproduction.

Culture Media

The fungi are heterotrophic organisms, so they get their food requirements necessary for the synthesis of protoplasm and the other living cellular formations from the surrounding medium where they can exploit the many natural materials that vary in the extent of the exploitation of fungi have depending on the content of nutrients, which are often the same complex chemical compositions as cellulose, starch.....etc. and other materials which can not soluble in water, but after hydrolysis by a group of fungal enzymes to materials with low molecular weights have the ability to disolve into the water ... the greater part of the compounds utilized by the fungi are modified or changed either before or after they are taken into the cells. Where it is outside the fungal cells; process of converting complex material into simple materials, or are these changes within the fungal cells through oxidation of nutrients into carbon dioxide, water, or to intermediate products. By this process the fungus obtains the chemical energy which it requires for the processes of synthesis.

The scientists were able to develop a set of different types of cultivation media of fungi in general as possible for germination of spores of fungi, as well as fungal strands.

The culture medium generally means any solution or solid material or the environment in which we can provide the living organism shall be sufficient for its food so that it can grow and reproduce and returns its life cycle or them.

Kinds of culture media

Firstly: There are three kinds of culture media according to its components:

1) Natural media

A natural medium is one which is composed entirely of complex natural materials of unknown composition. Among the natural substances so used are the following: Plant parts, malt, yeast, peptone, manure, bread, fruit and vegetables....etc.

Certain fungi have never been cultivated in laboratory; there are obligate parasites which live upon or within the living tissues of their host. These host plant when killed will no longer support growth of these fungi. However, many species which in the past were considered to be obligate parasites, have since been cultured on non-living media. Natural media have many advantages. They are cheap and easy to preparation but can not be relied upon to physiological studies of fungi.

2) Semi-synthetic media

A semisynthetic medium is one which is composed in part of natural materials of unknown composition. Such media are made by adding compounds of known composition to one or more natural materials. The widely used potato-glucose medium is an example of this type. The addition of agar to an otherwise synthetic medium introduces natural materials of unknown composition. Media which contain agar cannot be classed strictly as synthetic media. The composition of a given natural or semisynthetic medium is not

constant. Potatoes- glucose medium may vary greatly in composition depending upon whether or not the potatoes were peeled and upon the variety and age of the potatoes used.

3) Synthetic media

A synthetic medium is one of known composition, concentration and specific chemically as well as the role of each component of the elements of the media in the metabolic processes those take place within the fungal cells to a large extent. But it is considered a high-cost compared to other types of media and some chemicals may contain impurities can that have an impact on the microbes can not be neglected if we want to gather accurate information about the nutrition of fungi at the same time, the growth rate will be less if it is replaced by some other natural materials such as peptone or yeast extract, for example.

Synthetic media may be simple or complex but most contain the essential elements in utilizable form. The first synthetic medium was described by Raulin (1869) having the following composition:

Ammonium nitrate	4.0 g	Sucrose	70 g
Ammonium phosphate	0.6 g	Tartaric acid	4 g
Magnesium carbonate	0.4 g	Water	1.500 ml
Potassium carbonate	0.6 g		
Ammonium sulfate	0.25 g		
Zinc sulfate	0.07 g		
Iron sulfate	0.07 g		
Potassium silicate	0.07 g		

- Not enough information is given for the duplication of this medium:

a- Which ammonium phosohate (NH_4) H_2Po_4 or $(NH_4)_2$ HPo_4 was used?

b- Which zinc sulfate, $ZnSo_4.7H_2O$ or $ZnSo_4.H_2o$ was used?

c- Was the iron sulfate, $FeSO_4$, $FeSo_4.7H_2O$ or $Fe_2(SO_4)_3$?

d- Did D-tartaric, L-tartaric …..was used?

e- Did tap water or distilled water was used?

And other questions that are not answered by the result of negligence or lack of knowledge, or both.

Therefore, should be taken the necessary precautions at the mention of certain culture medium and so can be used to compare the results of them.

Secondely: There are two kinds of culture media according to its state:

1) Solid media

It is the culture media in which components are dissolved in water plus agar in order to become rigid or semi-rigid. Agar is obtained from various marine red algae which is a composite of Complex polysaccharide sulfate ester, which is chemically a polymer composed of molecules of galactose. It forms colloidal solutions at elevated temperatures (85 ºC) and solidifies in the form of sodium salt or potassium…..etc. and sets to a gel at temperature around between 32-40 ºC. Agar introduces physiologically active elements into media. It may contain essential elements such as zinc. Agar also contains growth factors such as thiamin. Many fungi make some growth on water–agar which indicates that agar or the impurities contained in it are utilized by fungi. Leaching agar with 5 % aqueous pyridine remove many of the physiologically active compounds.

Advantages of the solid medium:

a- Culture vessels can be freely handled without disturbing the fungus.

b- Microscopic examination is facilitated.

c- Any contamination can be detected for the pure culture.

d- Can be used to maintain stock cultures.

e- Single-spore isolations can be made more easily.

2) Liquid media

It is the culture medium in which components are dissolved in a known amount of water, which is more accurate for the solid medium for the following reasons:

a- The composition of the medium may be controlled and the amounts used measured accurately.

b- Culture may be aerated by shaking or by blowing sterile air through the media.

c- Weighing the mycelium is facilitated.

d- When is desired to study various metabolite deficiencies and many microbiological assays, it is almost necessary to use liquid media.

e- Isolation of by-product is less complicated.

Specific Metabolites

The material that can be consumed by the fungus to grow called **a metabolite** but when the fungus can not synthesize a specific metabolite in the growth period and should be added to culture medium to grow then the fungus called **Deficient**.

The following example will explain to us the role of specific metabolite in nutrition of fungi. In (1936) **Felows** studied the ability of *Ophiobolus graminis* to use the complex nitrogen sources as albumin, peptone and nucleic acids in the growth.

In (1941) **White** found that when adding some vitamins as thiamin and biotin, the former fungus grew in different media, which contain simple nitrogen sources of sodium nitrate and ammonium nitrate, and this is clear to us that thiamine and biotin are specific metabolites to the fungus and can not grow in the absence of the two previous vitamins.

Isolation, Cultivation and Preservation of Fungi

First: Isolation of Fungi

Fungi found everywhere there are none in the environment. they can be isolated from the soil, air, water and also from organic materials. The types of isolated fungi depending on the soil type and moisture content by as well depending on the type of crop grown on soil. In addition, we can isolate many of fungi from the surfaces of plants and animal and human skin and theses fungi are responsible for many of the diseases that affect plants in fields and warehouses, as well as causing preserved food products rich in organic matter damage.

Among the most famous types of fungi; yeasts, which play an important role in the fermentation process in many food materials, and fungi play an important role in nature, where it is attributed many environmental phenomena, which is a great benefit to the environment, some occurring while others considered extremely damage.

It is necessary to isolate and grow fungi in pure media and keep them in these media and the work of the various studies, such as:

1- Measurements of growth, sporulation and germination tests.

2- The study of the history of life to these fungi.

3- Methods of nutrition and parasitism.

There are many ways to isolate the fungi from their locations and preserve them to a pure state. There are different ways for isolation of fungi depending on type of isolated fungus and their environmental needs.

Selecting the appropriate method for isolating of fungi differs on several important factors such as:

1- The method of growing of fungi which parasitize externally plant can be isolated more easily than fungi that grow within or between plant tissues.
2- The stage of growth is it mycelium or spores or fruiting structures or others.
3- The type of culture medium is it liquid or solid.

One of the main difficulties which directs a researcher at the isolation procedure is the possibility of contamination of other microorganisms other than the microorganism that is to be isolated, therefore there must be precautions must be observed before the isolation process, such as sterilizing chamber, which the researcher to isolate the fungus where using formalin as well as the sterilization of all used tools in isolation process such as tweezers, needle, glass….atc and other tools also must be all the isolation and purification and transportation procedures in a sterile places to avoid contamination.

Purification of fungi from bacteria can be achieved as follows:

1- Acidification of the medium upon which the inoculum will be cultivated. This method depends on the fact that bacteria prefer a slightly alkaline medium whereas fungi could grow will in medium with low PH (acidic medium).
2- Addition of certain antibiotics to the growth medium. Among the most common antibiotics used in this field are oxytetracycline, cholrmphenicol...etc. Rose bengal can be also added to prevent the bacterial growth.
3- Using the method adopted by **Brown (1924)**. This method depends on the fact that hyphae tend to penetrate the medium. The contaminated fungus is allowed to grow for 1-5 days on a solid medium, then the growth together with agar under it is cut, inverted in the same place then left for few other days in the incubator, the upwards hyphae are picked up and cultivated in new medium.

