Elective/Special Course:
MYCOLOGY and PLANT PATHOLOGY

4102 [Special paper – I (Theoretical)]

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1. Fungal diversity in different ecosystems

The structure and composition of fungal cell, effect of environment on fungal growth and behavior

Diversity spectrum

- THE number of fungi recorded in India exceeds 27,000 species, the largest biotic community after insects1. The true fungi belong to kingdom Eukaryota which has four phyla, 103 orders, 484 families and 4979 genera. The eighth edition of *Dictionary of the Fungi2 has recognized eleven phyla.*
- The Deuteromycotina is not accepted as a formal taxonomic category. The number of fungal genera reported from the world and that from India between 1905 and 1995, are shown in Table 1. About 205 new genera have been described from India, of which 32% were discovered by C. V. Subramanian of the University of Madras. Of these, approximately 27,000 species are reported to colonize diversified habitats1. This indicates a ten-fold increase in the last 70 years.

Table 1. Fungal genera

Phyla	World	India
Myxomycotina	450	380
Mastigomycotina	308	205
Zygomycotina	55	50
Ascomycotina	2000	745
Basidiomycotina	357	232
Deuteromycotina	4100	468
Total	7270	2080

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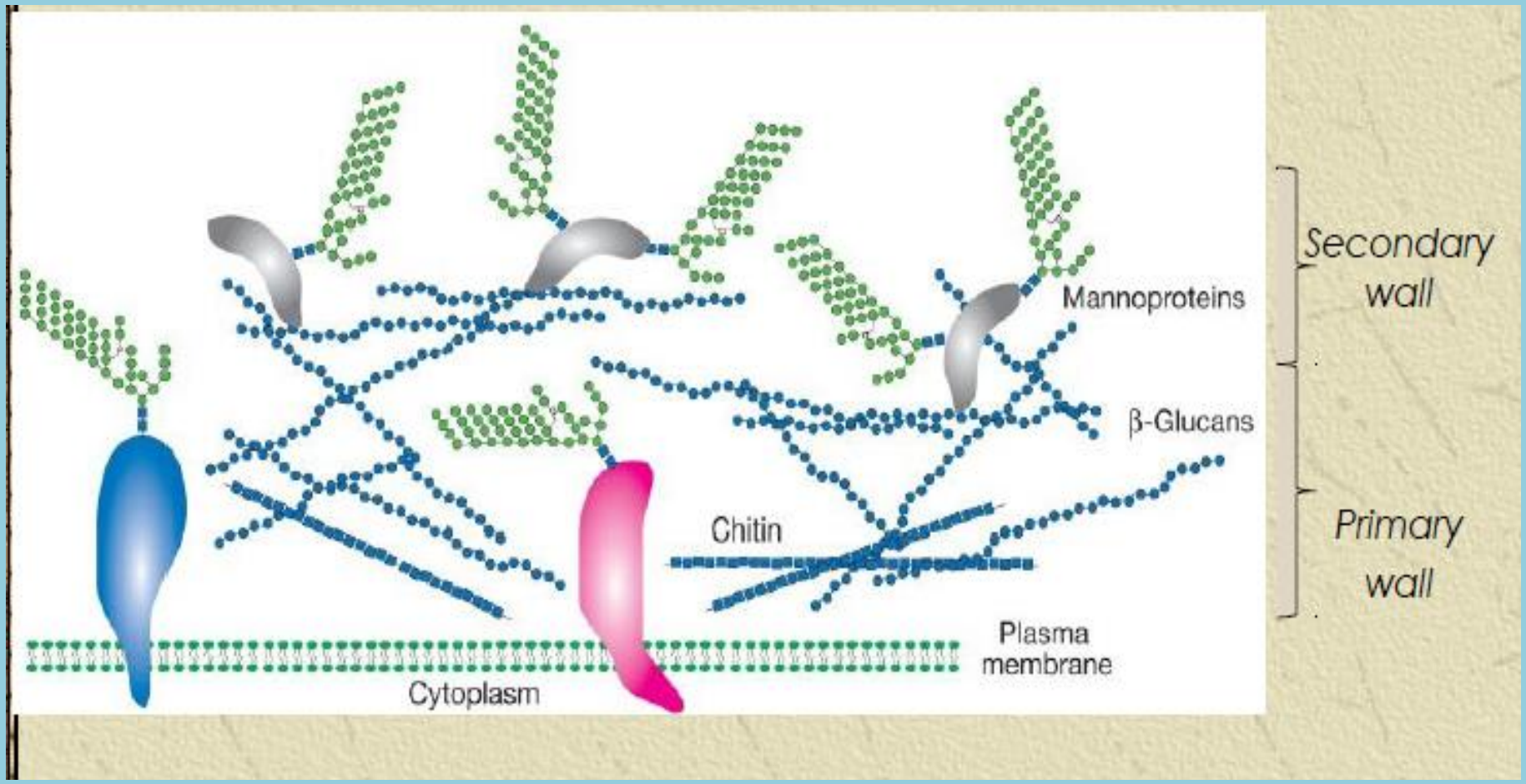
Characteristics of various subdivisions of fungal kingdom

Sub-division	Characteristics and remarks
Basidiomycota	This division contains the mushrooms and toadstools. Divided into 3 subphyla: Agaricomycotina, Ustilaginomycotina, Pucciniomycotina such as puffballs and stinkhorns, rusts and smuts, and gilled and pored fungi. Spores are produced on a characteristic cell called a basidium (plural basidia)
Ascomycota	Three subphyla : Taphrinomycotina, Saccharomycotina, Pezizomycotina. The largest number of species occurs in this group such as the cup fungi and flask fungi. Spores are produced in a sack like structure called an ascus (plural asci)
Zygomycota	Mostly microscopic species, the pin molds with coenocytic hyphae
Oomycota	The Oomycota includes the water molds and some important pathogens such as potato blight. Many produce motile spores during their life cycle which can swim. This group is now classified along with brown algae
Deuteromycota =Fungi imperfecti	Species for which sexual reproduction is not known such as molds (*Alternaria, Aspergillus*, *Penicillium).* Mainly basidiomycotina or ascomycotina anamorph
Microsporidiomycota	Spore forming unicellular parasites

Fungal cell wall

- Fungal cell wall is very different from insect exoskeletons or a plant cell walls in metabolic point of view.
- The cell wall is made up of:
  - 1) chitin (polymers of acetylated amino sugar N-acetyl-glucosamine)
  - 2) glucan (polymers of glucose)
  - 3) proteins (polymers of amino acids).
- Glucan and chitin are components of the primary wall.
- Proteins are components of the secondary wall.

Basic component of fungal cell wall



- Other components include chitosan, melanins and lipids.
- Enzymes include cellulase which acts on cellulose of plants.
- The outermost surface of the cell wall
  - 1) provides a medium between the cell and the environment
  - 2) a site where antigen and agglutinin gets attached to the substrate, host and other cells.

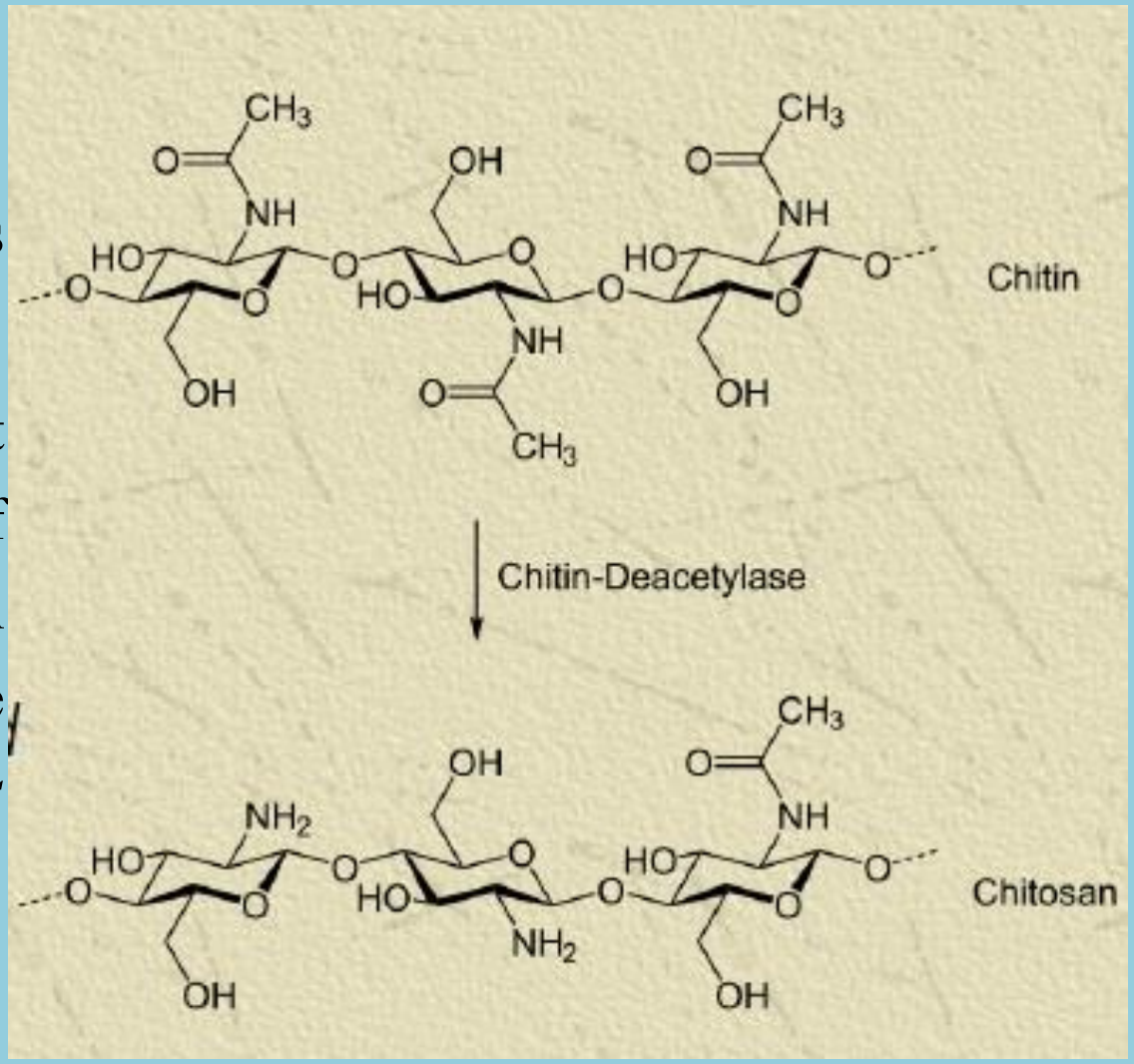
Chitin

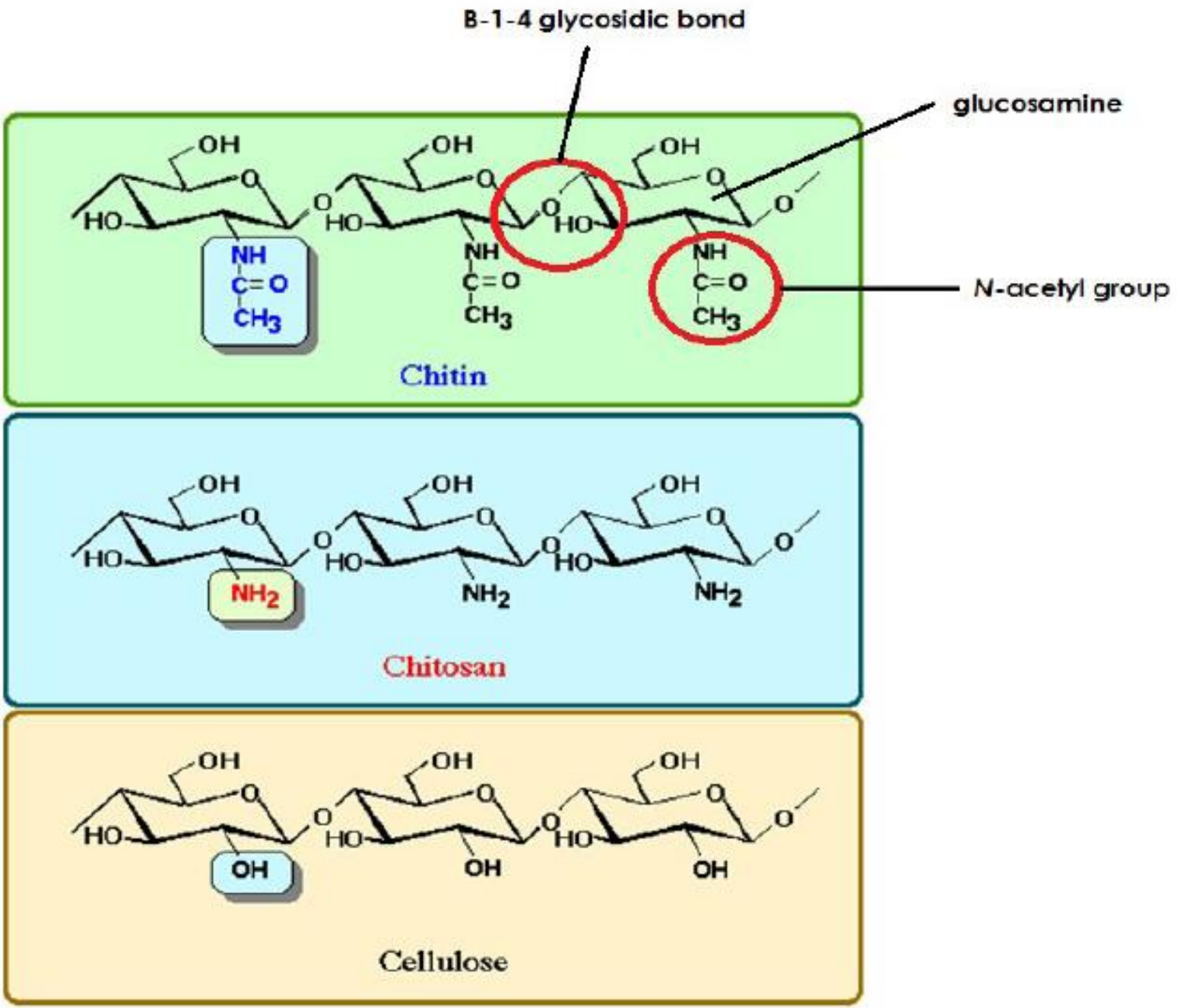
- Chained polymer β(1-4) N-acetyl-glucosamine.
- Found naturally as structural polysacharides in most fungal cell wall as cell wall components.
- Gives strength where each molecule contains a unit of sugar that is bonded by hydrogen bond to give it rigidity.
- Each microfibril of chitin gives the shape of the cell and gives strength to mature cell walls.
- Microfibrils can be of various shapes:
  - in yeast: short and thick
  - in hyphal wall: long and interwoven

- Septa is rich in chitin
- Septa can be stained with “calcoflour white”.
- *Saccharomyces cerevisiae* has low amount of chitin.

Chitosan

- Chained β(1-4) glucosamine.
- Result of continuous deacetylation of chitin.
- An important component in wall of Zygomycetes and can be found in ascospore walls of *Saccharomyces cerevisiae*.




B-1-4 glycosidic bond
glucosamine
N-acetyl group
Chitin
Chitosan
Cellulose

Glucan

- Most fungal walls contain β- chained glucan.
- Glucan are polysaccharides that contain only glucose as structural components, and are linked with β-(1-3) glycosidic bonds.
- Walls of Ascomycetes and Basidiomycetes contain glucan with branching of β(1-6) glucan.
- There are some fungi with a(1-4) glucan.

Glycoprotein and Protein

- Glycoproteins are protein that contain oligosaccharide chains (glycans) covalently attached to polypeptide side- chains.
- Glycoproteins (include mannoprotein, galactoprotein and xyloprotein) are important components of the matrixs of cell walls.
- Glycoprotein contain glucosamine and/or N-acetyl glucosamine.
- In parasitic fungi such as *Candida albicans* and *Aspergillus fumigatus,* the glycoproteins are antigens.
- Mannoprotein in S*accharomyces* cerevisiae are large molecules.

- Hydrophobin is a very hydrophobic protein and found in aerial hyphae of *Schizophyllum commune* and are components of rodlets.
- Rodlets are found on the surface of conidia of *Neurospora crassa* and *Aspergillus nidulans* and protects the hyphae from desiccation.

Characteristics of Fungal Cell Wall

1. Gives shape to fungi.
2. Gives strength to fungi.
3. Provides protection for the protoplasm from ultraviolet rays for the presence of melanins
4. Ability to resist lysis by organic solvents such as enzymes, toxins, osmotic integrity.
5. Ability to bind with metal ions.
6. Secretes enzymes from their walls (invertase hydrolyses sucrose to glucose and fructose) and so assisting in nutrition.

Fungal Cell Wall

•The Cell wall represents a dynamically forming exoskeleton that protects the fungal protoplast from the external environment and defines growth, cellular strength, shape and interactive properties.