4- The modified Brown's method, on which the contaminated fungus is grown on the proper medium for about 4 days then semisolid acidified medium (45ºC) is poured over the growing fungus. The plates are incubated for some other days and the hyphal tips penetrating upwards are picked up and transferred to new medium.

Single spore cultures

In some cases, to obtain pure culture developing from one mature spore of fungus, it requires a special technique where individual spores taken and grown on a petri-dish containing suitable culture medium. The used methods varied according to the type of fungus and the nature of sporulation; For example, in the case of fungi that are free spores on their surfaces can get pure culture as follows:

1- Preparation of spore suspension from the fungal culture.

2- Dipping a needle is inoculated with a loop in this suspension being Streaking on the surface of the agar medium poured in Petri dishes in the form of a zigzag.

3- 3-4 Dishes are streaked with the same inoculated needle and gently be streaking to prevent the excavation surface of agar.

4- The inoculated dishes flip upside down and examine under the microscope to identify the individual fungal spores, especially in the third or fourth plate.

5- Incubating the dishes on the proper temperature for the fungus that scan every 8-12 hours to control spore germination.

6- Movement of spores germinating into new agar medium to complete their growth.

7- Examination the resulting fungal cultures then replanataion of the surface of the agar. for preservation.

Hyphal tip cultures

This method is used in case of fungi that have no abundant spores, and the fungus grown on suitable medium for the growth until reaching a certain amount of growth.

1- Using a thin sterile Capillary glass tube to separate a small part of a single hypha of fungus after identifying the ends of hyphae under the microscope, which appear far apart as possible indistinguishable.
2- Mobility separated part of hypha to the suitable agar medium on petri dish and then incubated at the appropriate temperature for growth to get a new pure culture.

✍ Isolation of the parasitic fungi from their hosts is usually done by planting bits of host tissues directly on the desirable medium. The surface of the host tissues should be firstly sterilized or disinfected by means of the following disinfections:

1- 95% ethanol for few seconds and washing with sterile water several times each 5 min.
2- $HgCl_2$ solution (0.001 %) applied for 15 – 45 seconds and washing as above.
3- A solution of calcium hypochlorite applied for about one min and washing.
4- H_2O_2 solution (50%) applied for 15 seconds to 5 min and washing

✍ After the completion of the isolation process and get the pure fungal cultures, the isolated fungi are defined through morphological characteristics and microscopic characteristics of the fungal culture.

- **The morphological characteristics:**

The fungal culture is checked on solid medium and recorded distinctive morphological characteristics such as the color of the fungal culture, nature of fungal growth, intensity of fungal growth, the shape of the fungal colonies….etc. and then monitors the changes of color of fungal cultures as time passed.

- **The microscopic characteristics:**

Slides of fungal growth is prepared and pigmented using dyes as lactophenol, then examined under the microscope. The microscopic characteristics of the fungal culture are

recorded (the form of the fungal hyphae- the form of fungal spores- measurements of fungal spores).

Secondly: Cultivation of Fungi

Fungal cultures are usually grown on many types of media including:

1- Solid media:

Fungi grown on media containing agar through inoculation Petri dishes or agar slants where the fungal growth appears on the surface of agar but this method is not suitable for physiological studies of fungi.

2- Liquid media:

It is the best way to physiological studies of fungi, and fungal growth appears through inoculation of liquid media with spore suspension. Liquid cultures are classified according to whether the mycelium grows on the surface or is dipped inside the medium. Liquid cultures are either:

A- Surface cultures:

The constituents of the Liquid medium are dissolved in distilled water, distributed among conical flasks (usually 250 ml Erlenmeyer flasks each containing 50 ml of the medium are used) and sterilized. After cooling, these flasks are inoculated. The inoculum is a suspension of cells and spores of the organism under investigation. Agar slants usually used in growing the inoculum. The cultures are then incubated at suitable temperature for a period determined in a preliminary study.

At the end of the incubation period, the growth is separated from the metabolic solution. This method is easy and needs no complicated techniques. However, it need a prolonged period of incubation to obtain the maximal growth or the required product.

B- Submerged cultures:

Submerged cultures theoretically and practically offered the ideal method of studying fungal metabolism. Any biochemical process known to occur in surface cultures can be induced to take place under proper conditions of submerged culture.

Techniques of obtaining submerged growth:

Several different means can be used for performing submerged growth. In general, the methods commonly employed fall into two groups:

1) Submerged growth Methods without mechanical agitation

These methods rely upon passage of air through the culture liquid for the setting up of vigorous currents in the liquid sufficient to prevent the formation of a surface pad and to maintain the growth particles in a constant circulation throughout the liquid.

From the simplest but least efficient means consists of a tall narrow vessel tightly filled with a rubber stopper through which pass an inlet and outlet tubes for air. The air tubes reach to the bottom of the liquid while the outlet tube comes just pass the bottom of the stopper. The air is sterilized by filtration through tubes containing about 4 inches of sterile non absorbent cotton. Aeration is secured either by application of +ve pressure at the inlet or by suction at the outlet tube.

The advantages of these methods are its simplicity and suitability for small ordinary laboratory apparatus and equipment. However, they all have the **disadvantage** to various degrees of not providing the optimum aeration conditions.

Cultures of this type have a strong alcoholic odour in the exhaust of air indicative of anaerobic sugar fermentation. Increase of the amount of air in solution may overcome this disadvantage. Fine air bubbles cause foaming troubles which unless control cause the culture fluid overflows through the tube and ultimately to get contaminated.

Foaming is controlled in two ways:

a- Addition of foam breaking substances (antifoams) as soybean oil, castor oil, oleic acid...etc. Antifoams may be very toxic for different microorganisms if it's concentration is increased in the medium.

b- Reduction of air flow through the liquid. This reduction has very undesirable effects such as:

- reducing the total amount of growth.
- lower yields of oxidized metabolic products.
- retarding the rate of sugar consumption.

2) Submerged growth methods with mechanical agitation

This group requires a complicated and expensive machinery set up. Two types are used:

✍ **Shaking:** shaking machines are of two types:

a- Rotary shakers.

b- Reciprocal shakers.

This method allows the passage of air through cotton plugs due to the fermentation process ventilation and fit with the size of the solution.

✍ **Propeller:**

During the operation, a high speed motor turns the propeller which maintains the culture liquid throughout the growth cycle in a state of more or less violent agitation. Theoretically, this is the best way of aeration and largest scale industrial microbiological processes utilize the principle of propeller agitation (fermenters).

C- Replacement cultures:

Both surface and submerged cultures described above are **single batch cultures** in which the microbiological process is finished after certain incubation period which leads to the depletion of nutrients, time and effort, making it difficult to use on a large industrial

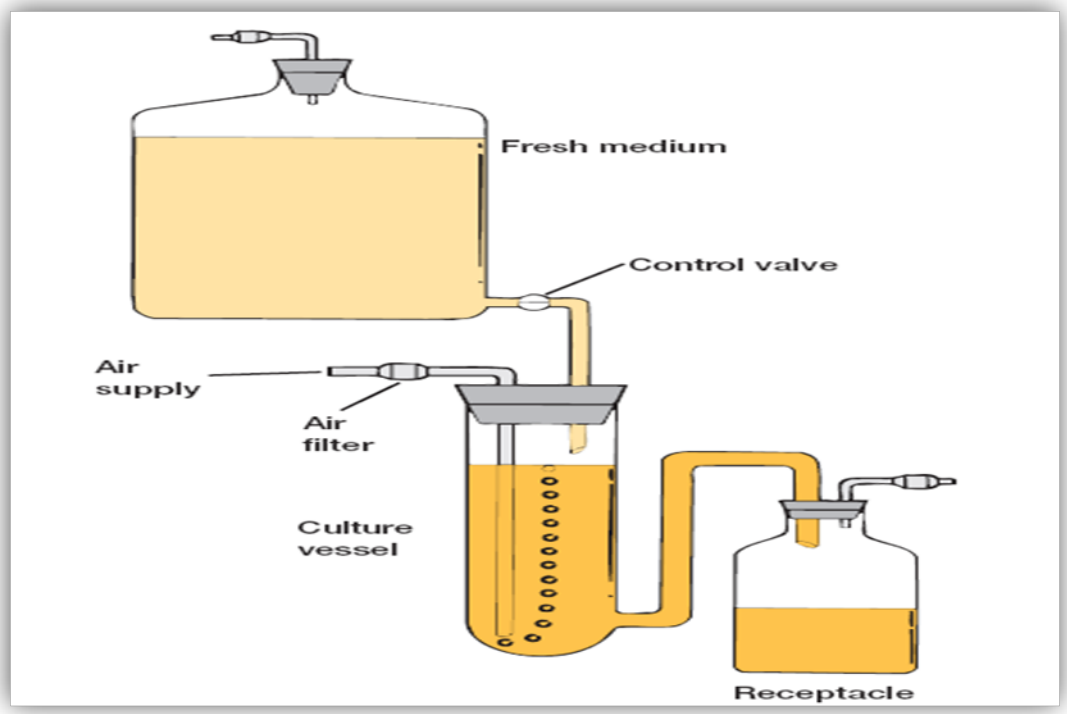
scale, as well as difficult from which to follow the transformation of a substance as it can be transformed into cellular material during growth.

In the replacement culture technique, the fungus is grown either in surface or submerged cultures for obtaining fully grow mats or pellets. The fluid is than gently poured out of vessel under aseptic conditions. Equal portions of newly prepared medium are introduced aseptically to replace the exhausted metabolic solutions. This process can be repeated several times.

In the transformation of certain substances, the fate of such substance can be followed after its introduction under the preformed mats or pellets where its conversion takes place essentially by the enzyme systems of the fungus.

D- Chemostat:

This system consists of a reservoir containing the sterilized medium which flows of it at a constant rate across controlling valve to a growth chamber. Three openings are connected to the growth chamber, one for aeration, the second for inoculation and air expel, and the



third acts as outlet for the overflow fluid by siphoning. The volume of liquid inside the growth chamber is kept constant throughout the microbiological process.

Thirdly: Preservation of Fungi

Preservation of fungi is one of the important subjects in this field. This is to maintain cultures with minimum genetic changes. The following are the most important methods used for the preservation of fungi:

1- Soil tube method:

Spore suspension of the fungus is transferred to test tubes containing sterile soil brought to pH 6.7 with calcium carbonate. The soil must be sterilized three successive times at 2.5 atm., each for 5 hours. Some fungi survive for 5 years in these soil tubes without any genetic changes.

2- Lyophilization:

As dense as possible spore suspension is prepared by growing the organism to be preserved in serum, milk or 3% lactose until reach the proper stage of maturity. 0.1-0.2 ml of the suspension is then transferred to vials and subjected to evacuate while frozen until completely desiccated. The vials containing the lyophilized cultures are sealed while evacuated.

Chemical composition of Fungal Cells

Fungi share the basic characteristics of eucaryotic organisms, where fungal cells almost contain all the organelles that distinguish eukaryotes such as Golgi Apparatus, vacuoles, endoplasmic reticulum, mitochondria and most of other parts of the cell. Fungal cell contains glycogen not starch as compared with the plant cell, also characterized by the

presence of chitin, a basic material in the structure of walls of eumycota (true fungi). The basic structures of nucleic acids, proteins, carbohydrates and lipids are general, but there are certain features of fungal composition that set them apart from other organisms. Chemical distinctions among fungal groups has been recorded and this led to use of molecular and physiological attributes of fungi as descriptors for taxonomy purposes.

(1) Water:

Growing fungal cells are largely watery. Water content reaches about 85 – 90 % of the fresh weight of mycelium and fleshy sporophores or conidiophores. The conidia of most fungi have a relatively low water content (17–40%). The proximal composition of the fungal cells is appeared from the following table (Griffin 1993).

Class of compounds	Dry weight (%)
Carbohydrates	16 – 85
Lipids	0.2-87
Proteins	14 – 44
RNA	1 – 10
DNA	0.15 – 0.3
Ash	1 – 29

(2) Inorganic electrolytes:

Inorganic electrolytes in fungi are ionic and therefore electrically responsive. They form an important component of the osmotic activity of the cytoplasm. Ash contents of fungi showed considerable variation among species and with conditions of growth. The common cations of the ash of some studied fungi are: Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Mn^{2+}. For anions, it has been found that various forms of phosphate (organic and inorganic) are the most common as well as carbonates and bicarbonates. The proportions of metal components are varied from fungal species to another, as they differ in one fungal type. It is worthy mentioning that the ash content of *Aspergillus niger* is 2.39% of the dry weight of mycelium, 5.5 % in *Rhizopus japonicus* and reached 7.19% in *A. oryzae*.

(3) Nucleic acids:

A- Deoxyribonucleic acid (DNA):

General structure of DNA in fungi is no different than in other eukaryotic organisms. It is noticed the very low DNA concentration in fungi as compared with that of in both bacteria and higher eucaryotes are about 10 times higher. DNA concentration varies not only among fungi but for the same fungus with different growth conditions, ages and stages of development.

There are three types of fungal DNA:

1- Nucleic DNA

It is found in nucleus. The nuclear genome constitutes about 80 % of the total DNA in *Sarccharomyces cerevisiae*.

2- Mitochondrial DNA

It is found in mitochondria and represents 10-20%. Size of the fungal mt DNA is intermediate between those of mammals and flowering plants. This type of DNA takes closed circles in eukaryotes and most fungi, but linear shape occurs in some fungi as *Pythium* and the slime mold *Physarum*.

3- plasmid DNA

It is found in nuclei or mitochondria and represents 1-5%. The best studied plasmids of *Hemiascomycetes* are nuclear, but the plasmids of filamentous fungi are primarily mitochandrial.

Considerable variation occurs among the different fungal groups in all of the previous genetic elements. This variation is used extensively to characterize species and strains of fungi genetically for taxonomy studies. Another variation is recorded between the different fungal groups in the content of guanine + cytosine (G + C). This content is calculated to be

41% of the total content of the nitrogen bases in *Zygomycetes*, 45 % in *Ascomycetes* and 55% in *Basidiomycetes* with on average of 48 % to all fungi.

B- Ribonucleic acid (RNA):

There is considerably more RNA in fungi than DNA. About 80 % of the RNA is associated with protein in ribonucleoprotein particles or ribosomes, in the cytoplasm. Transfer RNA (tRNA)is found also in the cytoplasm and account for about 15 % of the RNA. The messengar RNA (mRNA) molecules are free of protein and constitute about 5% of the RNA of the mycelium. In addition, a small nuclear RNA molecules are recorded in fungi. This type of RNA is common to eucaryotes but absent in procaryotes. The nuclear RNA lack any detectable function in fungi in contrast to that in the higher eucaryotes where it plays a role in information processing. Double stranded RNA molecules are often associated with virus–like particles.

(4) Proteins:

Fungal proteins are similar to proteins of other organisms, containing the 20 common amino acids linked together by peptide bonds. These proteins represent 60–70% of the total cellular nitrogen. Many proteins are covalently combined with carbohydrate to form glycoproteins and peptidoglycans those commonly found in the cell membrane or cell wall and are secreted as extracellular enzymes.

(5) Carbohydrates:

Carbohydrates are present in fungi primarily as polysaccharides. Sugars are generally present in very low concentration and they almost present as the phosphorylated derivatives that are important in primary metabolism. Fungi produce many polysaccharides that may be associated with the cell wall and cell membrane (extracellular) or they may be accumulated in the cytoplasm (intracellular). they may occur as homopolymers, heteropolymers, glycoproteins or peptidopolysaccharides.

These materials are found in fungi in many forms may be homopolymers such as glucan, mannan, galactan that contain sugar monomers, respectively, of glucose, mannose, galactose and both glucose and mannose. Heteropolymers such as glucomannan (glucose and mannose), glactomannan (galactose and mannose) and glycoproteins or peptidopolysaccharides. From the most important polysaccharides are cellulose, chitin, mycodextran and pullulan that are available in the fungal cells. The other polysaccharides have no known function. They may serve as storage reserves. Because of taxonomic interest in the occurrence of chitin and cellulose in fungi, much efforts were concentrated on the identification of these two fibrilar wall polysaccharides.

(6) Lipids:

The total lipids content of fungal mycelium is strongly affected by cultural conditions especially the concentration of sugar and the level of the nitrogen. The cultural conditions affect not only the quantitative but also the qualitative analysis of total lipids. Total lipids of some species of fungi were found to be 50 % or more of the mycelial dry weight.

Fungal lipids are characterized by the presence of high proportion of fatty acids that may sometimes up to 80% of lipids especially those that may contain 18 and 16 atoms of carbon and most important fatty acids are palmitic, oleic and linoleic.