•In filamentous fungi, cell wall formation and organization is intimately bound to the process of apical growth. Thus for Example, in *Neurospora crassa* the wall is thin (Approx. 50nm) at the apex but become thicker (Approx.125 nm) behind the tip.

•The Plasma membrane component of the fungal cell envelop is a phospholipid bilayer impregnated with globular proteins that dictates entry of nutrients and exit of metabolites and represents a selective barrier for their translocation.

•Ergosterol is the major sterol found in the membranes of fungi, in contrast to the cholessterol found in the membranes of animals and phytosterols in plants. This distinction is exploited during the use of certain antifungal agents used to treat some fungal infections and can be used as an assay tool to quantify fungal growth.

•The periplasm or periplasmic space is the region external to the plasmamembrane and internal to the cell wall. In yeast cell, it consist of secreted proteins (mannoproteins) and enzymes (such as invertase and acid phosphatase) that are unable to enter cell wall. In filamentous fungi, the cell membrane and wall may be intimately bound to form a compact hyphae. So, it is resistant to plasmolysis.

•Ultrastructural analysis of fungal cell wall reveals a thick, complex fibrillar network. The cell wall of filamentous fungi are mainly compost of different polysaccharides according to taxonomic groups.

•Generally, the **semi-crytalline microfibrillar** components are organized in a network mainly in the central of cell wall region and are embedded within an **amorphous matrix**.

Mejor polymer found in different taxonomic division of fungi with septation (adapted from Gooday in Gow & Gadd, 1995).

Division	Semi-crytalline microfibrillar network of **POLYMER in the Central of Cell Wall**	**Gel-like AMORPHOUS MATRIX COMPONENT**	**Perforated Septa Present or Absent**
Oomycetes	β-(1-3),β-(1-6) Glucan cellulose	Glucan	Absent
Chytridiomycetes	Chitin microfibrils [ß(1-4)-linked polymer of N-acetylglucosamine], Glucan	Glucan	Absent
Zygomycota	Chitin, Chitosan [ß(1-4)-linked polymer of glucosamine]	Polyglucuronic acid, Glucuronomannoproteins,	Absent
Ascomycota/ Deuteromyces	Chitin, β -(1-3), β-(1-6) Glucan [ß(1-3) and ß(1-6)] alkali insoluble	Galactomannoproteins, α (1-3) Glucan	Present mostly simple with large central pore
Basidiomycota	Chitin β -(1-3), β-(1-6) Glucan	Xylomannoproteins, α (1-3) Glucan	Present mostly Dolipore

Fungal cell wall components

- Approximately 80% of the fungal cell wall consists of **Polysaccharides**.

- Most fungi have a fibrillar structure built-up with **chitin**, **chitosan** (Zygomycota only), and **ß-glucans**, and a variety of **heteropolysaccharides**.

- **Proteins** constitute a small fraction of wall bound material, rarely more than 20%, and often as **glycoprotein,** which function as **mating, recognition**, **wall modification** and **nutrition**. A Class of hydrophobic proteins called **hydrophobins** are localized within the aerial growth or appresoria of certain fungi, **Hydrophobins** (may constitute up to 10% of total wall protein) are expressed constitutively, and become bound to the wall when the hyphae emerge in air.

- **Lipids** are found in walls, usually in very small concentrations.

- Walls also contain a range of other **minor components**, including **pigments, melanin** and **salts**. Melanin is important for protecting the hyphae and spores against biotic and abiotic **stress** (UV light),

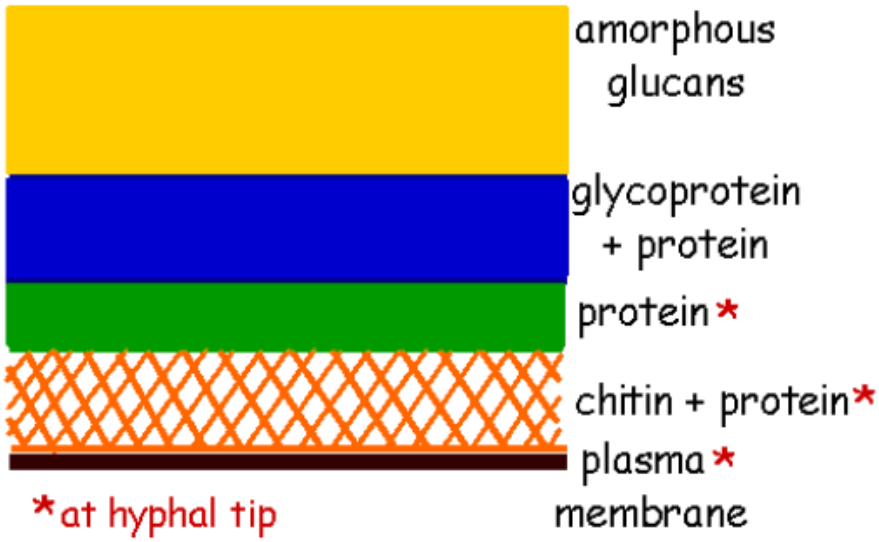
- The hyphae of higher fungi extend via tip growth followed by cross wall formation or septation, whereas the lower fungi remain aseptate. Septa may offer some structural support to hyphae.

- **EXTRA LAYERS** of wall material are deposited in the lateral walls behind the extending apex - strengthening the wall as the hypha matures.

- In the **oldest parts** of the hyphae (and in many fungal spores) **LIPIDS** and **PIGMENTS** may be deposited in the wall:
- **LIPIDS** serve as a nutrient reserve and help prevent desiccation of PIGMENTS, such as **MELANIN**, help protect the protoplast against the damaging effects of UV radiation.
- **Antigenic glycoproteins, agglutinans-** adhesions—on cell wall surface**.**

In the filamentous fungi, the wall of **HYPHAL TIP** is thinner and simpler in structure, consisting of only **TWO LAYERS –**

1. - an inner layer of fibrils (polymeric) embedded in protein and
2. - outer layer of mainly protein.

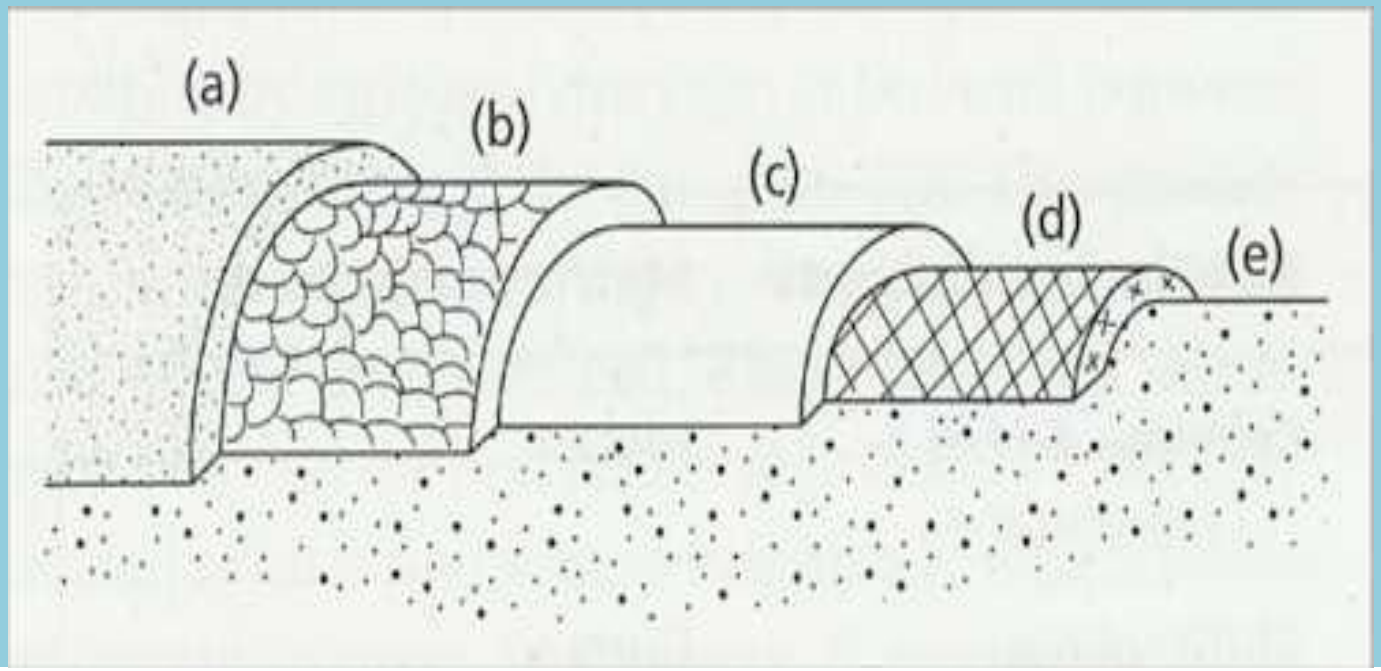


Arrangement of wall components
The diagram above represents a section through the mature lateral wall of hyphae of *Neurospora crassa*

- outer layer

-an inner layer

Diagram to illustrate the wall architecture in a 'mature' (subapical) region of a hypha of *Neurospora crassa* as evidenced by sequential enzymatic digestion.



(a) Outermost layer of amorphous ß-1,3-glucans and ß-1,6-glucans. (b) glycoprotein reticulum embedded in protein. (c) a more or less discrete protein layer, (d) chitin microfibrils embedded in protein, (e) plasma membrane. Based on Hunsley & Burnett, 1970. [© Jim Deacon]

Cell wall Functions:

- **PROTECTS** the underlying protoplasm;
- Determines **and MAINTAINS THE SHAPE** of the fungal cell or hypha; if you remove the wall the resulting protoplast will always assume a spherical shape;
- Acts as an **INTERFACE** between the fungus and its environment;
- Acts as a **BINDING SITE** for some enzymes;
- Possesses **ANTIGENIC** properties - which allow interactions with other organisms.

FUNCTION IN RELATION TO STRUCTURE

- Glycoproteins in Wall
- Hydrophobins in Walls
- Glomalin in Walls

Glycoproteins in Wall:

Different but **compatible glycoproteins**, called **agglutinins**, in the walls of each complementary hypha fuse to form a complex structure binding the cells together with release of **hormone-like compounds**.

Adhesion is also mediated by **fibrillar glyco-proteins** embedded in a **gel-like matrix**. The fibrils are highly specific and a **complementary protein** on the surface of the partner in pathogenic and mutualistic interactions.

Hydrophobins in Walls:

Hydrophobins may constitute up to 10% of total wall protein. Each molecule consists of a hydrophobic domain and a hydrophilic domain.

The protein is attached to the fungal wall by the hydrophilic end. The hydrophobic domain is exposed. Hydrophobins reduces movement of water through the wall of the hypha and providing some protection from desiccation. It may also increase the strength of the wall.

Glomalin in Walls

Some members of the **Glomeromycota** produce a putative glycoprotein in the walls. The compound has been called **glomalin**. Recent work suggests that the compound is related to the **Heat Shock Proteins (HSPs)** in **group 60**.

However, the structure and characterization of the function of glomalin are incomplete.

WORONIN BODIES and LARGE HEXAGONAL CRYSTALS OF PROTEIN

Septum of Ascomycota has spherical, membrane-bound organelles called **WORONIN BODIES.** First reported by the Russian mycologist **Mikhail Stepanovich Woronin** in the euascomycete *Ascobolus pulcherrimus* in 1864.

Not all fungi belonging to the Ascomycota possess Woronin bodies .
– those often possess **LARGE HEXAGONAL CRYSTALS OF PROTEIN (LHCPs)** in the cytoplasm that are capable of serving the same function, i.e. they can seal the septal pores of damaged or ageing hyphae.

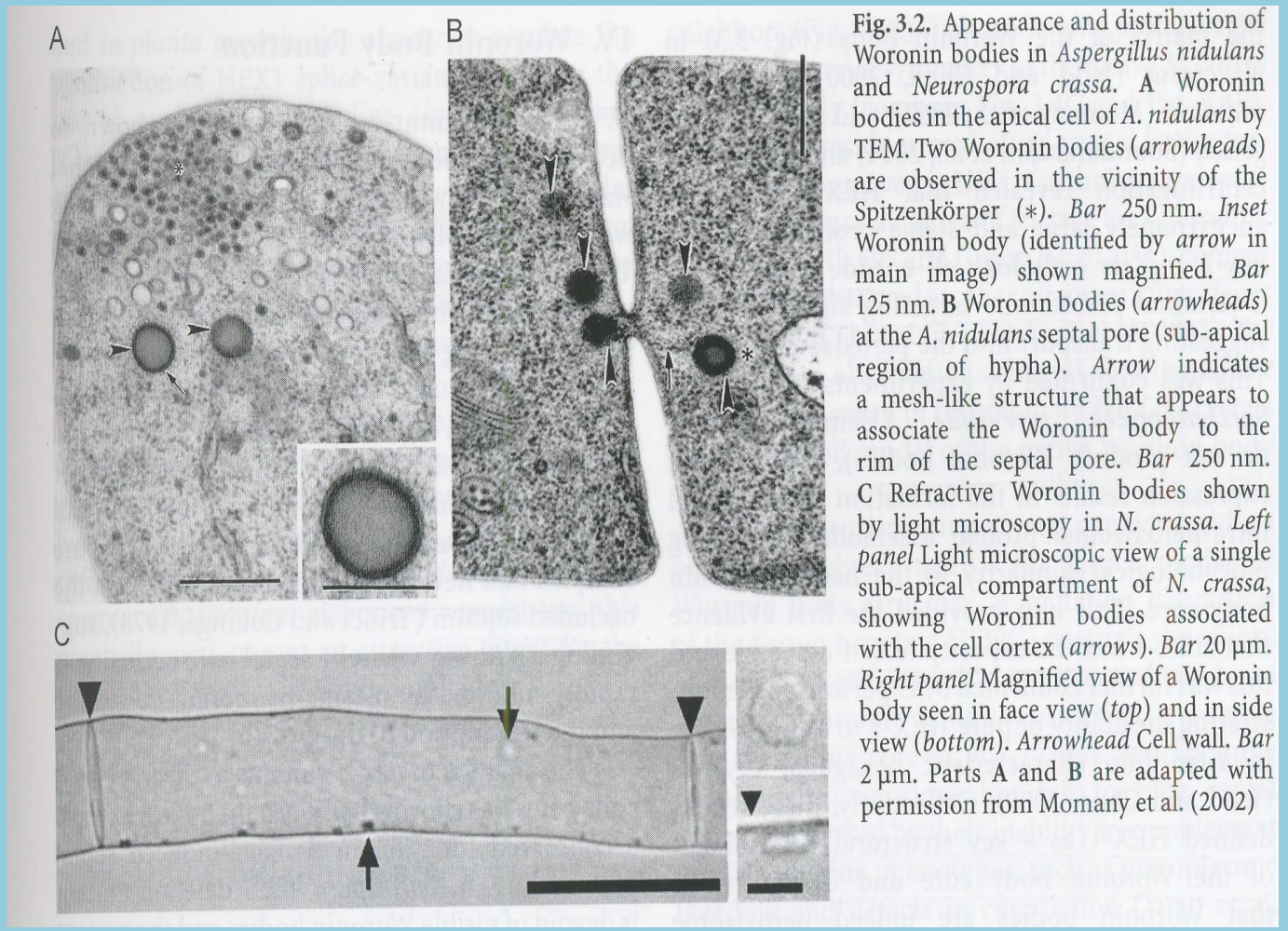


Fig. 3.2. Appearance and distribution of Woronin bodies in *Aspergillus nidulans* and *Neurospora crassa*. **A** Woronin bodies in the apical cell of *A. nidulans* by TEM. Two Woronin bodies (*arrowheads*) are observed in the vicinity of the Spitzenkörper (∗). *Bar* 250 nm. *Inset* Woronin body (identified by *arrow* in main image) shown magnified. *Bar* 125 nm. **B** Woronin bodies (*arrowheads*) at the *A. nidulans* septal pore (sub-apical region of hypha). *Arrow* indicates a mesh-like structure that appears to associate the Woronin body to the rim of the septal pore. *Bar* 250 nm. **C** Refractive Woronin bodies shown by light microscopy in *N. crassa*. *Left panel* Light microscopic view of a single sub-apical compartment of *N. crassa*, showing Woronin bodies associated with the cell cortex (*arrows*). *Bar* 20 µm. *Right panel* Magnified view of a Woronin body seen in face view (*top*) and in side view (*bottom*). *Arrowhead* Cell wall. *Bar* 2 µm. Parts **A** and **B** are adapted with permission from Momany et al. (2002)

Associated with each septum are spherical, membrane-bound organelles called **WORONIN BODIES**

THE FORMATION OF WORONIN BODY AS ADAPTATION

A **Woronin body** (named after the **Russian botanist Mikhail Stepanovich Woronin**) is a peroxisome derived, dense core microbody with a unit membrane found near the septae that divide hyphal compartments in filamentous Ascomycota. One established function of Woronin bodies is the plugging of the septal pores after hyphal wounding, which restricts the loss of cytoplasm to the sites of injury

Fungal HYPHAE

Cylindrical, branching filaments composed of a **tubular cell wall** filled with **cytoplasm and organelles**. Most fungal hyphae are 2-10 μm diameter. Each HYPHA (singular) is essentially a tube - consisting of a **rigid wall** and containing protoplasm **tapered at its tip** - this is the **region of active growth** (i.e. the extension zone). Hyphae tend to form a larger network of cells called a **MYCELIUM**.