The fatty acids may be:

A- linked with glycerol composed glycerides which are mono-, di- and triglycerides. Triglycerides are reported to occur in fungi as the major constituents of oil droplets suspended in the cytoplasm of mycelium or fungal spores. They are considered secondary components of lipids involved in the cell membranes and cell walls. Their primary function is storage materials.

B- combined with long-chain amino alcohol sphingosine (C18) forming sphingolipids. A sphingosine derivative called phytosphingosine is recorded in lipids of some fungi e.g

Penicillium natatum. This derivative is a characteristic of these compounds in the higher plants.

Phospholipids are usually less than 10 % of the total lipids, but some species of *Aspergillus* contain high percentage of Phospholipids. The majority of these compounds are identified as lecithin and cephalin.

Interesting differences in the distribution of sterols among fungi have been observed, including the absence of sterols from the *peronosporales* (*Oomycetes*) and the presence of the unusual sterol brassicasterol in *Taphrina* (*Hemiascomycetes*). Ergosterol is recorded to be the principal fungal steroid. Sterol content in the fungi is usually about 1 % or less of the fungal dry weight.

(7) Cell Wall:

Chemical structure of cell wall is highly variable among different fungal groups and has taxonomic significance. Polysaccharides are the main components of the fungal cell walls (80–90% of the dry weight of cell wall). A fluctuating amounts of lipids, proteins and ash are also recorded in different fungal groups.

✍**Polysaccharides can be classified into:**

a-The alkali-soluble fraction: such as glucan and glycoproteins. A significant part of the polysaccharide in the fungal cell wall is associated with polypeptides forming glycoprotein or peptidopolysaccharides as in mannan which found in cell walls of many Eumycota forming heteropolymers with the other sugars i.e. glucuronomannan (*Zygomycetes*), glactomannan (*Ascomycetes*) and xylomannan (*Basidiomycetes*).

The most important associations are glucuronomannanoprotein (*Zygomycetes*), galactomannanoprotein (*Ascomycetes*) and xylomannanoprotein (*Basidiomycetes*). These

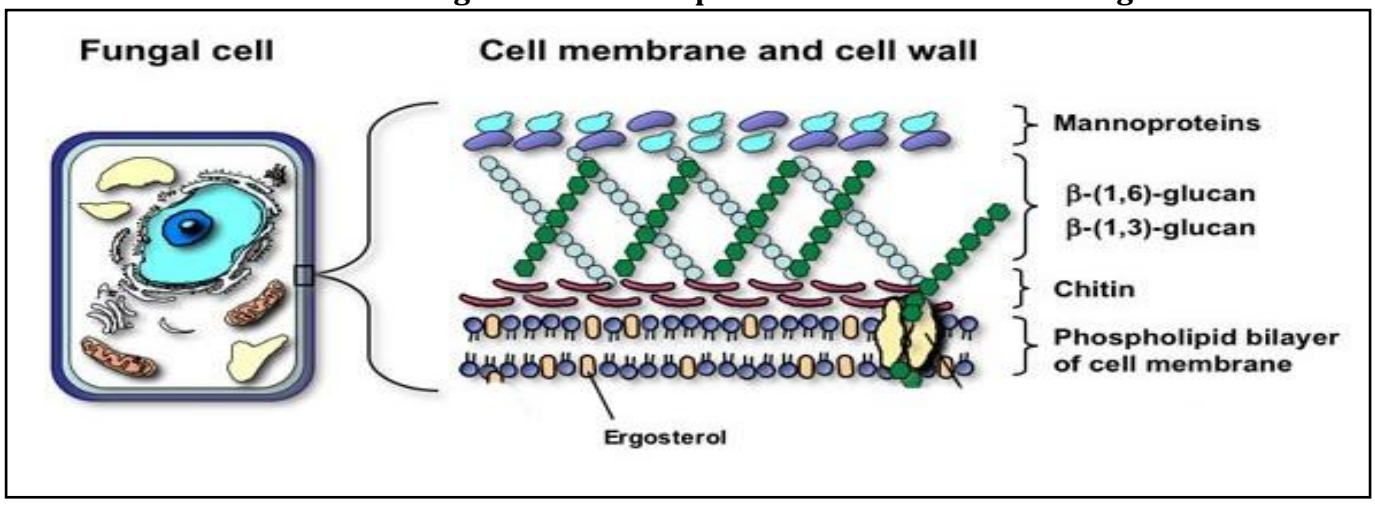
polymers are also called peptidoglucuronomannan, peptidogalactomannan and peptidoxylomannan, respectively.

b- alkali-insoluble fraction: it forms filamentous network of the fungal cellular wall and gives its distinctive shape also give it strength and durability. The alkali-insoluble fraction contains chitin and/or cellulose and insoluble glucan. It is noted that the structure of chitin-like cellulose in some respects as it consists of Linear polysaccharides of repeating units of 1,4- linked b-N-acetyl glucosamine residues. Chitin takes filamentous forms thickness ranges between 10 to 25 nanometers.

The majority of fungi, *Eumycota*, had chitin in their walls, but a small group of Oomycetes had cellulose and lack chitin. Some fungi contain both chitin and cellulose in their walls and these fungi belonging to *leptomitaceae* (*Oomycetes*) and *Hyphochytridimoycetes*) such as *Rhizidiomyces*. Alkali-insoluble B-glucan is the predominant in the cell walls of *Oomycetes* (except *Leptomitaceae*), *Ascomycetes*, *Basidiomycetes*, *Deuteromycetes* in addition to *Chytridiomycetes*. Polyglucuronic acid is recorded in *Zygomycetes*.

The fungal cell wall contains some fungal pigments such as melanin. These pigments protect the fungi from radiation such as UV radiation as well as against some enzymes.

"The structure of fungal cell wall and plasma membrane in the fungal cell"



✍The wide variation among fungi with respect to polysaccharides of the cell wall is shown in the following table (griffin, 1993).

Class	Genus	Dry weight of wall (%)		
		Chitin	Cellulose	Other
Chytridiomycetes	*Allomyces*	58	-	16
Zygomycetes	*Mucor*	9	-	44
Ascomycetes	*Saccharomyces*	± 1	-	60
Deuteromycetes	*Fusarium*	39	-	29
Basidiomycetes	*Schizophyllum*	5	-	81
Basidiomycetes	*Corprinus*	33	-	50
Oomycetes	*Phytophthora*	-	25	65
Oomycetes	*Leptomites*	14	7	74

Growth of Fungi

Growth

Growth may be considered either as an increase in cell number or as an increase in mass. Because it is sometimes possible to increase the number of cells does not increase weight.

Example:

Fungus cells may divide and form new cells without an increase in mass. A spore may germinate in distilled water and give rise to a germ tube, but in the absence of nutrients this process soon stops. A few cell divisions exhaust the reserve materials originally present in the spore, and growth soon ceases unless these new cells obtain nutrients from the external environment. Under certain conditions fungus cells may increase their store of reserve materials, and thus their mass, without an increase in cell number, but this process is also

limited. Growth, excluding the limited meanings given above, involves an increase in both the number and the mass of cells.

One question comes to mind so that if we want to know to understand the complex nature of the growth Would we should extend every cell needs in other words, everything that can be found when the analysis of chemicals until growth is occurring. Since the cell consists of complex structure of protoplasm and the nucleus that contains the genes, as well as enzymes and other vitamin compounds and other hormonal and so on, but we in fact extend the cell with a limited number of components and materials, but with this fungus grows and if analyzed and found the material did not interfere in the composition of the matrix, which outgrew it.

Then, **the growth in the fungi** is a synthesis of a complex set of materials that resulted from a series of interactions of the few and simple materials supplied by medium, so we find that **the Synthesis** is the basis of the growth process in all organisms.