Fungal hyphae have a number of unique features:

1. **SEPTA (cross-walls)**, possess one of more **PORES** - such septa divide up the hyphae into a series of interconnected **HYPHAL COMPARTMENTS**, rather than separate, discrete cells.

2. Each hyphal cell or compartment normally contains one or more **NUCLEI** able to pass between adjacent compartments, via the central septal pore.

3. The **GROWING TIP** is structurally and functionally very different from the rest of the hypha, Its cytoplasm appears more dense there are no major organelles at the extreme tip

4. At the extreme tip there is an accumulation of membrane-bound vesicles - the **APICAL VESICULAR CLUSTER (COMPLEX) (AVC)** - which plays an important role in apical growth.

5. **VACUOLES** may be visible in sub-apical hyphal compartments - although small at first, they **grow larger and merge with one another**; they store and recycle cellular metabolites, e.g. enzymes and nutrients.

6. **Mycorrhiza**l species **store phosphate** in the form of **polyphosphate**. **Calcium storage for intracellular signaling**. **Proteases** for breakdown of cellular proteins and recycling of amino acids. **Regulatio**n of cellular pH.

APICAL VESICULAR CLUSTER (AVC):

The growing hyphal tip is structurally and functionally different from the rest of the hypha with **thinner and simpler in structure** than the mature lateral wall of the hypha and the presence of **vesicles** which form the **APICAL VESICULAR CLUSTER (AVC)**:

When a hypha stops growing, these vesicles disappear, when growth of the hypha resumes, the vesicles reappear.

When a hypha is growing straight ahead, the vesicles are positioned in the centre of the hyphal tip, movement of the vesicles to the left or right side of the hyphal tip is accompanied by a change in direction of growth of the hypha.

Vesicles of the AVC contain:

- **Wall PRECURSORS** - the sub-units or building blocks of the wall polymers - e.g. uridine diphosphate N-acetylglucosamine, the sub-unit of chitin wall.
- **LYTIC ENZYMES** - which help breakdown and separate wall components - e.g. chitinase, glucanase.
- **wall SYNTHASE ENZYMES** - which help assemble new wall components and so increase the size of the wall - e.g. chitin synthase, glucan synthase.

Vesicles of the AVC contain:

- The **AVC** is found at the actively growing hyphal tip (the apex) and consists of a mass of vesicles surrounding the **Spitzenkörper**, an opaque structure which can be seen with phase contrast microscopy. Further back from the centre of the AVC, several **mitochondria** are seen.
- The AVC can be seen in **ascomycetes, basidiomycetes and oomycetes**; in fact, in all the fungal species which grow as **mycelia**.

NUCLEI

- ✓ Nuclei are always present in living cells.
- ✓ In the fungi nuclei are **1 - 3 µm in diameter**, **somewhat smaller** than most other eukaryotic organisms, where they range from 3 - 10 µm.
- ✓ **Contain 3--40 chromosomes which are small** and difficult to visualize in stained and squashed preparations.

Up to **13--40 Mb** (million base pairs) DNA coding for 6,000 to 13,000 genes

Usually **haploid** [*Schizophyllum commune* (6)
Neurospora crassa (7),
Emericella (*Aspergillus nidulans(8*),
Saccharomyces cerevisiae (16),
Ustilago maydis (20);

Naturally diploid - *Candida albicans* and members of Oomycota or exist

as polyploid species - *Allomyces* and several sp. of *Phytophthora*

✓**Nuclear membrane persists** during division, unlike plants and animals. The process of formation of 2 sister nuclei by constriction in the middle of the nuclear membrane-**Karyochoresis**)

✓**No clear metaphase plate**, chromosomes randomly dispersed, at anaphase daughter chromatids pull apart along two tracks on spindle fibres of different lengths.

✓**Various types of spindle pole bodies**; significance of the differences unclear, needed to ensure that chromosomes separate correctly during nuclear division.

✓**Nuclear associated organelles (NAOs):** Associated with the nuclear envelope; function as microtubule-organizing centers during mitosis and meiosis.

✓**Spindle pole bodies (SPBs):** In fungi that lack a flagellated stage in lifecycle,

- ➢disc shaped -Ascomycota and Mitosporic fungi;
- ➢two globular ends connected by a bridge-Basidiomycota

✓**Centrioles:** In fungi and other organisms possessing flagellated stage in lifecycle.

ENDOPLASMIC RETICULUM

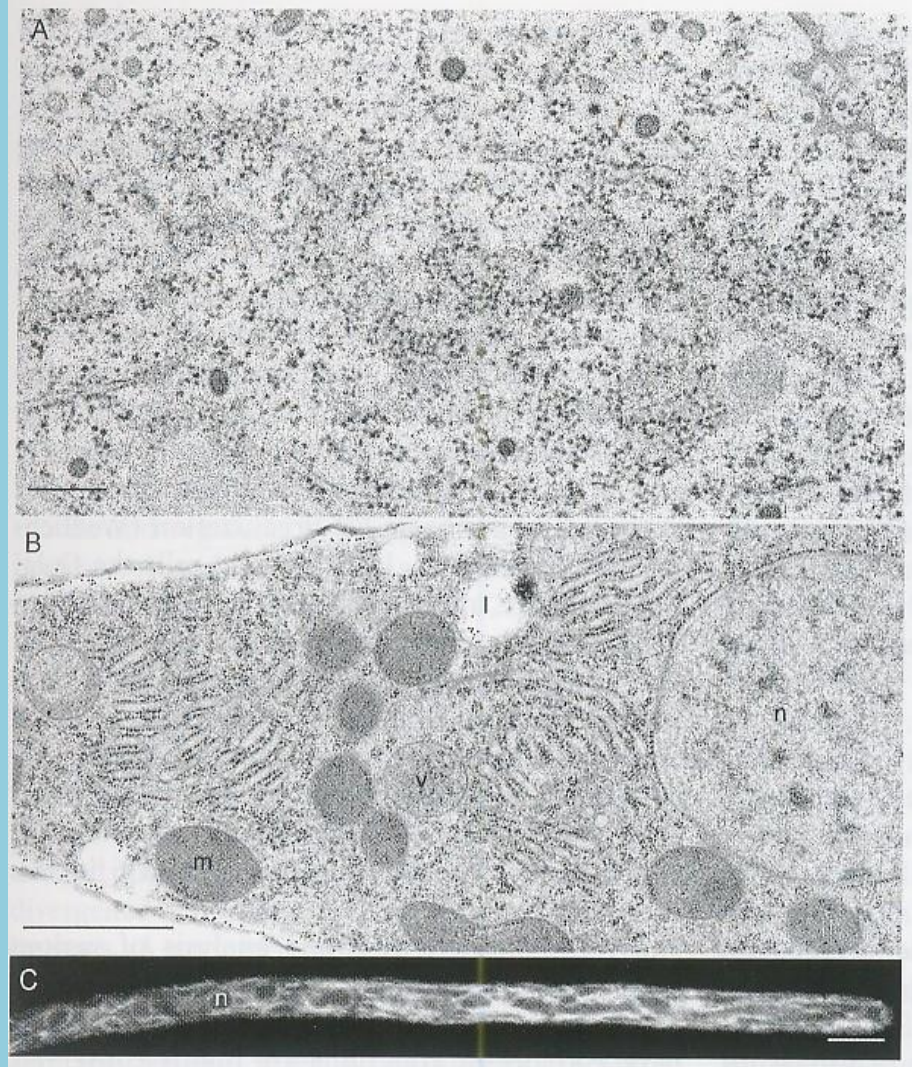


Fig. 1.2. Endoplasmic reticulum (ER) and nuclear envelope. **A** The ER in fungal cells is most often observed as sheets that, when tangentially sectioned, reveal a concentration of polysomes comprised of a spiral array of individual ribosomes as observed here in a vegetative hypha of *Aspergillus nidulans*. *Bar* 250 nm. **B** Note that the concanavalin A binding sites marked by gold particles are absent in this dilated ER in an apical conidial cell of *Magnaporthe grisea*. The continuity of the ER with the nuclear envelope is illustrated by the ribosome-studded outer membrane of the nuclear envelope. *l* Lipid body, *m* mitochondrion, *n* nucleus, *v* vacuole. *Bar* 1.0 µm. **C** ER in a living cell of a multinucleate *Fusarium verticillioides* hypha targeted with a fluorescent protein, using the KDEL ER-retention sequence. In this single optical laser scanning confocal section, the nuclear envelope is apparent as is the abundance of ER in this metabolically active tip cell. *n* Nucleus. *Bar* 5.0 µm. In **A**, **B**, cells were prepared by freeze substitution.

ENDOPLASMIC RETICULUM

ER is a single interconnected membrane system subdivided into a no. of functional systems.

It is continuous with the nuclear envelope and intracellulary forms a contiguous system of both non-fenestrated sheets and variously branched tubules. The latter predominates at the cell periphery where the tubules are closely associated with and may possess molecular links to the plasma membrane.

ER persists throughout the cell cycle.

The type of ER network varies in yeast and filamentous fungi (tubular & distributed towards the periphery vs. cisternal sheets often stacked into parallel arrays and found throughout the radius.

Prominent peripheral ER network in rust fungus), during stages of specific development (**tubular vesicular network)** mostly rough with polyribosomes.

•The ER is highly motile. In *Ustilago maydis* cytoplasmic **dynein** and **microtubules** were shown to be required for ER motility but not for maintaining basic ER organization.

FUNCTION

Essential role in both Protein and Lipid pathways as the site of biosynthesis of nearly all cellular membrane and trans membrane proteins regardless of ultimate destination.

Luminal proteins destined for Golgi apparatus and Vacoules are co-translationally delivered into the ER lumen

ER lumen stores calcium ions to be released into cytosol upon induction via appropriate signalling.

GOLGI APPARATUS

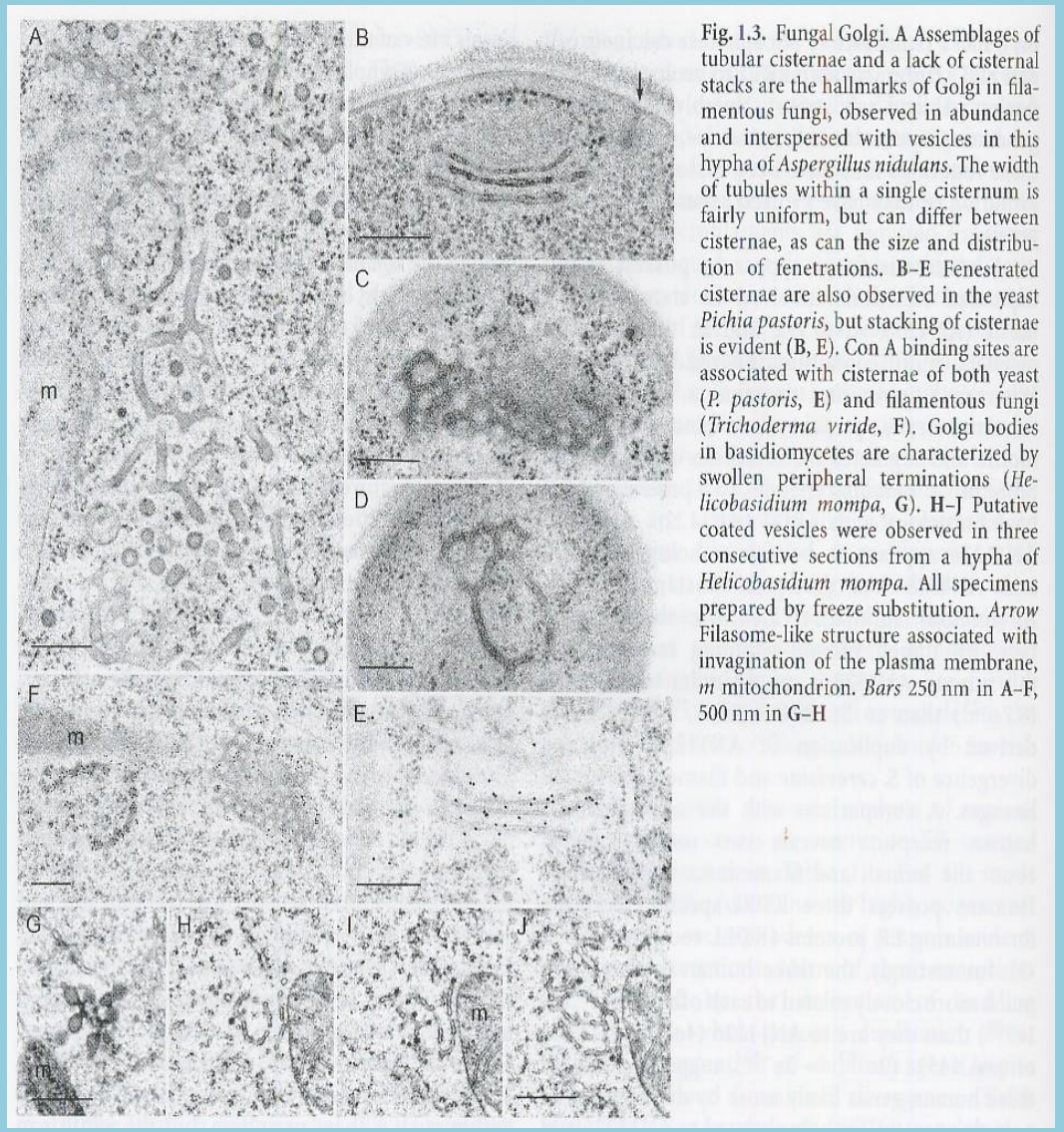


Fig. 1.3. Fungal Golgi. A Assemblages of tubular cisternae and a lack of cisternal stacks are the hallmarks of Golgi in filamentous fungi, observed in abundance and interspersed with vesicles in this hypha of *Aspergillus nidulans*. The width of tubules within a single cisternum is fairly uniform, but can differ between cisternae, as can the size and distribution of fenetrations. B–E Fenestrated cisternae are also observed in the yeast *Pichia pastoris*, but stacking of cisternae is evident (B, E). Con A binding sites are associated with cisternae of both yeast (*P. pastoris*, E) and filamentous fungi (*Trichoderma viride*, F). Golgi bodies in basidiomycetes are characterized by swollen peripheral terminations (*Helicobasidium mompa*, G). H–J Putative coated vesicles were observed in three consecutive sections from a hypha of *Helicobasidium mompa*. All specimens prepared by freeze substitution. *Arrow* Filasome-like structure associated with invagination of the plasma membrane, *m* mitochondrion. *Bars* 250 nm in A–F, 500 nm in G–H

A- Assemblages of tubular cisternae and a lack of cistarnal stacks are the hallmarks of Golgi in filamentous fungi, observed interspersed with vesicle in hyphae of *Aspergillus nidulans*. The width of tubules of a single cisternum is fairly uniform. Golgibody of basidiomycetes are characterized by Swollen peripheral terminals

GOLGI APPARATUS

In true fungi, golgi bodies are morphologically very simple. Lacks the stacks of flattened cisternae or dictyosomes and consists of single cisternal elements.

Golgi equivalents are individual organelles, consisting of

(i) Fenestrated sheets with tubular extensions that are dispersed throughout the hyphae

OR

(ii)Fenestrated hollow spheres or sheets wrapped closely around mitochondria. The width of cisternal tubules is generally uniform within such a sheet or sphere,

TUBULES, VESICLES/MULTIVESICULAR BODIES & VACOULES

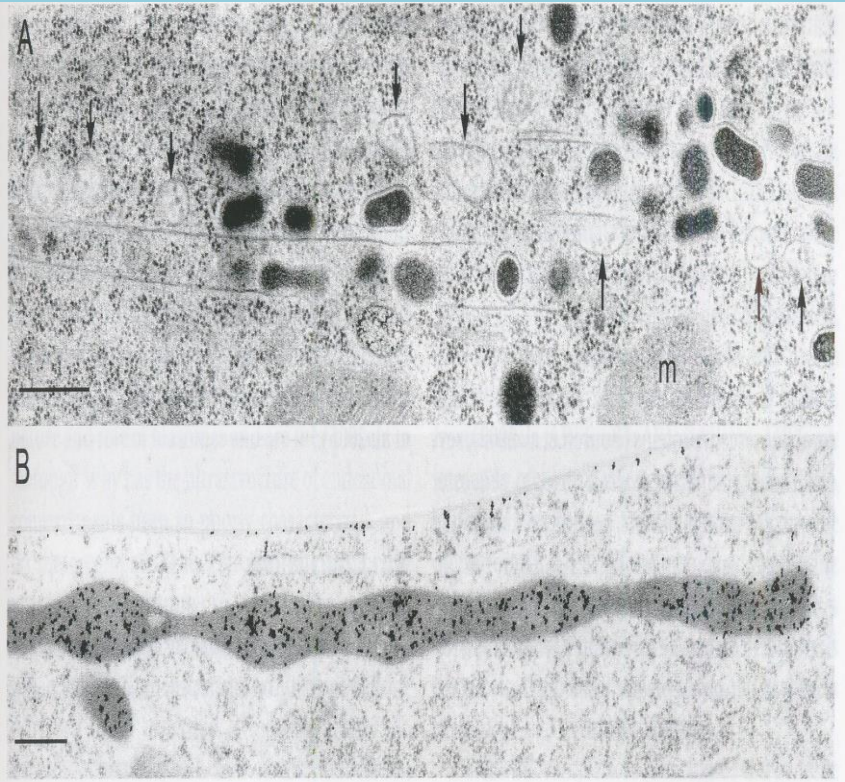


Fig. 1.6. Vacuoles and multivesicular bodies. A Multivesicular bodies are thought to be intermediate compartments between early and late endosomes and can be very abundant in hyphae (*arrows*) as illustrated here in *Gilbertella persicaria*. They are interspersed with microtubules and vesicles that exhibit bidirectional movement. B Fungal vacuoles are an assemblage of pleiomorphic entities that can exist as elongated tubular structures with Con A binding sites as illustrated here in *Magnaporthe grisea*. Specimens prepared by freeze substitution. *m* Mitochondrion. *Bars* 500 nm

A- Multivesicular bodies are thought to be intermeadiate compartment between early and late **endosome** and can be very abundant in hyphae in *Giberella persicaria*. They are inter-spread with microtubules and vesicles that exhibit bidirectional movement.

B- Fungal vacuoles are an assemblage of pleiomorphic entities that can exist as elongated tubular structure.

HYDROGENOSOMES:

In some obligately anaerobic organisms, including fungi, an organelle is observed which appears to produce hydrogenase and pyruvate oxidoreductase. The enzymes function in the anaerobic conversion of organic carbon to energy. The organelle is called a hydrogenosome. Hydrogenosomes have been observed in the Chytridiomycota found in the rumen of herbivores; these Chytrids lack mitochondria.

FILASOMES:

Relatively little is known about them. Appear as tiny vesicles that are coated with a dense filamentous material. They are numerous in tips of actively growing hyphae (peripheral subapical region) where they are characteristically found in close association with the plasma membrane.

PLASMALEMMASOMES AND LOMASOMES

Plasmalemmasomes are the various membrane configurations which are external to the plasmalemma, often in a pocket projecting into the cytoplasm, and less obviously embedded in wall material.

Lomasome has been defined as membranous vesicular material embedded in the wall external to the line of the plasmalemma. There is a gradation between these two structures.

CYTOPLASM

The cytoplasm is typical is all respects of a eukaryotic cell. Of particular interest is the presence of **plasmids**. These have been characterized in yeasts.

As many as one hundred plasmids are found in yeast cells. Plasmids are also found in filamentous fungi, where some are associated with disease virulence.

Typical fungal cytoplasmic constituents are **Multivesicular bodies** (MVBs), **Woronin bodies**, **Filasomes**, Glycogen storage particles, microbodies, Golgibodies (Golgi equivalents) strands of Endoplasmic Reticulum and the **microtubules** and **microfilaments** that comprise the **fungal cytoskeleton**.

MITOCHONDRIA

•Aside from Nuclei it is the most conspicuous structure. Barely visible in light microscopy as tiny thread or rod like structures, at ultrastructural level appear as electron-dense structures.

•The mitochondria of fungi are clearly recognizable. They have a double bilayer membrane and contain complex internal membranes.

•They differ from other eukaryotic organisms in that the mitochondria are commonly elongate, oriented along the hyphal axis.

The flattened or plate-like mitochondrial cristae in Fungi is similar to that of animals. *Contrast with* Oomycota-*Tubular*.

•The membranes are organised as parallel lamellae usually oriented along the long axis. This orientation is particularly common in older regions of the hypha where vacuoles comprise a large proportion of each compartment, and the cytoplasm is between the vacuole and the wall.

Giant, branched mitochondria have been observed in yeasts, and intermediate forms occur in cells transforming from yeast to filamentous growth.

The flagellum

Among the true fungi only chytrids have flagella. The motile zoospores have one posterior flagellum (opisthokont) with no tinsel (whiplash). The flagellum has 9+2 microtubules arranged in the typical eukaryotic pattern. The chytrid rumen fungi have posteriorly multiflagellate (up to 16 whiplash flagella) zoospores. The organization of the flagellar apparatus at the base of the flagellum is a characteristic feature of different groups of the chytrids.

Principal factors influencing fungal growth and mycotoxin production

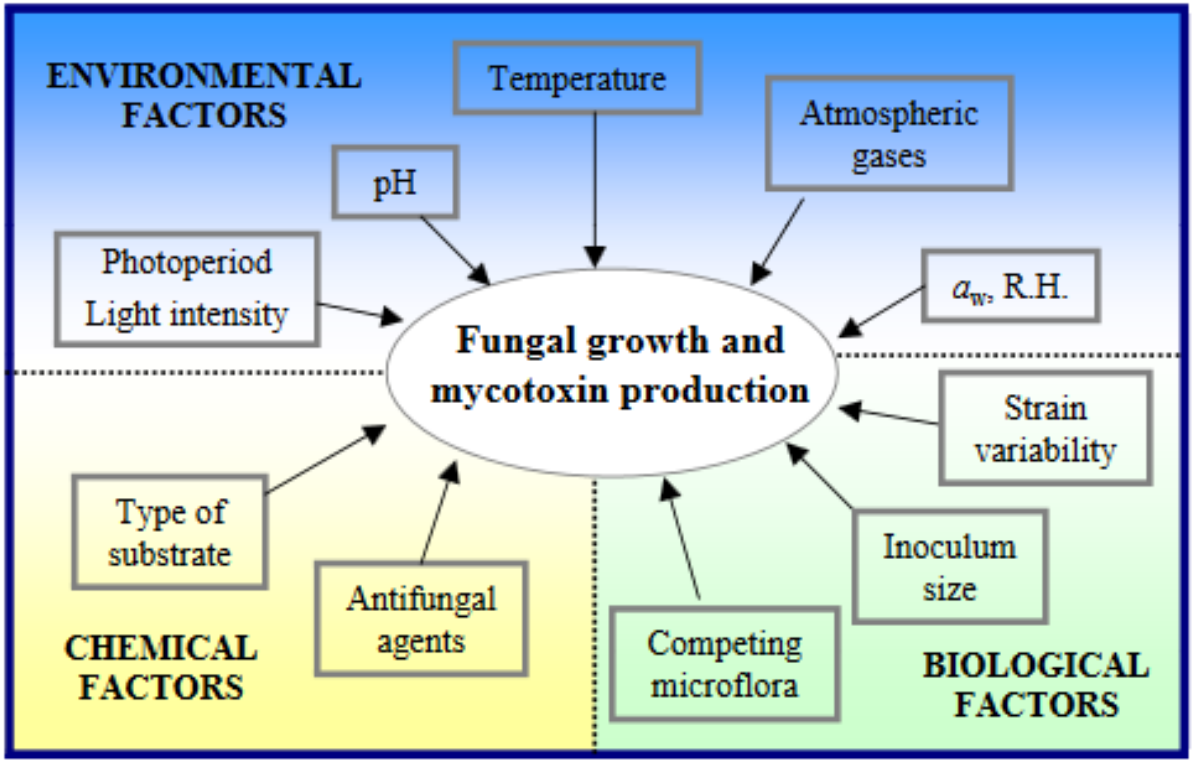


Figure 49. Principal factors influencing fungal growth and mycotoxin production (modified from EC, 1994).

2. Fermentation technology

Feedstock for fermentation process, fermentor design and operation, solid substrate fermentations.

What is Fermentation?

- The word Fermentation is derived from Latin word *fervere which means to boil.*
- But the conventional definition of Fermentation is to break down of larger molecules into smaller and simple molecules using microorganisms.
- In Biotechnology, Fermentation means any process by which microorganisms are grown in large quantities to produce any type of useful materials.

Microorganisms used in Fermentation

- Microorganisms used in Fermentation include bacteria, fungi, algae and actinomycetes. The commonly used species are-
- **Bacteria:** *Acetobacter lacti, Acetobacter woodi, Bacillus subtilis, Bacillus polymyxa, Clostridium etc.*
- **Algae:** *Spirulina maxima, Chlorella sorokiniana etc.*
- **Fungi:** *Aspergillus oryzae, Aspergillus niger, Saccharomyces cervisae, Saccharomyces lipolytica etc.*
- **Actinomycetes:** *Streptomyces griseus, Streptomyces noursei etc.*

Culturing the Microorganisms

- After isolation of microorganisms they are grown in culture medium. Different types of microbial cultures are used for different purposes. Some of the common types of cultures are-
  - 1. Batch culture
  - 2. Continuous culture
  - 3. Fed-Batch culture

Batch Culture:

- It is the simplest method of culturing the microorganisms in which the microorganisms are grown on a limited amount of medium until essential nutrients are exhausted to toxic byproducts inhibit the growth. In a batch culture, the microbes pass through a number of stages during their growth.

A. Lag phase: The growth of microorganisms will not occur immediately after inoculation. They take some time to adjust or adapt to the medium. This time is called Lag phase. The Lag phase can be reduced by using relatively large amount of exponentially growing inoculum which is grown in a medium having similar composition as that used in the fermentation.

B. Exponential or Log phase: In this phase, the microbes grow in an exponential manner consuming the nutrients present in the medium.

C. Stationary or Deceleration phase: As soon as the level of nutrients is reduced or exhausted in the medium, the growth of culture gradually slows down. This may also occur due to accumulation of toxic metabolites which inhibits the growth. During this phase, the microorganisms can not grow and hence their biomass can not increase.

D. Death or Decline phase: In this phase the nutrients in the medium exhaust completely and there will be accumulation of toxic materials which leads to death of microbial cells.

Growth Curve

A- Lag phase
B- Exponential/Log phase
C- Stationary phase
D- Death/Decline phase

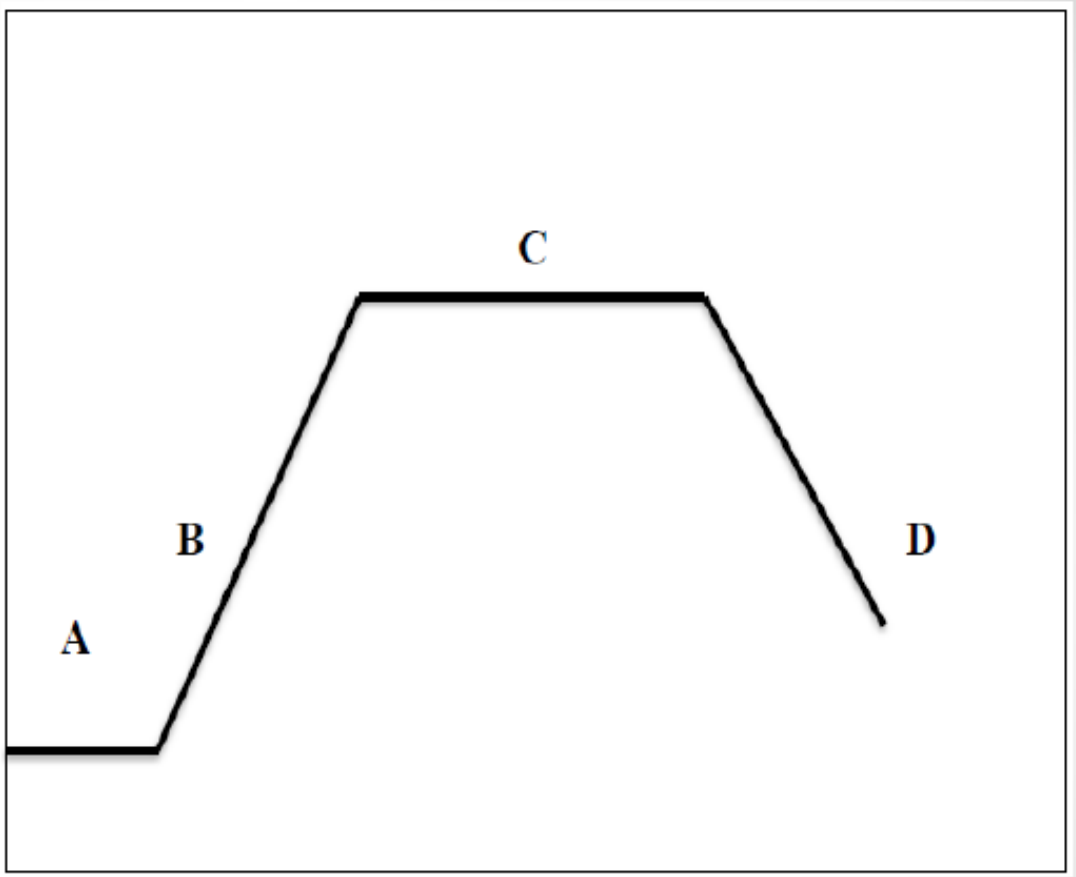


Fig: Growth curve of microorganisms

Continuous culture

- If the culture medium is designed such that the cessation of growth is due to depletion of nutrients rather than by accumulation of toxins, the exponential growth in the batch culture can be prolonged by the addition of fresh medium to the culture vessel. If the addition of fresh medium displaces an equal amount of culture, then continuous production of cells can be achieved. If the medium is added continuously to such a system at a suitable rate, the displacement of culture can be balanced by the production of new biomass and a steady state can be achieved.

Fed-Batch Culture

- It is also the batch culture which is fed continuously with fresh medium with fresh medium without the removal of original culture from the fermenter. The volume of medium in the fermenter increases continuously.

Improvement of industrial strains of microorganisms

- Improvement of industrial strains means improvement of the productivity of the microorganisms those are used in industrial production of fermented products.
- This can be done by two techniques-
  - 1. Mutation
  - 2. Recombination

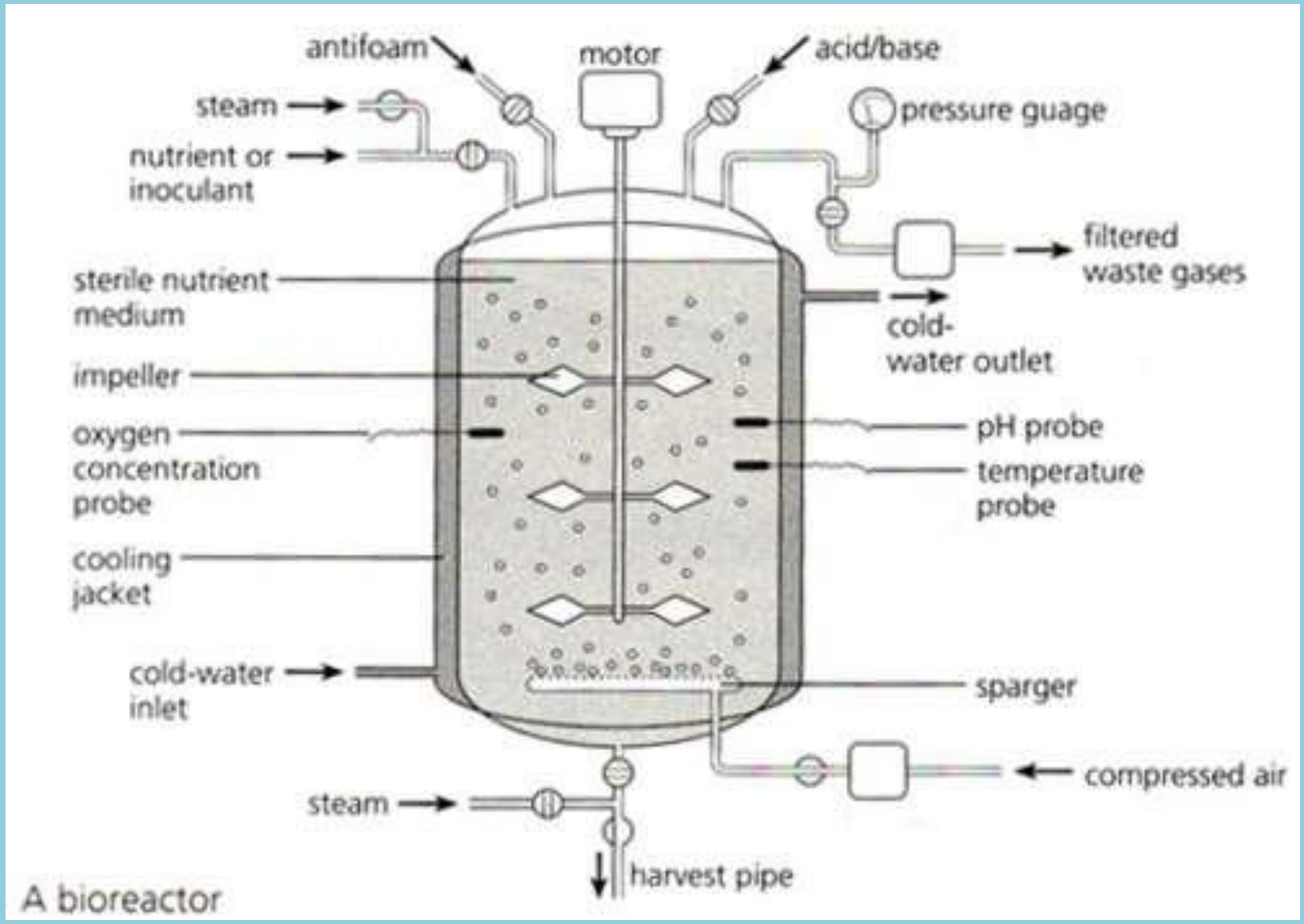
- **1. Mutation:** The changes those occur in the nucleotide sequence of DNA are called as mutation. This change is inheritable. The strain that exhibits altered characters due to mutation is called mutant.
- **2. Recombination:** The process of recombination helps to generate new combination of genes from different individuals. The process of recombination is applicable to fungi and bacteria. Recombination can be done by two techniques-
  - Protoplast fusion
  - In vitro rDNA technology.