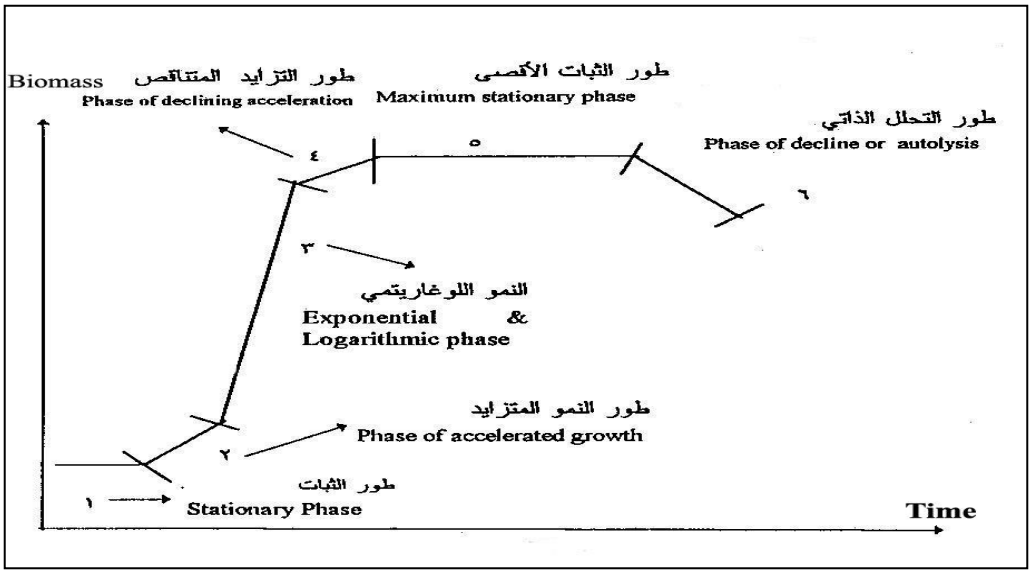
Phases of fungal growth

Growth in the fungi depends upon the species and the environmental and nutritional conditions. In the present discussion, it will be assumed that the external conditions are favorable and that growth takes place in a limited volume of medium. The growth rate defines the amount of growth in unit time.

Growth in the fungi as is the case in other organisms, like bacteria, and the phases of growth in unicellular fungi as yeast can be summarized as follows:

1- Stationary phase (lag phase)

When cells are inoculated into a medium, there is a period of time following inoculation when there appears to be no change in number. The stationary phase may be long or short depending upon the age and vigor of the inoculum, the medium and other factors.



"Phases of growth in unicellular fungi (Yeast)"

2- Phase of accelerated growth:

This phase is characterized by an increase in the rate of cell division i.e the generation time is decreasing.

3- Exponential phase (logarithmic phase):

This phase is characterized by a constant generation time. If the log of the cell number is plotted against time, the curve is a straight line. also this phase characterized by Continuous growth with maximum rate under the optimum growth conditions.

4- Phase of declining acceleration:

As the nutrients become exhausted or as toxic by-products accumulate. The average generation time increases. A combination of these and other factors results in a lessened rate of growth. If fresh medium were continuously supplied and toxic by-products removed, it is possible that this phase would never be attained.

5- Maximum stationary phase:

This marks the attainment of maximum weight or numbers of living cells. It is quite likely that the death of old cells is balanced by new growth. The duration of this phase is dependent upon the organism and upon the composition of the medium at this time.

6- Phase of decline (Phase of autolysis):

Sooner or later, following attainment of maximum development, autolysis sets in. As the cells die, the cellular enzymes begin to digest the various cell components. Only the more resistant portions of the cell remain. Microscopic examination at this time reveals that many cells are devoid of protoplasm. It is quite possible that some of the materials released by autolysis are used by the remaining living cells.

Growth phases of Filamentous Fungi

With exception of the third phase of growth discussed above, the filamentous fungi follow the same order of development as the yeasts. Filamentous fungi fail to attain an exponential rate of growth. Usually, the exponential phase is replaced by a more or less linear phase of growth.

Methods of measuring growth

In choosing a method of measuring growth or any other physiological process, the accuracy and type of information desired must be kept in mind. For some purposes the simplest methods are satisfactory; for others the most accurate methods should be chosen.

(1) Visual inspection

The simplest way to measure growth is by inspection and comparison. The value of this method lies in the speed with which growth measurements are made. Elaborate equipment is not needed. Test tubes and Petri dishes are satisfactory culture vessels. This method has the further advantage that the same cultures may be kept under observation. It is frequently the method of choice for preliminary experiments.

(2) Linear growth

A widely used method of measuring growth consists in growing fungi in Petri dishes and measuring either the diameter or the area of the colony. This is a useful method in some instances but almost useless in other. In this method, the diameter, radius or the area of a colony is used to express the amount of growth while the daily increase represents the rate of growth. It is obvious that this method neglects the thickness of the colony.

Worley (1939) has proposed to take the thickness of the mycelium into account when growth is measured by this method. Such measurements are difficult and neglect the mycelium buried in the agar. The rate of the linear growth of some fungi has little relation to the composition of the medium. The rapid extension of mycelium on water–agar medium may serve as a familiar example.

Fries (1943) grown *Ophiostoma ulmi* on a solid culture media for five days and then measured radii of developing colonies as well as the weights of colonies after removal of agar and he has been found that the average rate of the radius of the fungal culture, which has not added to vitamin Pyridoxine is 16.3 mm, while the middle of the fungal culture, which added to Pyridoxine is 12.3 mm while the weights of mycelium in the above two cases is 5.2 mg, 18.1 mg, respectively. From this example it is clear the difference between the two methods and longitudinal measurement method may have given misleading results.

Many researchers assumed that the fungi grow at a constant rate at constant environmental conditions, however, this is not true, there are a lot of fungi have limited growth to the presence of two factors, **first factor** is the change in the concentration of the components of culture media due to the distribution and exploit the fungi to these components. **The second factor** is the secretion of inhibitory materials during the growth of the fungi. It is possible to grow fungi on a constant rate of a certain temperature, not at

another temperature and this constant rate of growth may be changed as the optimal temperature changes.

(3) Dry weight

By weighing the mycelium and spores produced, an accurate and objective measure of growth is obtained. The mycelium may be filtered from the culture medium by use of a finely woven cloth or filter paper and then transferred to weighing bottles. The excess medium should be removed by washing and pressing the mycelium, which is then dried to constant weight at 80–100ºC. After the mycelium is dried, it is weighted on a sensitive balance.

This technique should be more widely used. This method commonly used in liquid culture media but sometimes it used in case of solid media as some fungi make better growth and sporulate more readily on solid agar medium than in liquid medium. Some authors have obtained the dry weight of cultures grown on agar as follows:

a- The mycelium is removed from agar by autoclaving the cultures.

b- filtering off the mycelial mats and washing with hot water or the mat can be removed from the melted agar with a pair of forceps instead of filtering and washing.

It should be noted that, autoclaving removes some soluble components from the mycelium, but if a uniform procedure is adopted, the results are comparable. From the problems of this method is that the gelatinous growth takes a long time during the filteration and can overcome this problem for the fungi by using a **centrifuge** to separate the fungal growth from the other components of medium.

(4) Indirect methods

a- Determination of the content of certain cellular components. It is well known that the growth is based on the synthesis of protoplasm and known that protoplasm consists mainly of protein which contains 16 % nitrogen. By Nitrogen estimation of the fungal growth can measure the growth.

b- Determination of the total nitrogen content of the mycelium can be used as general index of the active cells.

c- **Righelato (1967)** found a value of 2.56:1 for the ratio of consumed glucose: dry cells produced for *Penicillium chrysogenum*. However, in many cases sugar may be converted to other substances as gluconic acid and large amounts of sugar can disappear without any increase in cell weight.

d- Respiratory rate is also possible index of growth.

Measuring yeast growth

The growth of yeasts may be measured by four methods:

a- Yeast cells may be counted in an aliquot of the medium using a hemocytometer or other counting chamber.

b- The volume of yeast cells in a given volume of medium may be measured in special graduated centrifuge tubes. Yeast cells are large and easily separated from the medium by centrifuging.

c- Turbidity may be used to measure the amount of yeast growth. Accurate determinations by this method require the use of a photoelectric photometer. This method is rapid and sufficiently accurate for many purposes.

✍ **Lindegren and Raut (1947)** have cultivated yeasts in colorimeter tubes and have followed the rate and amount of growth for as long as desired.

d- Yeast cells may be filtered under vacuum, washed, dried and weighed. Using procelain crucibles with fitted bottoms are suitable. This method is accurate but somewhat time - consuming.

Factors Affecting Growth of Fungi

All the separate factors comprising the internal and external environment may affect either the rate or the amount of growth, or both.

Firstly: Internal Factors

1 – Genetic Constitution:

It is one of the most important factors affecting the growth of fungi. One species differs from another and even one isolate of a species may differ from another in genetic constitution and can improve that capability by controlling the external environmental conditions and changing the genotype of the fungus. Many mutations have been produced in the laboratory by the action of x rays, ultraviolet rays and certain chemicals as nitrous acid and mustard gas. These mutations of a single species differ from the parent type in one or more biochemical or morphological characteristics.