Common features of typical fermenter:

- 1. They should be strong enough to withstand the pressure exerted by large volume of the medium.
- 2. The materials used for the construction of fermenter should not be corroded by the fermentation product and it should not yield toxic ion to the medium.
- 3. The fermenter should have provision for the control and prevention of the growth of contaminating microorganisms because industrial fermentation requires pure culture.
- 4. If aerobic organisms are used in the process, there should be provision for rapid incorporation of sterile air into the medium so that the oxygen is immediately dissolved in the medium and available to the microorganisms.
- 5. The Carbon dioxide produced by the microorganisms should be removed from the medium.

- 6. Stirring is necessary to mix the organisms with the medium and to make nutrients and oxygen available to individual microbe.
- 7. The fermenter should provide provision for the addition of antifoaming agents intermittently depending on the foaming status of the medium.
- 8. Thermostatic system should be available to maintain constant temperature in the fermenter.
- 9. There should be provision for aseptic withdrawal of culture during fermentation and also for the aseptic introduction of inoculum at the starting of the fermentation process.
- 10. A system should be available for detection of pH of the culture medium and also for its adjustment.

A typical fermenter.

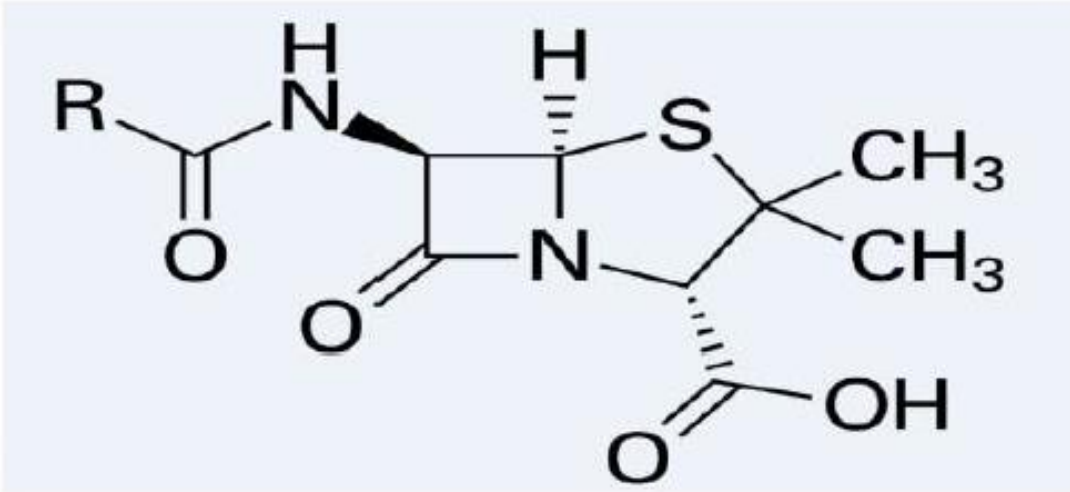


Biologicals obtained from fermentation

- 1. Antibiotics
  - Penicillin
  - Streptomycin
  - Tetracycline
- 2. Solvents
  - Ethanol
  - Glycerol
- 3. Vitamins
  - Riboflavin
  - Cyanocobalamine
- 4. Organic acids
  - Lactic acid
  - Citric acid

Production of Penicillin by Fermentation

- Penicillin is a beta-lactum thiozolidine ring. Different penicillins are produced in presence of different precursors.



Precursor	Radical-R	General name
Phenyl acetic acid	C6H5CH2-	Penicillin G (Benzyl Penicillin)
Phenoxy acetic acid	C6H5O-CH2-	Penicillin V (Phenoxy methyl Penicillin)

Formerly, *Penicillium notatum was used for penicillin fermentation.* Now-a-days, the principal organism for the commercial production is *Penicillium chrysogenum*

Production

- Initially, the strain of microorganism is grown in a sporulation medium and subsequently, the inoculum is transferred into the fermentation medium. After inoculation, incubation is done for 5-7 days to allow spore production. Later, the spores are transferred to the liquid medium in shake flask to allow vegetative growth.
- Finally, appropriate volume of inoculum is added to final fermenter containing the sterile medium of which consists of lactose, glucose, $NaNO_3$, $ZnSO_4$, $CaCO_3$, phenylacetic acid and vegetable oil etc.
- The pH is maintained at 7.0 using $CaCO_3$ which is optimum for the production of penicillin. The medium is maintained at a temperature of 22-25*C.
- After 48-96 hours of cultivation when optimum production of penicillin is obtained, the fermented broth containing about 1% of penicillin is processed. The crude antibiotic is subjected to the extraction procedure.

Solid-State Fermentation Technology

- Solid-state fermentation has long been applied to the food industry. SSFs are processes carried out with microbes growing on nutrient impregnated solid substrate with little or no free water. The growth of koji, an enzyme-rich mold grown on shallow trays of steamed rice, is a classical example of SSF. Solid state fermentation (SSF) can be directly carried out with abundant low-cost biomaterials (starch, cellulose, lignin, hemicellulose, chitin, etc.) with minimal or no pretreatment, and thus is relatively simple, uses less energy than submerged fermentation (SmF), and can provide unique micro environments conducive to microbial growth and metabolic activities. Currently, SSF is undergoing a renewed surge of interest, primarily because of the opportunities that SSF affords for increased productivity and product concentration as compared to SmF new product possibilities, cheaper product recovery, and the prospect of using a wide range of agri-industry commodities and waste streams as substrates.

- Large amounts of excess plant biomass are produced by the agri-industry. It is desirable to use this as a renewable resource for sustainable chemical production via microbial cultivation. If not used to generate a value-added product, the biomass would remain in the waste stream and require expensive disposal or treatments. The major reason that Western industry is reluctant to use SSF is a lack of knowledge and of scalable bioreactor technologies. There are very few data on growth and product formation kinetics, reactor design, or process control in SSF available in the literature. However, with the increased interest in SSF with the goal of developing industrially applicable SSF systems, progress is being made. This chapter will review the recent advances in SSF system research and development.

Advantages and Disadvantages of Traditional Solid-State Fermentation Technology

- These are the advantages of solid-state fermentation:
- 1.The medium is cheap. Cereals, wheat bran, and other agricultural products can be used.
- 2.Special products can be produced. For example, red pigments, whose production is enhanced by solid-state fermentation.
- 3.The purification, recovery, and disposal of downstream processes in solid-state fermentation are usually simpler than in liquid fermentation.
- 4.Solid-state fermentation can produce food with a special flavor and improve nutritional value. For example, tempeh can be used as a substitute for meat, and its amino acid and fatty acids can be easily digested.
- 5.There is no wastewater discharge in solid-state fermentation.

3. Enzyme technology

Fungal enzymes of commercial importance, production of fungal enzymes, free and immobilized cells and enzymes.

Fungal enzymes

- Enzymes are the biocatalysts synthesized by living cells. They are complex protein molecules that bring about chemical reactions concerned with life. It is fortunate that enzymes continue to function (bring out catalysis) when they are separated from the cells i.e. in vitro.
- Basically, enzymes are nontoxic and biodegradable. They can be produced in large amounts by microorganisms for industrial applications.
- The role of industrially produced enzymes increases every day with the development of native and recombinant proteins in modern biotechnology.
- Enzyme technology broadly involves production, isolation, purification and use of enzymes (in soluble or immobilized form) for the ultimate benefit of humankind. In addition, recombinant DNA technology and protein engineering involved in the production of more efficient and useful enzymes are also a part of enzyme technology.

Applications of Enzymes:

- Microbial enzymes have been utilized for many centuries without knowing them fully. The first enzyme produced industrially was taka-diastase (a fungal amylase) in 1896, in United States. It was used as a pharmaceutical agent to cure digestive disorders.
- Enzymes have wide range of applications. These include their use in food production, food processing and preservation, washing powders, textile manufacture, leather industry, paper industry, medical applications, and improvement of environment and in scientific research.
- As per recent estimates, a great majority of industrially produced enzymes are useful in processes related to foods (45%), detergents (35%), textiles (10%) and leather (3%). For details on the applications of individual enzymes.

- The utilization of enzymes (chiefly proteases) for laundry purposes started in 1915. However, it was not continued due to allergic reactions of impurities in enzymes. Now special techniques are available for manufacture, and use of enzymes in washing powders (without allergic reactions). Commercial enzymes can be produced from a wide range of biological sources. At present, a great majority (80%) of them are from microbial sources.

The different organisms and their relative contribution

Fungi – 60%
Bacteria – 24%
Yeast – 4%
Streptomyces – 2%
Higher animals – 6%

Enzymes from microbial sources

- Microorganisms are the most significant and convenient sources of commercial enzymes. They can be made to produce abundant quantities of enzymes under suitable growth conditions. Microorganisms can be cultivated by using inexpensive media and production can take place in a short period.
- In addition, it is easy to manipulate microorganisms in genetic engineering techniques to increase the production of desired enzymes. Recovery, isolation and purification processes are easy with microbial enzymes than that with animal or plant sources.
- In fact, most enzymes of industrial applications have been successfully produced by microorganisms. Various fungi, bacteria and yeasts are employed for this purpose

Various fungi, bacteria and yeasts are employed for enzyme production

Enzyme	Source(s)	Application(s)
α-Amylase	*Aspergillus oryzae* *Aspergillus niger* *Bacillus subtilus* *Bacillus licheniforms*	Production of beer and alcohol, Preparation of glucose syrups, As a digestive aid Removal of starch sizes
Amyloglucosidase	*Aspergillus niger* *Rhizopus niveus*	Starch hydrolysis
Cellulase	*Aspergillus niger* *Tricoderma koningi*	Alcohol and glucose production
Glucoamylase	*Aspergillus niger* *Bacillus amyloliquefaciens*	Production of beer and alcohol Starch hydrolysis
Glucose isomerase	*Arthrobacter sp* *Bacillus sp*	Manufacture of high fructose syrups
Glucose oxidase	*Aspergillus niger*	Antioxidant in prepared foods
Invertase	*Saccharomyces cerevisiae*	Surcose inversion Preparation of aritficial honey, confectionaries

Keratinase	*Streptomyces fradiae*	Removal of hair from hides
Lactase	*Kluyveromyus* sp	Lactose hydrolysis
	Saccharomyces fragilis	Removal of lactose from whey
Lipase	*Candida lipolytica*	Preparation of cheese
	Asperigillus niger	Flavour production
Pectinase	*Aspergillus* sp	Clarification of fruit juices and wines
	Sclerotina libertina	Alcohol production, coffee concentration
Penicillin acylase	*Escherichia coli*	Production of 6-aminopenicillanic acid
Penicillanase	*Bacillus subtilis*	Removal of penicillin
Protease, acid	*Aspergillus niger*	Digestive aid
		Substitute for calf rennet
Protease, neutral	*Bacillus amyloliquefaciens*	Fish and meat tenderiser
Protease, alkaline	*Aspergillus oryzae*	Meat tenderiser
	Streptomyces griseus	Detergent additive
	Bacillus sp	Beer stabilizer
Pollulanase	*Klebsiella aerogens*	Hydrolysis of starch
Takadiastase	*Aspergillus oryzae*	Supplement to bread,
		Digestive aid

Aspergillus niger— A unique organism for production of bulk enzymes

- Among the microorganisms, *A. niger* (a fungus) occupies a special position for the manufacture of a large number of enzymes in good quantities. There are well over 40 commercial enzymes that are conveniently produced by *A. niger*. These include a-amylase, cellulase, protease, lipase, pectinase, phytase, catalase and insulinase.

Commercial recombinant enzymes

- Recombinant fungi are one of the main sources of enzymes for industrial applications. The industrial enzyme market reached $1.6 billion in 1998 for the following application areas (excluding diagnostic and therapeutic enzymes).

Aspartic proteinase

- A recombinant strain of *Aspergillus oryz*ae producing an aspartic proteinase from *Rhizomucor miehei* has been approved by **Food and Drug Administration** (FDA) for cheese production.

Lipases

- Lipases are extremely important in the detergent industry. They are extensively used in household detergents, industrial cleaners, and leather processing, where they can be combined with proteases, oxidases, and peroxidases. To be suitable, lipases should be alkalophilic, able to work at temperatures above 45 Degree C and at pH values of about 10, and capable of functioning in the presence of the various components of wash-product formulations, such as oxidants and surfactants. In 1994, Novo Nordisk introduced Lipolase, the first commercial recombinant lipase for use in a detergent, by cloning the *Humicola lanuginos*e lipase gene into the *A. oryzae* genome

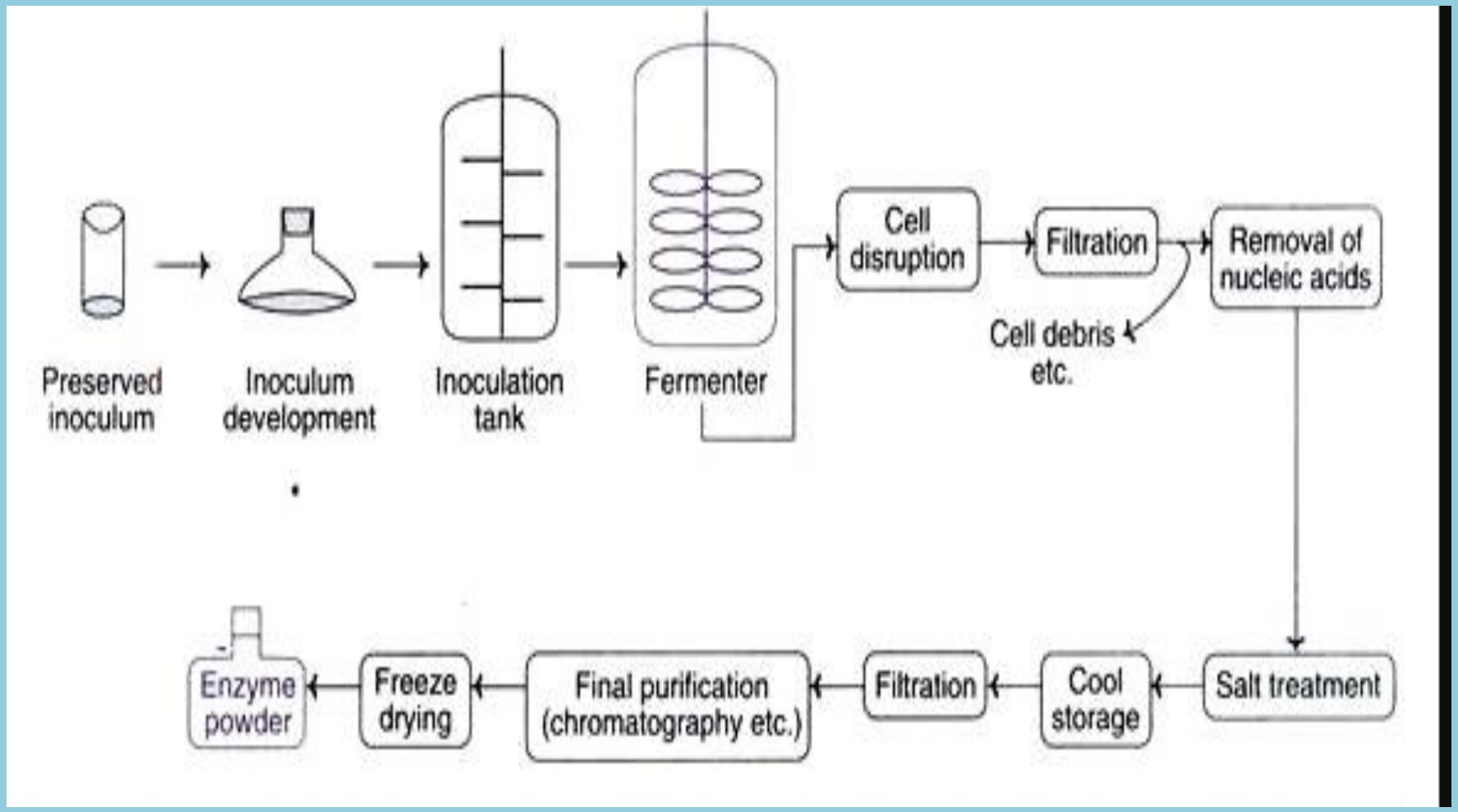
Microbial lipases

- Microbial lipases have a huge potential in areas such as food technology, biomedical sciences, and chemical industries since they are: (1) stable in organic solvents, (2) possess broad substrate specificity, (3) do not require cofactors, and (4) exhibit high enantioselectivity.
- In the food industry, lipases are commonly used in the production of fruit juices, baked foods, desirable flavors in cheeses, and interesterification of fats and oils to produce modified acylglycerols. There are three fungal recombinant lipases currently used in the food industry, *Rhizomucor miehi*, *Thermomyces lanuginosus* and *Fusarium oxysporum*, all of which are produced in *A. oryzae*

The Technology of Enzyme Production

- In general, the techniques employed for microbial production of enzymes are comparable to the methods used for manufacture of other industrial products .The salient features are briefly described.
  - 1. Selection of organisms
  - 2. Formulation of medium
  - 3. Production process
  - 4. Recovery and purification of enzymes.