2- Inoculum:

Age, history and kind of the inoculum may influence the rate and amount of growth and other functions of the fungi. It was found that youngest cells of inoculum have a greater ability to grow than oldest cells, and that the amount of inoculum has an impact on the growth of fungus speed. Certain species are difficult to maintain in culture unless they are frequently subcultured. Among these are various species of *Pythium* and *Phytophthora*.

3- Production of certain metabolites:

Many fungi have latent abilities to synthesize various essential metabolites as vitamins and other growth materials. In the virtual absence of these compounds in the medium and after a shorter or longer period of incubation, a fungus may begin to synthesize these essential metabolites and grow so the fungal growth takes the normal way. Some fungi can't synthesize these materials and must be added to the medium as well as other fungi may produce some inhibitory materials for growth at certain conditions so it may decrease or delay.

4- Ability to cause infection (pathogenicity):

Many fungi lose their pathogenicity when cultured for a long time on artificial culture medium. Host passage frequently restores pathogenicity.

Secondly: External Factors

These factors can be classified into two types, physical and chemical factors.

A- Physical Factors

1-Temperature

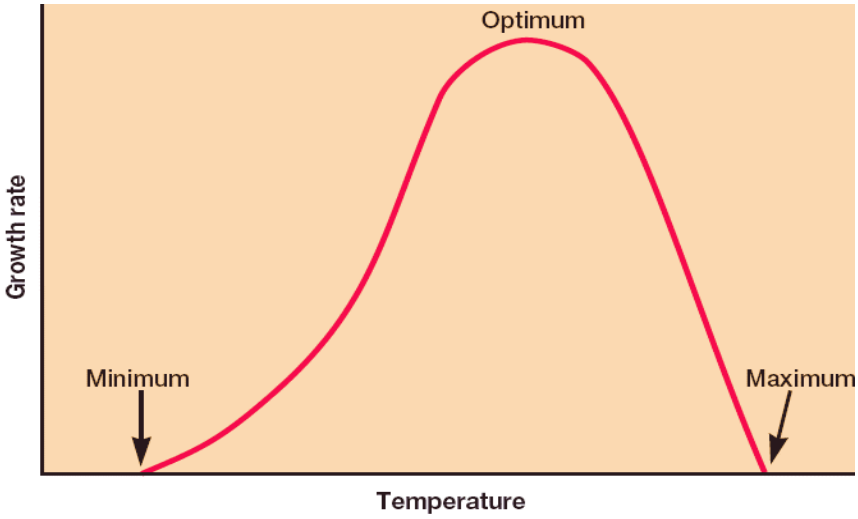
Temperature significantly affect the growth of fungi due to its impact on the vitality of fungi and thus determine the amount of fungal growth and average apparent impact on most of the various physiological processes such as the absorption of water and nutrients from the food environment.

✍ **For each fungus, there is three definite temperatures:**

a- Minimum: Below which the fungus will not grow. A minimum temperature of most fungi ranges between zero to 55 ºC, but there are some fungi can grow in temperatures as low as – 10 ºC.

b- Optimum: At which the fungal growth is at its maximum. Optimum temperature of most of fungi ranges between 25-30 ºC, but it changes according to changing of conditions.

c- Maximum: Above which fungal growth ceases. The fungus can not grow at all if the temperature increases, ranging maximum fungal growth between 27-50 ºC but the thermophilic fungi can grow at 50 ºC or more at the same time it can not grow at temperatures below 20 ºC.



"Tepmerature Profile of Fungal Growth"

Fungi are classified into three groups according to their temperature profiles as follows:

Fungal group	Temperatures			Examples
	Minimum	Optimum	Maximum	
Psychrophiles	Zero or below	Zero-17ºC	20ºC	***Cladosporium sp.***
Mesophiles	> zero	15 – 40ºC	< 50ºC	***Most fungi***
Thermophiles	20 or above	The higher half of that range	50 or above	***Humicola stellata, Mucor pusillus M. Miehei Chaetomium thermophile***

* The results are extracted from fungal physiology (Griffin 1993)

It is worth mentioning that some fungi do not follow the former classification such as *Aspergillus fumigatus* which has a temperature range of 12 – 52 ºC might be called a thermotolerant mesophile.

It has been found that the temperature change affects the shape and structure of fungal spores. It is well known that there is a temperature range for fungal growth varied between 5 and 30 ºC. The temperature range which allowed sporulation was more narrow than the range for growth.

Zonation is a phenomenon produced as a result of swinging the incubation temperature for Periods are sufficient to produce spores makes some fungi form areas of spores alternated with areas of fungal growth, which leads to a change in the external appearance of the growth of fungus. For this phenomenon occurs to be the availability of certain conditions such as the stability of the amount of exposed light for fungus and the difference between the fluctuating temperatures fluctuating more than 10 ºC for sufficient period at each grade.

Marked morphological differences were found in strains of *Fusarium* subjected to different exposures of temperatures. Regions of condensed conidia followed by regions of few conidia are formed when the difference between temperature is at least 10 ºC with the incubation at the new temperature for sufficient period. The constancy of light is an additional condition for the performance of this phenomenon.

✍The effect of temperature on fungal growth

Lowering the temperature below the optimum temperature cause decreasing the metabolic activity of cells with remarkable speed and thus decrease enzymatic activity as well as cell growth causing a state of dormancy for the fungal cells. When the temperature below the freezing point; the metabolic activity completely stopped as a result of direct stopping of enzymatic activity and also lack the ability of cells to absorb the necessary

water for metabolic interactions and mineral elements, as well as waste disposal. Fungal cells may be ruptured mechanically as a result of slow freezing. Fast freezing is found less injurious as the slow freezing causes large acute ice crystals rupture the cells while, the fast freezing leads to formation of small snow crystals with a shape and feel of cotton. Certain fungi tolerate the freezing for prolonged periods.

On the other hand, the temperature higher than the maximum oftenly causing the increase of the speed of cellular metabolic processes and thus the metabolic activities that lead to the damage of the cellular and enzymatic proteins also resulting in damage and death of fungal cells due to a lethal effect by denaturing the cellular protein as well as the enzymes. The moist heat is more effective than the dry heat as it facilitates the penetration of heat so the fast destruction of proteins. The temperature that increases than the maximum temperature Called **Thermal death point** which is defined as a lower temperature that kills the fungus if exposured for a period of 10 minutes when the life of the fungal culture is 24 hours.

2- pH

Hydrogen and hydroxyl ions are present in all media and in substrates upon which fungi grow in nature. The pH of the medium affects the rate and amount of fungal growth and many other life processes as the production of pigments, vitamins, antibiotics, organic acids….etc.

Initial pH ranges of 4-8 is satisfactory for the majority of the fungi with an optimum at pH 5 or 6, where most of the fungi prefer neutral environments with light acidity. Fungi usually can't grow at pH 3 or below as well as in the alkaline range at 8 or 9. However, the pH 3 was reported to be the optimum for *Lenzites seapiraia*, *Fomes roseus and Coniophora cerebella*. Moreover, the lower pH limit for *Acontium velatum* was 0.5. On the other hand, the upper pH limit pH of *penicillium variable* is 11.1.

The incubation temperature may influence the optimum pH of a fungus. The optimum pH for *Phacidium infestans* is 4.5 at 5 ºC, 5 at 10 ºC, 5.5 at 15 ºC and 6 at 20 ºC.

The pH of the nutrient medium may be changed as a result of the metabolic activities of a fungus. Fungi have the ability to utilize cations such as ammonium ions for the synthesis of protoplasm or for any other purpose Also, they could utilize nitrate ions or other anions such as phosphate or sulfate. Fungi produce acids from non-acidic nutrients such as carbohydrates. Ammonium is the most common basic substance produced by fungi. *Piricularia oryzae* produces ammonium in considerable amounts. The various cations and anions may combine to form insoluble compounds at certain pH values. The concentrations of H^+ and OH^- in culture medium change during growth and may act to regulate the adsorption of other ions. The pH of the culture medium may alter the relative adsorption of other ions which are essential to nutrition or which are toxic.

It has been found that the acidic soil rich in fungi more than rich alkaline soils of different types of bacteria. Therefore, the decomposition of organic material in the more acidic soil attributed to the activity of fungi, while the decomposition of humus in the alkaline soil attributed to the activity of the bacteria. It was found that some pathogenic fungi thrive in an acidic soil and some in alkaline soil depending on the adaptation of pathogen at a certain degree of hydrogen ion of soil and as well as the degree of hydrogen ion concentration in the host tissues. The advantage of that to resist these pathogenic fungi by choosing the type of fertilizers alter the pH of the soil and makes it inappropriate for the fungal growth.

✍ The effect of pH on the growth of fungi

Severe acidity or alkalinity has a detrimental effect on the enzymatic protein of cell. There is a close relationship between the concentration of hydrogen ion and the different enzyme activities. Every enzyme has an optimum pH at which its activity reached the

maximum. Also, there is a relationship between pH and the permeability of plasma membrane of different fungi which increases the absorption of ions necessary for growth and reduce adsorption of toxic or harmful ions on the fungal growth.

3- Light

Effect of light on fungi varies according to its strength and duration as well as its wave length.

Indirect light has little effect on the vegetative growth of most fungi, while the **direct sun light** may kill the cells or at least decrease their growth to great extent. Light affect greatly on the shape and characteristics of the spores or conidia as for *Fusarium* and *Helminthosporium*. Generally, the effect of light on the fungi have been concerned with reproduction than vegetative growth.

Strong daylight was found to depress the growth of *Penicillium glaucum*. The amount of inhibition was least when the culture medium contained complex nutrients such as peptone. Greater inhibition resulted when the media contained glucose. mannitol and malic acid. On the other hand, *Karlingia rosa* (Chytridiomycetes) produced twice the amount of dry weight when cultured in light than when the cultures were kept in darkness.

From the important effects of light on the fungi what called **tropism**. There are different tropisms studies in fungi from which we will discus the phototropism and gravitropism.

There is no convincing evidence that light affect the growth of undifferentiated fungal thalli directly, but phototropism of reproductive organs has been clearly demonstrated. **Phototropism** is caused by unequal growth on the proximal and distal parts for lighting. In order to obtain positive photropism, the growth of the distal part must be faster than that of the proximal part. From fungal parts that show positive phototropism include the sporangiophore of the *Zygomycetes* (e.g. *Phycomyces* and *Pilobolus*), the perithecial necks

of the *ascomycets* (e.g. *Sordaria*) and the elongating stipes of *Basidiomycetes* (e.g. *Polyporus* and *Coprinus*).

Gravitropism has been identified in many fungi, but it is also limited in reproductive hyphae only. It has been found to elongate the pedicles of *Basidiomycetes* represents negative gravitropism may be the reason that it has a strong optical response.

4- Water activity

Relative humidity can be defined as the ratio between the amount of water vapour in a volume of atmosphere at certain temperature and the amount of water vapour required to saturate the same volume at the same temperature. Relative humidity is usually expressed as a percentage. An interrelation is present between the relative humidity and the water activity (a_w). If the relative humidity is 80 %, the water activity will be 0.8. It is worth mentioning that all the vital activities of microorganisms completely stop when the water content of at least about 0.55, where the changing nature of DNA which is known Denaturation.

According the water activity, fungi can be classified into three groups as follow:

a- Xerophobic

Xerophobic fungi are unable to grow at a_w below 0.97 as in members of *Mastigomycotina* such as *Pythium*, *Saprolegnia* and *Blastocladia*.

b- Xerotolerant

The optimal growth of these fungi require a_w of 0.98 or above and have a minium at 0.9 or below. Many of the *Accomycetes*, *Basidiomycetes* and *Deuteromycetes* are examples of this group such as: *Aspergillus*, *Penicillium* and many yeasts.

c- Xerophillic

These fungi are unable to grow at a_w above 0.9 and can tolerate a_w between 0.65 and 0.75. e.g *Xeromyces* sp.

Most fungi also be able to resist long periods of drought by formation of thick walls of spores or sclerotia as in *Rizoctonia soloni*. Also they can tolerate the unsuitable temperature in drought conditions. Air humidity affects the form of conidiophores and conidia as in the fungus *Botrytis cinerea* where, it bears long conidiophores in the presence of moisture and very short conidiophores in the presence of dry air.

5- Aeration

Filamentous fungi are highly dependent on oxygen in their respiration, so number of fungi in soil is gradually decreased with the depth due to the decrease of oxygen. On the other hand, yeasts, can grow in the anaerobic conditions producing the required energy from the fermentation process. However, these yeasts loss their activity after a period due to accumulation of ethanol and CO_2.

Aeration increases the sugar consumption, respiration rate and fungal growth. However, the high aeration produces an inhibitory effect on the previous activities. This may be due to the cooling effect of the air i.e. Pasteur effect.

The inhibitory effect of Co_2 on growth and sporulation is found to increase with the decrease of temperature. This property can be used in control of certain plant pathogens.

6- Osmotic Pressure

Osmotic pressure is the force necessary for the movement of water from low concentration of salt solution to more concentrated solution through a semipermeable membrane. The osmotic pressure of any solution depends on the number of ions and molecules of solute contained in unit volume of this solution.

The ability of fungi to grow in solutions having high osmotic pressure is varied from fungus to other. Many fungi can grow in medium with high osmotic pressures and these called osmophilic fungi. These fungi can grow in saturated lithium chloride (1000 atm.) e.g. *Torula* and saturated sodium chloride solution e.g. *Aspergillus glaucus*.

7- Type and volume of used equipments

The maximum weight of mycelium which is obtained from a given volume of medium depends upon the type and size of the culture vessels used. The rate of growth is also affected.

These results appear to be due mainly to differences in aeration. The effect of depth of medium on rate and amount of growth in non-agitated cultures may be demonstrated by using a constant volume of medium in different-sized flasks, or by varying the volume of medium in flasks of the same size. Using of glass vessels in the cultivation of fungi is different from the use of metallic vessels. Type of the vessels must be chosen with great care especially in the industrial fermentation.

PRACTICAL PART

Practical Physiology of Fungi

B414

Fourth-year Biology

Prepared By

Dr.Medhat tahon

Assistant Prof. of Microbiology

General Guidelines

Dealing with microorganisms requires us to take into account care and caution while dealing with it so it should take into account the following:

Safety

1- Deal with all microorganisms as if it were pathogenic.
2- Tracking techniques always work under sterile conditions, as you learned in the previous years.
3- Must wear the coat Buffer (petticoat).
4- Before the start of work must cover all wounds with Medical Adhesive for avoiding non-contamination of these wounds.
5- Work in groups inside the lab without jowl with your colleagues in order to prevent any harm.
6- Be sure to label or encrypt fungal colonies without the slightest doubt in scanned or loss, such as the use of fixed colored pencils or adhesive tape..... etc.
7- Should not be open fungal colonies except in the case of work only under sterile conditions as previously learned.

Techniques

1- Choose the work area away from air currents.
2- Before starting to work don't forget to scan workbench with cotton moistened ethylic alcohol 70% .
3- Prepare the required tools in front of you .
4-Work always in the region between two Benzene flames.
5- Don't forget always leave your workplace clean inside the lab.

Methods of measuring the rate of growth in fungi

Growth:

It is the increase in the number of nuclei, the number of cells and the size or the amount of structural living materials.

Methods of measuring growth:

1- Change in the number and size of cells.

2- Linear measurement of the diameter colony.

3- Measuring the amount of certain cellular components such as the amount of phenolic substances (secondary outcomes).

4- Measuring the dry weight of the cell mass.

Laboratory methods for measuring the rate of growth in fungi:

To measure the growth rate of fungi in the solid cultivations we use measuring the diameter of the colony, but on the way in liquid cultivations we use dry weight method.

1) Linear growth method

It is one of the most common methods are made through inoculation center of petri dishes containing suitable cultivation medium such as Czapek's medium by regular discs of fungal colonies from 7 day old and then incubated at a temperature of 30°C or in an atmosphere of the lab for a week, taking into account measuring the diameter of the colony on at regular intervals through the work of the two lines perpendicular to the base of the dish and measure both of them and taking the average expressed in centimeters.

2) Dry weight method

This method is more accurate and it leaves the fungus to grow for a limited period which is measured in as the dry weight of the fungus at regular intervals and in the following manner:

1- Prepare a three of 250 ml Erlenmeyer flasks containing 50 ml of liquid cultivation medium such as Czapek's medium.

2- Inoculate each flask by two discs of pure fungal isolate from 7 old days under conditions of sterilization.

3- Incubate a set of Erlenmeyer flasks at a temperature of 30°C.

4- After week of incubation; filter each flask using glass funnel on filter paper known weight.

5- Place the fungal growth in the temperature of the oven (50-60°C) for 48 hours until Constant weight.