An outline of the flow chart for enzyme production by microorganisms



Selection of organism

- The most important criteria for selecting the microorganism are that the organism should produce the maximum quantities of desired enzyme in a short time while the amounts of other metabolite produced are minimal. Once the organism is selected, strain improvement for optimising the enzyme production can be done by appropriate methods (mutagens, UV rays). From the organism chosen, inoculum can be prepared in a liquid medium.

Formulation of medium:

- The culture medium chosen should contain all the nutrients to support adequate growth of microorganisms that will ultimately result in good quantities of enzyme production. The ingredients of the medium should be readily available at low cost and are nutritionally safe. Some of the commonly used substrates for the medium are starch hydrolysate, molasses, corn steep liquor, yeast extract, whey, and soy bean meal. Some cereals (wheat) and pulses (peanut) have also been used. The pH of the medium should be kept optimal for good microbial growth and enzyme production.

Production process:

- Industrial production of enzymes is mostly carried out by submerged liquid conditions and to a lesser extent by solid-substrate fermentation. However, solid substrate fermentation is historically important and still in use for the production of fungal enzymes e.g. amylases, cellulases, proteases and pectinases.
- The medium can be sterilized by employing batch or continuous sterilization techniques. The fermentation is started by inoculating the medium. The growth conditions (pH, temperature, O_2 supply, nutrient addition) are maintained at optimal levels.
- The bioreactor system must be maintained sterile throughout the fermentation process. The duration of fermentation is variable around 2-7 days, in most production processes. Besides the desired enzyme(s), several other metabolites are also produced. The enzyme(s) have to be recovered and purified.

Recovery and purification of enzymes:

- The desired enzyme produced may be excreted into the culture medium (extracellular enzymes) or may be present within the cells (intracellular enzymes). Depending on the requirement, the commercial enzyme may be crude or highly purified. Further, it may be in the solid or liquid form. The steps involved in downstream processing i.e. recovery and purification steps employed will depend on the nature of the enzyme and the degree of purity desired.
- In general, recovery of an extracellular enzyme which is present in the broth is relatively simpler compared to an intracellular enzyme. For the release of intracellular enzymes, special techniques are needed for cell disruption by physical means (sonication, high pressure, glass beads). The cell walls of bacteria can be lysed by the enzyme lysozyme. For yeasts, the enzyme β-glucanase is used. However, enzymatic methods are expensive.
- The recovery and purification steps will be the same for both intracellular and extracellular enzymes, once the cells are disrupted and intracellular enzymes are released. The most important consideration is to minimise the loss of desired enzyme activity.

Recovery and purification of enzymes

- **Removal of cell debris-** Filtration or centrifugation can be used to remove cell debris.
- **Removal of nucleic acids-** Nucleic acids interfere with the recovery and purification of enzymes. They can be precipitated and removed by adding poly-cations such as polyamines, streptomycin and polyethyleneimine.
- **Enzyme precipitation-**Enzymes can be precipitated by using salts (ammonium sulfate) organic solvents (isopropanol, ethanol, and acetone). Precipitation is advantageous since the precipitated enzyme can be dissolved in a minimal volume to concentrate the enzyme.
- **Liquid-liquid partition-** Further concentration of desired enzymes can be achieved by liquid-liquid extraction using polyethylene glycol or polyamines.
- **Separation by chromatography-**There are several chromatographic techniques for separation and purification of enzymes. These include ion-exchange, size exclusion, affinity, hydrophobic interaction and dye ligand chromatography .Among these, ion-exchange chromatography is the most commonly used for enzyme purification.
- **Drying and packing-**The concentrated form of the enzyme can be obtained by drying. This can be done by film evaporators or freeze dryers (lyophilizers). The dried enzyme can be packed and marketed. For certain enzymes, stability can be achieved by keeping them in ammonium sulfate suspensions.
- All the enzymes used in foods or medical treatments must be of high grade purity, and must meet the required specifications by the regulatory bodies. These enzymes should be totally free from toxic materials, harmful microorganisms and should not cause allergic reactions.

Immobilized enzyme

- An **immobilized enzyme** is an enzyme attached to an inert, insoluble material—such as calcium alginate (produced by reacting a mixture of sodium alginate solution and enzyme solution with calcium chloride). This can provide increased resistance to changes in conditions such as pH or temperature. It also lets enzymes be held in place throughout the reaction, following which they are easily separated from the products and may be used again - a far more efficient process and so is widely used in industry for enzyme catalysed reactions. An alternative to enzyme immobilization is whole cell immobilization.
- Immobilized enzymes are very important for commercial uses as they possess many benefits to the expenses and processes of the reaction of which include:
- **Economy**: The immobilized enzyme is easily removed from the reaction making it easy to recycle the biocatalyst. This is particularly useful in processes such as the production of Lactose Free Milk, as the milk can be drained from a container leaving the enzyme (Lactase) inside ready for the next batch.
- **Stability**: Immobilized enzymes typically have greater thermal and operational stability than the soluble form of the enzyme.

Immobilization of an Enzyme

- There are various ways by which one can immobilize an enzyme. Various methods used for immobilization of enzymes are adsorption, covalent binding, entrapment, Affinity-tag binding and Cross-linkage :
- **Affinity-tag binding:** Enzymes may be immobilized to a surface, e.g. in a porous material, using non-covalent or covalent Protein tags. This technology has been established for protein purification purposes.
- **Adsorption on glass, alginate beads or matrix:** Enzyme is attached to the outside of an inert material. In general, this method is the slowest among those listed here as the active site of the immobilized enzyme may be blocked by the matrix or bead, greatly reducing the activity of the enzyme.
- **Entrapment:** The enzyme is trapped in insoluble beads or microspheres, such as calcium alginate beads.
- **Cross-linkage**: Enzyme molecules are covalently bonded to each other to create a matrix consisting of almost only enzyme. The reaction ensures that the binding site does not cover the enzyme's active site, the activity of the enzyme is only affected by immobility.
- **Covalent bond:** The enzyme is bound covalentely to an insoluble support (such as silica gel or macroporous polymer beads with epoxide groups). This approach provides the strongest enzyme/support interaction, and so the lowest protein leakage during catalysis.

Immobilization of enzymes

- Various types of gels are used for entrapment of enzymes. The enzyme may be entrapped within polymeric mesh such as agar, polyacrylamide gel or calcium alginate by carrying out the polymerization reaction and /or cross – linking reaction in the presence of enzyme.

Polyacrylamide

- Polyacrylamide gel is the most commonly used material for entrapment. For the preparation of 15% gel, 7.5 g acrylamide, 0.5 g bisacrylamide, 50 mg ammonium persulfate was added to 25 ml of phosphate buffer, pH 6.8 and mixed to dissolve these solids. Then 25 ml of amylase solution added. Mixed properly and added 50 µl of TEMED. Mixed gently and poured into glass Petri dishes, or gel casting vertical electrophoresis unit in order to get the gel of uniform and desired thickness. Polymerization was done at room temperature for 1 h. The gel was cut into small pieces and suspended in 0.1 M phosphate buffer till further use.

Entrapment in calcium alginate gel

- For calcium alginate beads, 25 ml of 0.5, 1, 2, 3 and 4% solutions of sodium alginate were prepared and mixed with equal volume of amylase solution to get the final concentration of sodium alginate 0.25, 0.5, 1, 1.5 and 2%, respectively. Entrapment of enzyme in calcium alginate gel was done by modifying the method of Kierstan and Bucke. Different $CaCl_2$ concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 M) were used to optimize the best concentration. For 50 ml sodium alginate- enzyme mixture, 500 ml of $CaCl_2$ solution was used.

Immobilized whole cell

- **Immobilized whole cell** system is an alternative to enzyme immobilization. Unlike enzyme immobilization, where the enzyme is attached to a solid support (such as calcium alginate) in immobilized whole cell systems, the target cell is immobilized. Such methods may be implemented when the enzymes required are difficult or expensive to extract, an example being intracellular enzymes. Also, if a series of enzymes are required in the reaction; whole cell immobilization may be used for convenience.This is only done on a commercial basis when the need for the product is more justified.

4. Fungal toxins

Mycotoxicoses- fungi in dermatomycosis, aspergillosis and fungi allergenic to man and animal.

Mycotoxicoses

Mycotoxicoses is defined as an illness of man or animal due to ingestion of pre-formed substances produced by the action of certain molds or filamentous fungi on particular foodstuff.

MYCOTOXINS

- Secondary metabolites produced by food-borne filamentous fungi
- Vary in their severity :Carcinogen/allergen
- Non-volatile & low molecular weight
- Some are lethal
- Cause identifiable diseases
- Weaken the immune system without producing symptoms

Feeds Most Susceptible to Fungi producing Mycotoxins

- Cottonseed
- Peanut meal
- Rye
- Bread
- Corn
- Wheat
- Oats
- Barley
- Sorghum

Fungi responsible for mycotoxicoses

- Most of the significant fungi producing mycotoxicoses mainly belong to toxigenic species of 3 genera:

Dermatophytes

- Dermatophytes are aerobic fungi that can invade and infect the keratinized layers of skin, hair, and nails. Three genera of fungi, *Trichophyton*, *Microsporum*, and *Epidermophyton*, account for most dermatophytic infections.
- These fungi are found worldwide and infection is acquired by contact with infected humans or animals, or from exposure to contaminated soil or fomites (e.g., combs, brushes). Dermatophytes often are classified by their host preferences – anthropophilic and zoophilic species primarily infect human and animal species, respectively; geophilic species reside in soil.
- Direct contact can result in transmission of zoophilic or geophilic dermatophytes to humans. Dermatophytes do not cause invasive disease except in immunocompromised hosts. The clinical disease attributable to dermatophytes varies by organism, site of infection, and host immunologic responses.

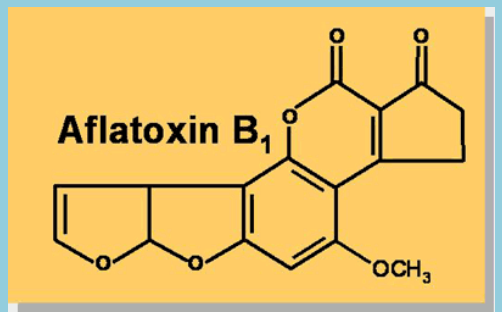
MEDICALLY SIGNIFICANT MYCOTOXINS

1. Aflatoxins
2. Fumonisins
3. Trichothecenes
4. Ochratoxins
5. Cyclopiazonic Acid
6. Zearalenone
7. Patulin

MYCOTOXINS	FUNGAL SPECIES	SOURCE OF EXPOSURE	CLINICAL CONDITIONS
Aflatoxins	A.flavus,parasiticus, nomium,P.puberulum	Nuts,Maize	Aflatoxicosis, Rey'sSyndrome
Fumonisins	Fusarium moniliforme	Maize	ELEM,PPE
Trichothecens	Fusarium graminearum, F.sporotrichioides	Maize,Sorghum	HumanToxicosis,
Ochratoxins	A.ochraceus,A.niger, P.verrucosum	Cereals,coffee-Beans,Bread	Nephropathies
Cyclopiazonic Acid	A.flavus,A.versicolor, A.oryzae,P.cyclopium	Groundnut, Corn,Meat	Co-contaminant, Kodua Poisoning
Zearalenones	F.Graminearum	Wheat,Maize, Barley,Sorghu	Genital disorders in animals ie, Pigs
Patulin	P.patulum/griseofulvum	m Thought to be the antiviral	

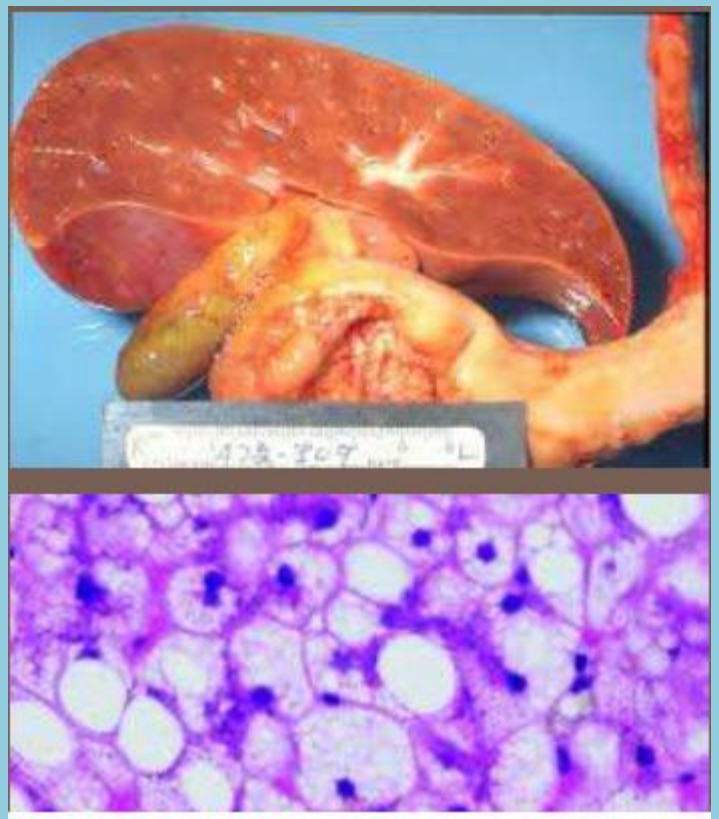
AFLATOXINS(Aflatoxicosis)

- These toxins were discovered as a cause of mysterious disease termed as Turkey-X-disease during 1960s in England.
- Killed approx.1,00,000 birds and ruined turkey industry.
- Aflatoxins most commonly produced by *A. flavus* are B1 and B2.
- *A.parasiticus* produces G1and G2.
- These are designated as B & G on the basis of their metabolites which exhibits blue(B) and green(G) fluorescence under U.V. light on TLC plates.
- Aflatoxin B1 is the most important biologically active mycotoxin because of its common occurrence in food items and is highly toxic and carcinogenic.
- Poisoning that results from ingesting aflatoxins 2 forms:
  - Acute severe intoxication: Result in severe liver damage, And subsequent illness or death.
  - Chronic subsymptomatic aflatoxicosis: signs and symptoms are lethargy, anorexia and muscle weakness followed by spasm.



Reye's syndrome

- It is an acute aflatoxicosis in which patient presents with signs and symptoms of encephalopathy and fatty degeneration of viscera.
- This is an endemic disease of children in developing countries.



Reye's syndrome
Fatty degeneration of liver

FUMONOSINS

- Secondary metabolites produced by various species of fusarium.
- They are toxic and carcinogenic
- Common contaminants of maize ,corn and their products.
- Cause fatal illness in animals like equine leukoencephalomalacia(ELEM)fatal disease in horses,Porcine pulmonary edema(PPE) in pigs,and hepatotoxic and carcinogenic effects in rats.
- Cause toxicity by blocking ceramide synthetase which converts sphingosine & acetyl CoA to ceramide.
- An outbreak has been reported in 1999 in poultry due to consumption of fumonisin-contaminated feed containing rain –damaged maize.

OCHRATOXINS

- □Naturally occuring mycotoxin and is produced by various species of *Aspergillus* and *Pencillium*.
- □Originally isolated from *Aspergillus* ochraceous hence named ochratoxin.
- □Out of many ochratoxins, ochratoxin A is medically significant.
- □Natural occurrence of these toxins in grains and other plant products.
- **Human exposure to ochratoxin**
- Direct – consumption of contaminated plant food
- Indirect – consumption of animal tissues exposed to contaminated materials.
- It produces fatal renal disease called as endemic nephropathy and urinary tract tumors.

CYCLOPIAZONIC ACID

- □Produced by genous *Aspergillus* and *Pencillium*
- □Occurs naturally in agriculture products such as ground nut and corn.
  - Co-contaminant with aflatoxin
  - Clinical symptoms are: loss of weight,weakness, vomiting,diarrhoea,dehydration,convulsions and death.
- □Causing symptoms of kodua poisonin (consumed kodo millet seed as staple food)

ZEARALENONE

- Produced by *Fusarium* species
- Found in variety of infected cereals like maize, barley, wheat grains and sorghum
- Cause genital disorders in domestic animals

PATULIN

- Derived from *Pencillium patulum*
- It was initially thought to be the antiviral drug which relieve the symptoms of common cold Subsequently realized as mycotoxin

Toxin hypothesis

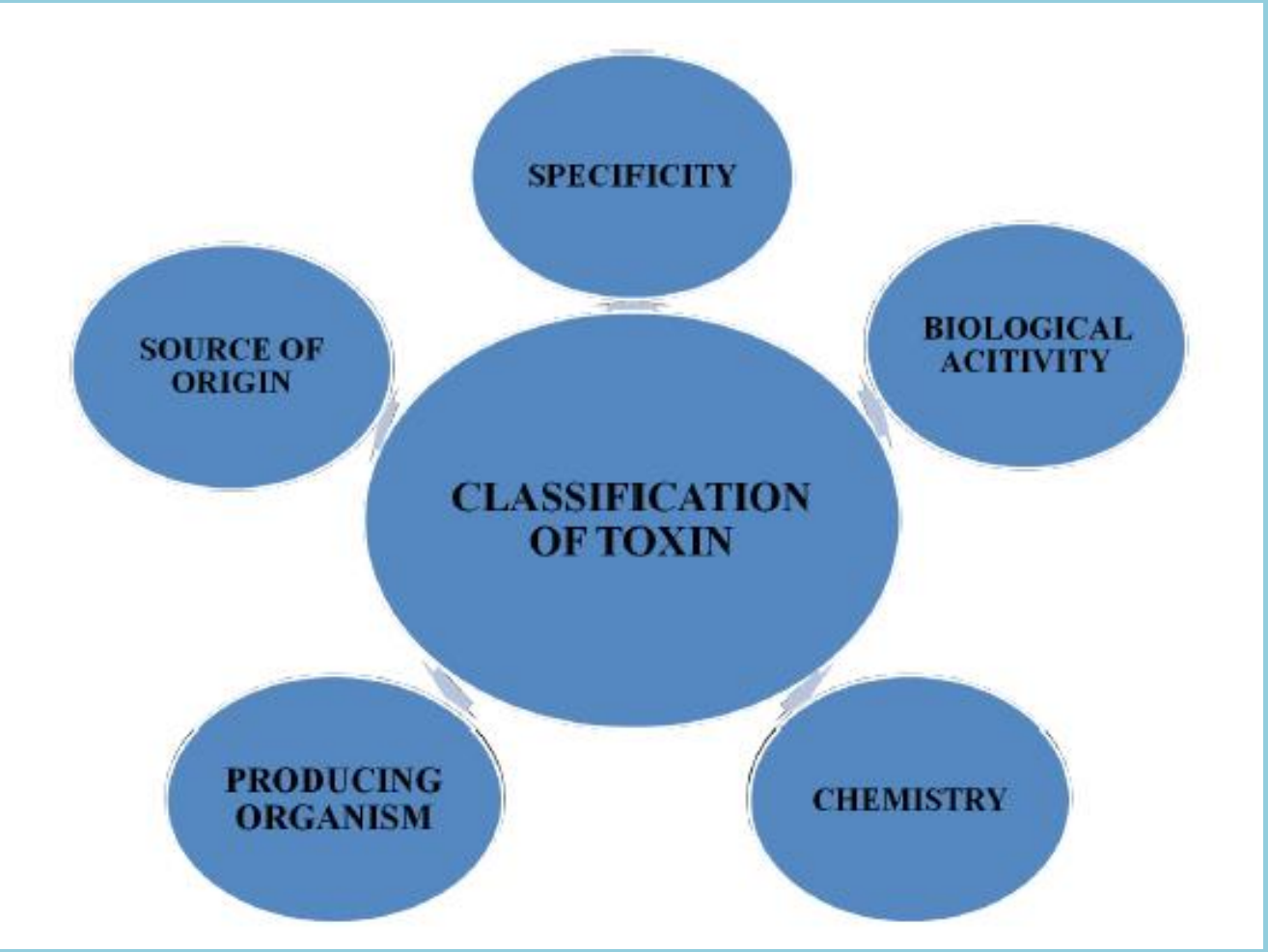
1. A toxin should produce all symptoms characteristic of the disease.

2. Sensitivity to toxin will be correlated with susceptibility to pathogen.

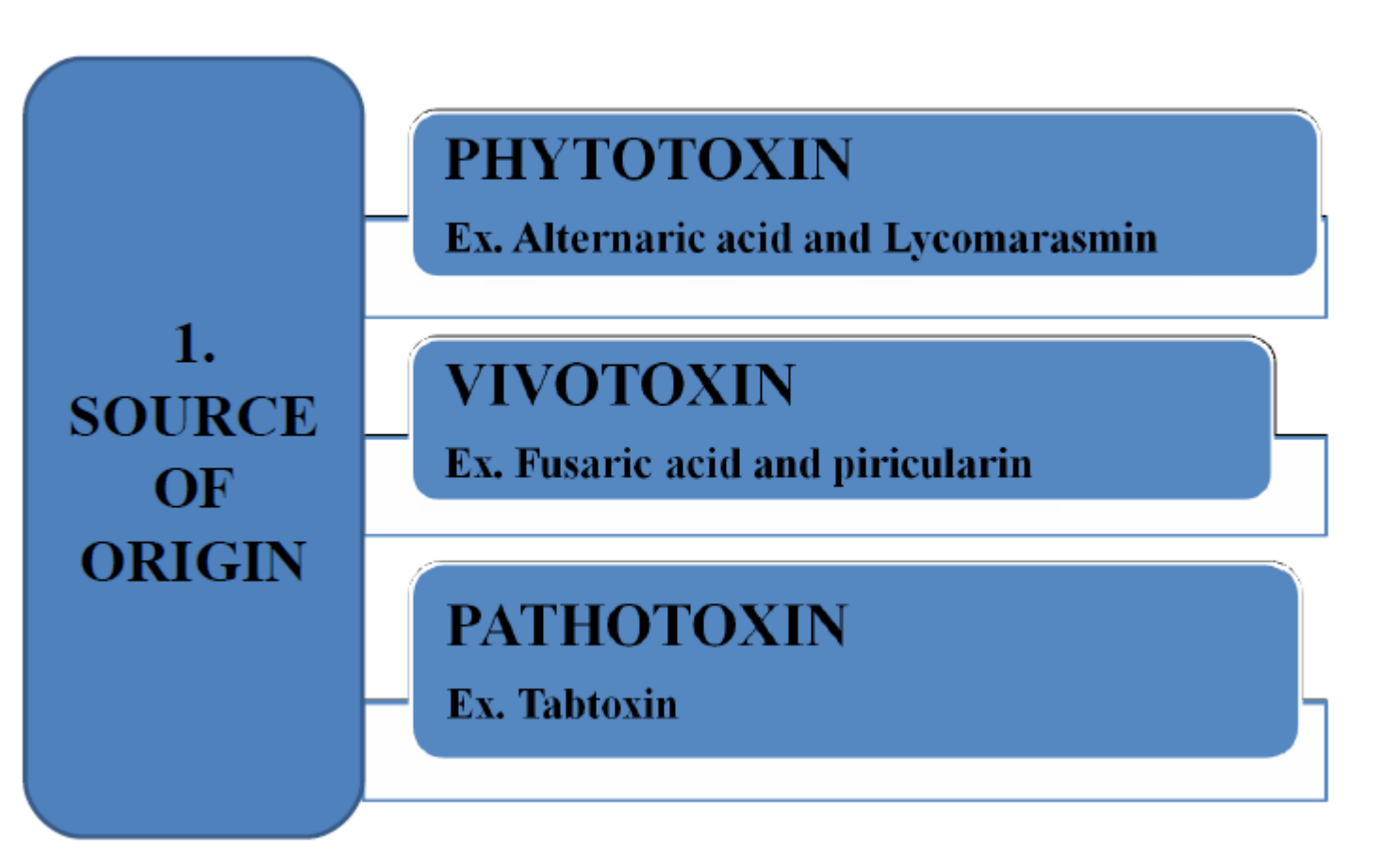
3. Toxin production by the pathogen will be directly related to its ability to cause disease.

- Except, victorin, the toxic metabolite of *Cochliobolus victoriae, the* vast majority of toxins associated with plant diseases fail to exhibit all the above characters.

Classification of Toxin



Source of Origin



1. Pathotoxins (Wheeler and Luke, 1963)

-Tab-toxin; HMT toxin

-causal role in the disease

2. Vivo toxins (Diamond & Waggoner, 1953)

-partial role in disease

-Fusaric acid, pyricularin

3. Phytotoxins

-Role is suspected in disease

-Alternaric aid; cochliobolin.

Pathotoxins (Wheeler and Luke, 1963)

- Those toxins which plays an important causal role in disease and are produced by the pathogen or interaction between host and pathogen

- Criteria for any toxin to be a pathotoxin:

-When applied on a susceptible host in low concconcentration, should produce all or nearly all symptoms of the disease

-Toxin and the pathogen should have same host range; same resistance/ susceptibility spectrum

-The pathogenicity of the pathogen should be correlated with its capacity to produce the toxin.

e.g. Tab-toxin: *Pseudomonas tabaci;* HMT toxin: *Dreschlera maydis race T*

Vivotoxins (Diamond & Waggoner, 1953)

Those toxins produced in the infected plant /host by the pathogen that function in the production of the partial disease.

Criteria/ characteristics of vivotoxins

•---Reproducible separation of the toxin from the diseased host

•---Purification and chemical characterization and

•---Induction of atleast a part of disease syndrome when applied on healthy plant

These are generally non-specific

•Fusaric acid: Fusarium spp.

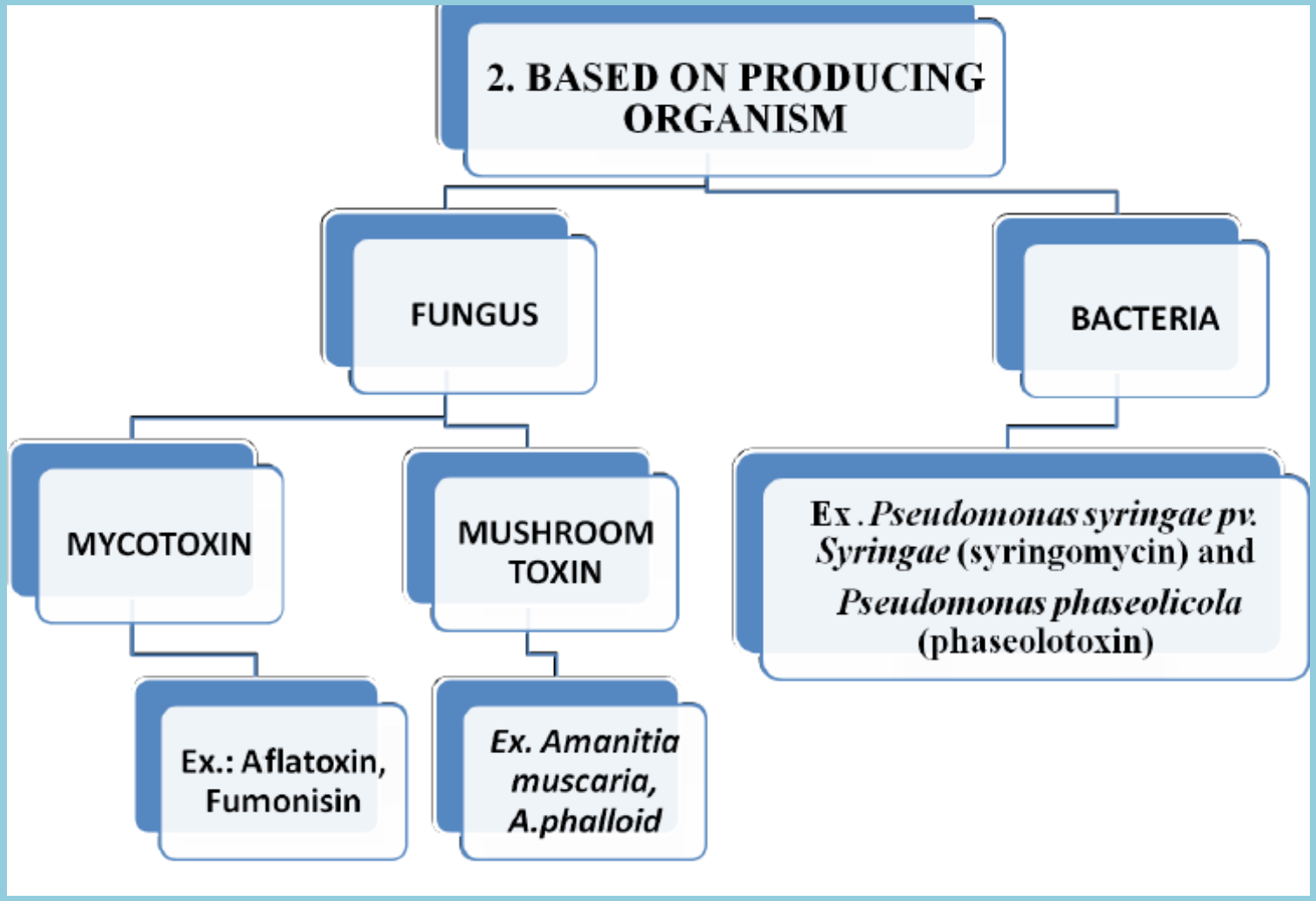
•Pyricularin: *Pyricularia oryzae*

Phytotoxins

Phytotoxin are toxic substances produced by living organism and whose role in disease is merely suspected rather than established.

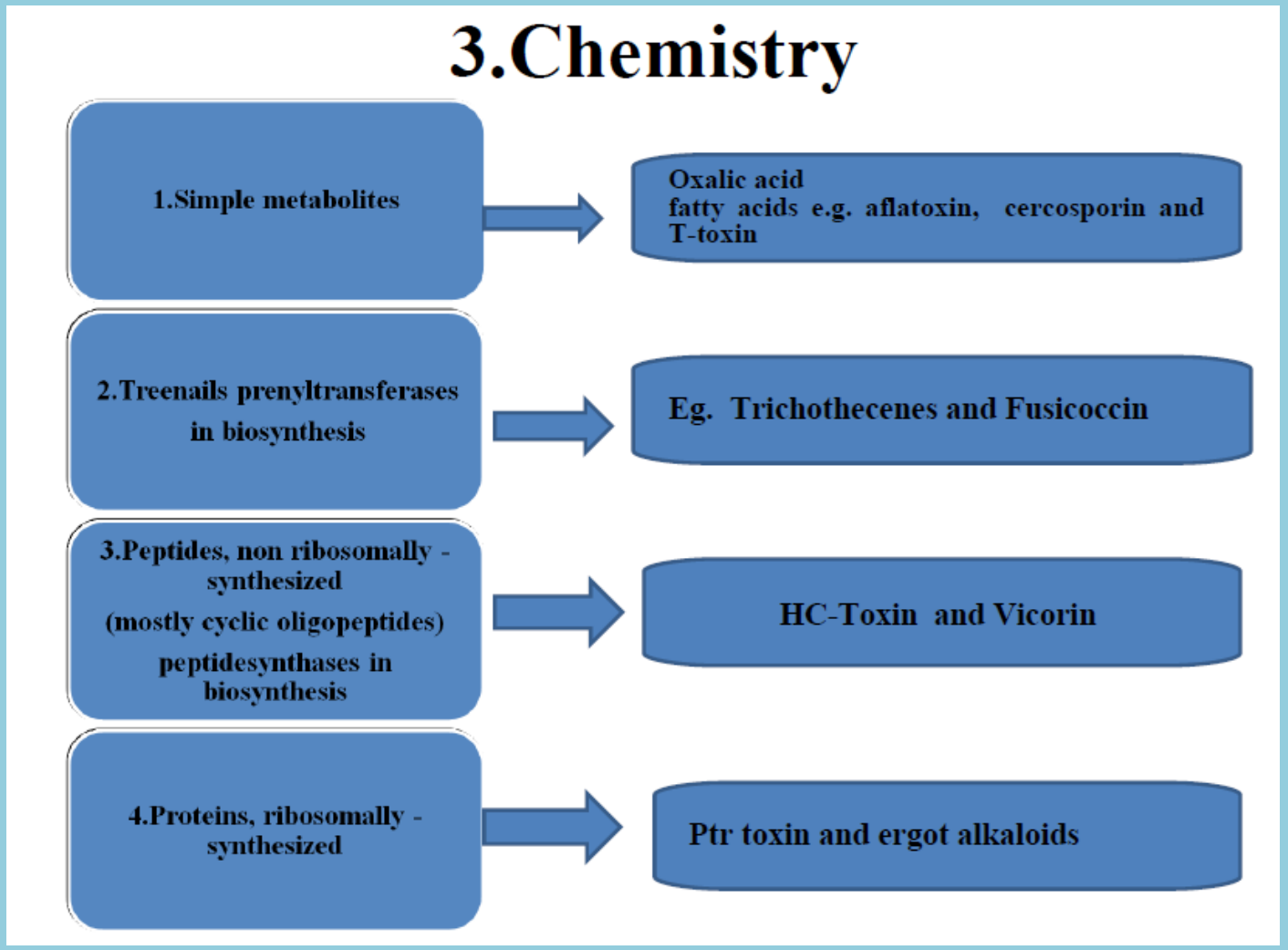
- Role is suspected in disease
- Alternaric acid; cochliobolin.