6- After dryness of fungal growth; calculate the dry weight of fungal growth as follows:

Dry weight of fungal growth =

(weight of the filter paper + fresh weight of the fungal growth) - the weight of the filter paper

Effect of different culture media (liquid&solid) on growth of fungi

Steps:

1- Prepare a number of cultivation media such as Czapek, Dox, Waxman and Richard media.

2- Prepare two equal amounts of each nutrient medium, one liquid and the other solid, then divide each one in 250 mL conical flask (50 ml each flask) in the case of liquid media, and about 50 ml in the case of the solid one.

3- Sterilize the cultivation media in autoclave, and then pour the solid one in sterilized petri dishes under conditions of sterilization.

4- Inoculate the solid and liquid media with 1ml spore suspension of the fungus under study.

5- Incubate for 7 days at 30°C.

6- Determine the rate of growth (Cm or mg/50 ml), then draw it graphically by columns method.

Results:

Medium	Liquid				Solid			
	R1	R2	R3	M	R1	R2	R3	M
Czapex								
Dox								
Waxman								
Richard								

Comment:

The Second Practical Lab

The effect of nutrient deficiency on the rate of growth of fungi

The nutritional elements constitute a great importance to the growth of fungi in the case of a shortage or a change in the concentration of these elements; they affect the growth of fungi.

Steps:

1- Prepare a set of petri dishes and conical flasks (250 mL) containing each of them on a suitable cultivation medium for the growth of fungi resulting from previous experiment; in each group are eliminated one of the essential macro-elements and another set of cultivation medium containing all the nutrients for comparison (Control).

2- Sterilize the cultivation media, and then pour the solid one in sterilized petri dishes under conditions of sterilization.

3- Inoculate the solid and liquid media with 1 ml spore suspension of the fungus under study and incubate it for 7 days at 30°C.

5- Determine the rate of growth (Cm or mg/50 ml), then draw it graphically.

Results

Medium	Liquid				Solid			
	R1	R2	R3	M	R1	R2	R3	M
Sucrose (-C)								
$NaNo_3$ (-N)								
$MgSO_4$ (-Mg)								
KCl (-K)								
KH_2PO_4 (-p)								
Control								

Comment:

The effect of Incubation period on the rate of growth of fungi

Steps:

1- Prepare six sets of a suitable cultivation medium for the growth of fungi (solid and liquid).

2- Sterilize the cultivation media, and then pour the solid one in sterilized petri dishes under conditions of sterilization.

3- Inoculate the solid and liquid media with 1 ml spore suspension of the fungus under study.

4- Incubate from 3-8 days at 30°C.

5- Determine the rate of growth (Cm or mg/50 ml), then draw it graphically.

Results

Incubation period (day)	Liquid				Solid			
	R1	R2	R3	M	R1	R2	R3	M
3								
4								
5								
6								
7								
8								

Comment:

The Third Practical Lab

The effect of some environmental factors on the rate of fungi growth

1) The Effect of Incubation Temperature:

The aim of experiment: illustrate the difference between fungi in the temperature range required for the growth and determine the optimum temperature for growth.

Steps:

1- Prepare seven sets of a suitable cultivation medium for the growth of fungi (solid and liquid).

2- Sterilize the cultivation media, and then pour the solid one in sterilized petri dishes under conditions of sterilization.

3- Inoculate the solid and liquid media with spore suspension of the fungus under study.

4- Incubate at 20, 25, 30, 35, 40, 45 and 50°C for optimum incubation period resulting from the previous experiment.

5- Determine the rate of growth (Cm or mg/50 ml), then draw it graphically.

Results

Temp. (°C)	Liquid				Solid			
	R1	R2	R3	M	R1	R2	R3	M
20								
25								
30								
35								
40								
45								
50								

Comment:

2) The Effect of Initial pH:

The aim of experiment: determining the optimum pH for the growth of fungi.

Steps:

1- Prepare seven sets of a suitable cultivation medium for the growth of fungi (solid and liquid).

2- By using NaOH (0.1N) and HCl (0.1N); adjust the initial pH as shown in the following table using a pH meter.

3- Sterilize the cultivation media, and then pour the solid one in sterilized petri dishes under conditions of sterilization.

3- Inoculate the solid and liquid media with 1 ml spore suspension of the fungus under study.

4- Incubate at both optimum incubation period and temperature.

5- Determine the rate of growth (Cm or mg/50 ml), then draw it graphically.

Results

pH	Liquid				Solid			
	R1	R2	R3	M	R1	R2	R3	M
3								
4								
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Comment:

The fourth Practical Lab

The effect of carbon sources on the rate of fungi growth

Steps:

1- Prepare six sets of a suitable cultivation medium for the growth of fungi (solid and liquid) taking into account the lack of carbon source addition and replaced in Equimolecular weights of the following sources (Galactose- Fructose- Maltose- Starch- cellulose) with a cultivation medium containing main carbon source for comparison (control) and adjust the pH of the media.

2- Sterilize the cultivation media, and then pour the solid one in sterilized petri dishes under conditions of sterilization.

3- Inoculate the solid and liquid media with 1 ml spore suspension of the fungus under study.

4- Incubate at both optimum incubation period and temperature.

5- Determine the rate of growth (Cm or mg/50 ml), then draw it graphically.

Results

Carbon source	Liquid				Solid			
	R1	R2	R3	M	R1	R2	R3	M
Galactose								
Fructose								
Maltose								
Starch								
Cellulose								
Control								

Comment:

The effect of nitrogen sources on the rate of fungi growth

Steps:

1- Prepare six sets of a suitable cultivation medium for the growth of fungi (solid and liquid) taking into account the lack of nitrogen source addition and replaced in Equimolecular weights of the following sources (Ammonium sulphate- Amm. chloride- Amm. nitrate- Peptone- Yeast extract) with a cultivation medium containing main nitrogen source for comparison (control) and adjust the pH of the media.

2- Sterilize the cultivation media, and then pour the solid one in sterilized petri dishes under conditions of sterilization.

3- Inoculate the solid and liquid media with 1 ml spore suspension of the fungus under study.

4- Incubate at both optimum incubation period and temperature.

5- Determine the rate of growth (Cm or mg/50 ml), then draw it graphically.

Results

Nitrogen source	Liquid				Solid			
	R1	R2	R3	M	R1	R2	R3	M
Ammonium sulphate								
Ammonium chloride								
Ammonium nitrate								
Peptone								
Yeast extract								
Control								

Comment:

The fifth Practical Lab

Effect of some growth inhibitors on the rate of growth of fungi

Growth inhibitory substances are substances that stop microorganism activity and growth or cause to kill him, may be inhibitory substance antibiotics or heavy metals or disinfectants or even natural materials and others.

Steps:

1-Prepare a suitable amount of solid cultivation medium for the growth of fungi, sterilize and then pour it in sterilized petri dishes under conditions of sterilization.

2- Inoculate the solid media with 1 ml spore suspension of the fungus under study.

3- Create a central hole in the center of the dish using a sterile cork polar then remove the disk from the position.

4- Under conditions of sterilization; Put in the central hole one of the inhibitory substance such as disinfectants (Dettol), alcohols (alcohol ethylic 100%), natural materials (garlic extract), heavy metals (silver nitrate, copper sulphate), Pigment (Crystal violet).

5- Incubate at both optimum incubation period and temperature.

6- Determine the clear zone in each treatment and record the results.

Results

Inhibitory substance	Solid			
	R1	R2	R3	M
Dettol				
alcohol ethylic 100%				
garlic extract				
silver nitrate				
copper sulphate				
Crystal violet				

Comment:

APPENDIX

Czapek's medium

$NaNO_3$	2.0 g
K_2HPO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
Sucrose	30.0 g
KCl	0.5 g
Agar	20.0 g
Tap water	1000 ml

Dox's medium

$NaNO_3$	2.0 g
K_2HPO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
Sucrose	15.0 g
KCl	0.5 g
Agar	20.0 g
Tap water	1000 ml

Richard's medium

KNO_3	10.0 g
KH_2PO_4	5.0 g
$MgSO_4.7H_2O$	2.5 g
Sucrose	35.0 g
$FeCl_3$	Traces
Agar	20.0 g
Tap water	1000 ml

Waksman's medium

Peptone	5.0 g
Dextrose	10.0 g
KH_2PO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
Agar	20.0 g
Tap water	1000 ml

Glucose-Asparagine medium

glucose	20.0 g
L-Asparagine	5.0 g

Weekly report on the performance of the student in laboratory

Date	Experiment	Evaluation