Based on producing organism

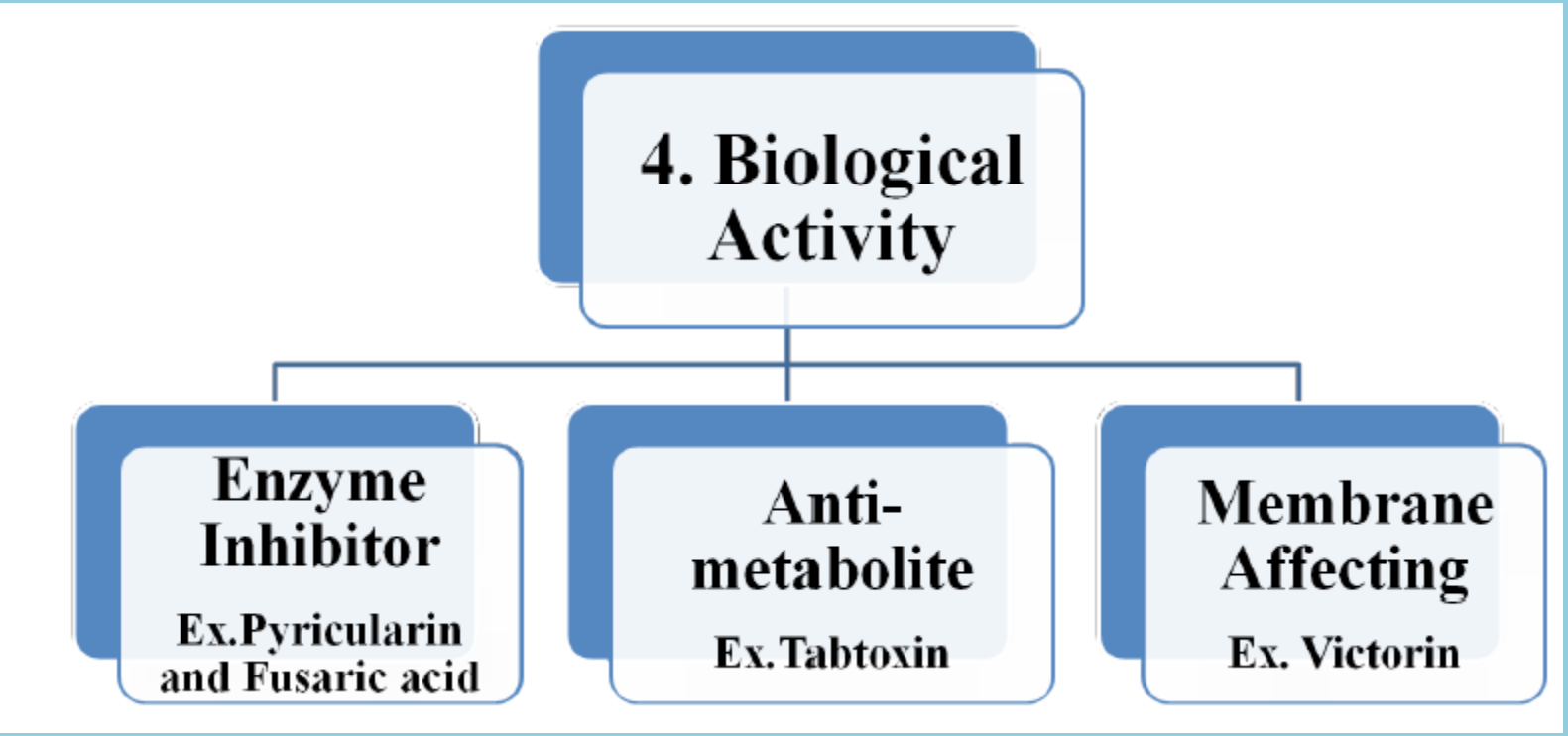


Sheffer & Briggs, 1981

Based on Chemistry

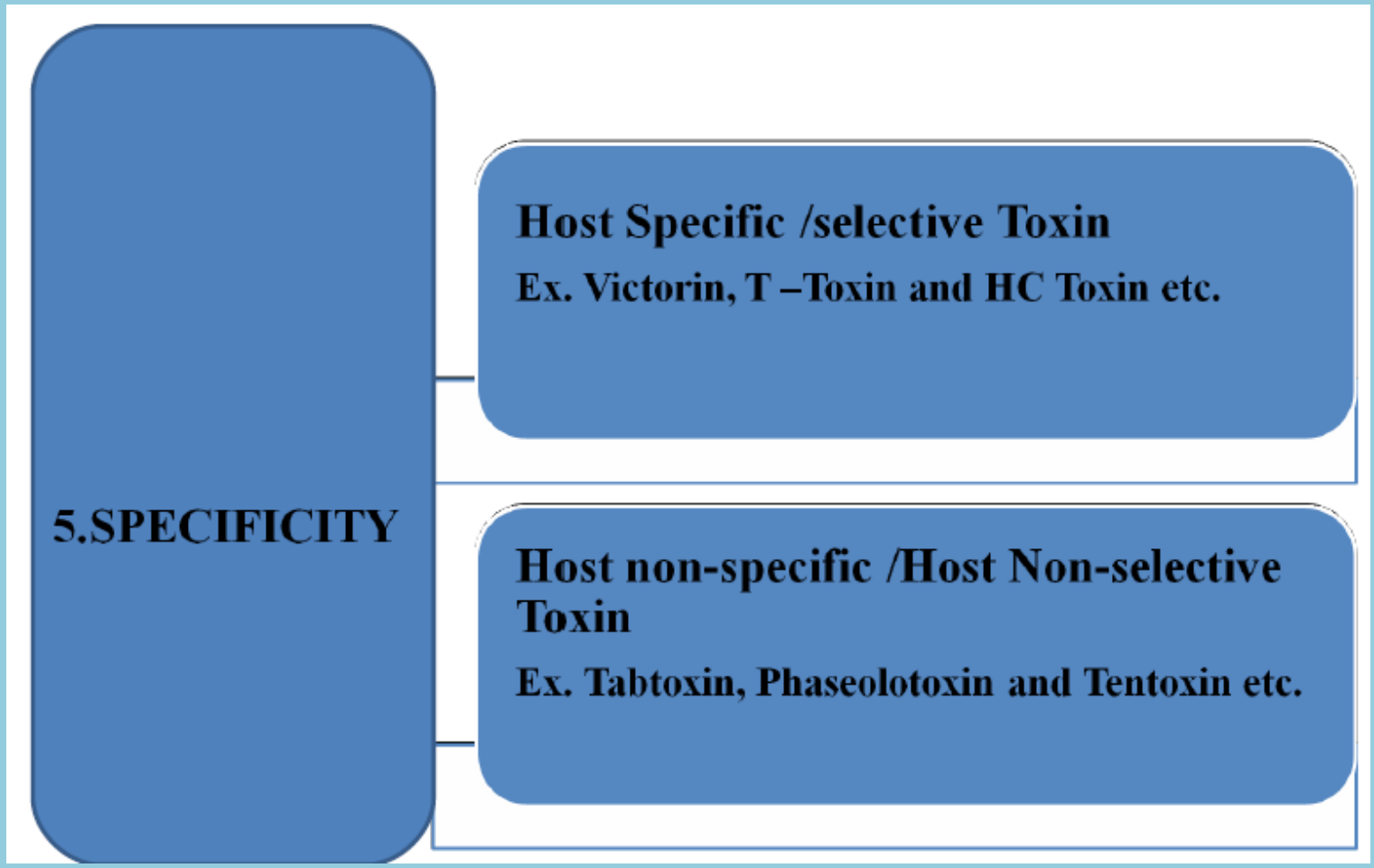


Based on Biological Activity



Scheffer and Briggs, 1981

Based on Specificity



Effect of toxins on host tissue

- Changes cell permeability
- Disruption of normal metabolic activity
- Loss of salts from protoplasm which increases
  - **Respiration** – tab toxinine
  - **Uncoupling of oxidative phosphorylation** – victorin
  - **Inhibition of host enzyme** - tab toxin inhibit normal host enzymes
  - **Affect cellular transport system** e.g. H+/K+ exchange at the cell membrane
- Other mechanisms
  - **Interfere with growth regulatory system** e.g. inhibition of root growth (*F. moniliforme*)

Host selective(specific) toxins

Host-Specific (selective) Toxins

- Produced by fungi
- Almost all are produced by loculoascomycetes
  - *-Alternaria spp. and Cochliobolus spp. primarily*
- First toxins characterized - AKT-toxin (Japan 1933), Victorin (USA,1947), HC-toxin (USA,1965)
- Toxins reproduce all disease symptoms when applied to a susceptible host.
- Scheffer & Nelson – 1960's – single genetic loci control toxin production in *Cochliobolus spp. E.g.*
  - *-Cochliobolus heterostrophus – T-toxin – TOX1*
  - *-C. carbonum – HC-toxin – TOX2*
  - *-C. victoriae – Victorin – TOX3*

Specific/ selective toxins

- Are those toxins which adversely affect the specific host of the pathogen
- Are normally essential for pathogenicity
- 20 such toxins has been recognized mainly produced by *Alternaria and Cochliobolus spp.*
  - Victorin
  - T-toxin,
  - HC-toxin,
  - AAL-toxin

VICTORIN

- Victoria blight of oats -*Cochliobolus victoriae*
- A host-specific toxin – victorin: a cyclic pentapeptides
- Toxin reproduces all disease symptoms when applied to a susceptible host
- Susceptibility to toxin conferred by dominant allele at *Vb locus*
- Victorin causes premature senescence of leaves

T-toxin

- *Produced by Helminthosporium maydis race T (Cochliobolus heterostrophus race T)*
- Southern Corn Leaf Blight
- Toxin is a polyketide
- Toxin causes swelling, uncoupling of oxidative phosphorylation, stimulation of respiration, leakage of Ca2+ and NAD in mitochondria
- single genetic locus is involved in toxin production

HC-toxin

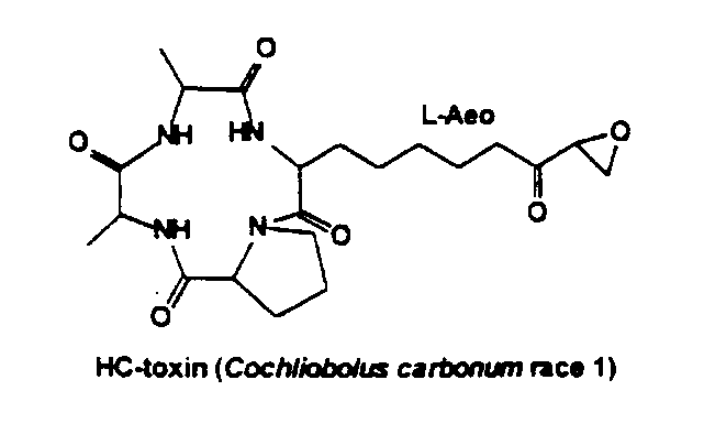
•Produced by *Cochliobolus carbonum (Helminotsporium carbonum)– causes Northern Leaf blight of Corn*

•Cyclic peptide

•Mode of action may be different than T-toxin - inhibiting defense reactions rather than causing cell death

•*Hm1 was the first plant resistance gene to be cloned and characterized in 1992 by Johal & Briggs Science 258: 985.*

•*Hm1 encodes a NADPH-dependent reductase enzyme (HC-toxin reductase - HCTR) that detoxify the toxin .*



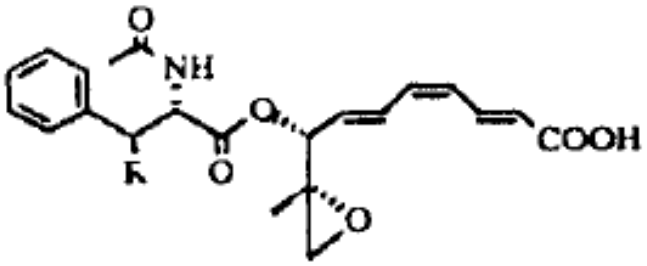
HC-toxin (*Cochliobolus carbonum* race 1)

Alternaria toxins

- Produced by closely related *Alternaria spp. Alternaria alternata*.
- All are low molecular weight, secondary metabolites and are structurally similar
- Toxins affects
  - -Plasma membrane of susceptible hosts
  - - electrolyte loss, membrane invaginations (Kohmoto et al. 1993)
  - -One specific genotype of each host is susceptible to toxin - very narrow host range
- Tangerine pathotype – ACT-toxin
- Strawberry pathotype – AF-toxin
- Japanese pear pathotype – AKT-toxin

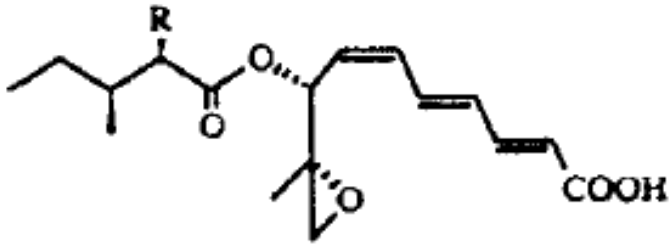
AKT-toxin

A. alternata Japanese pear pathotype



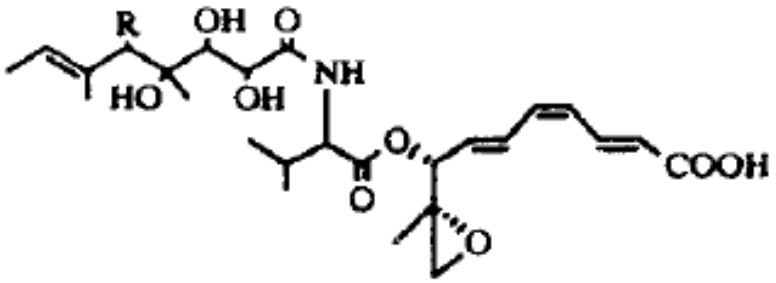
AF-toxin

A. alternata strawberry pathotype



ACT-toxin

A. alternata tangerine pathotype



Non host selective (non-specific) toxins

Non-Specific/ selective

Are those toxins that affect the protoplast of many unrelated plant species in addition to the main host of the pathogen producing the toxins

- **E.g. Tab toxin, phseolotoxin, tentoxib Cercosporin**
- These toxins affect virulence of the pathogen but are not essential for pathogenicity.

Tab toxin (Non-Selective)

Produced by *Pseudomonas syringae pv. tabaci causes wild fire disease in tobacco*.

- Toxin producing strains cause necrotic spots on leaves surrounded by yellow halo.
- Tab toxin is a dipeptide composed of amino acid threonine and tabtoxinine
- The toxin as such is not toxic but in the cell it get hydrolysed and release aminoacid tabtoxinine which is toxic
- Act by

  -inhibiting / inactivating the enzyme glutamine synthetase

  -Uncoupling of phosphorylation and photorespiration,

  -destroy the thylakoid membrane of the chloroplast thus causes chlorosis and then necrosis.

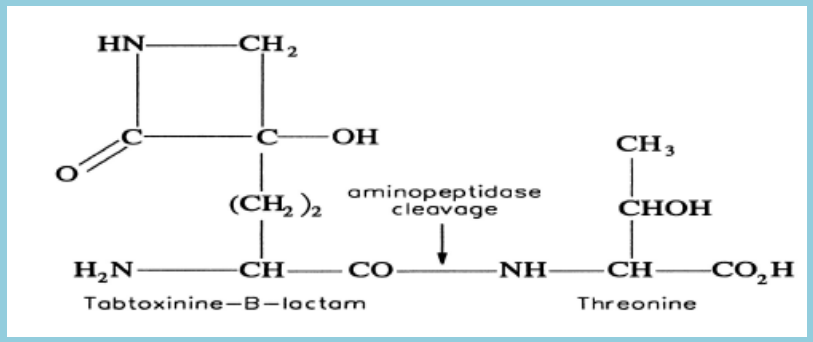
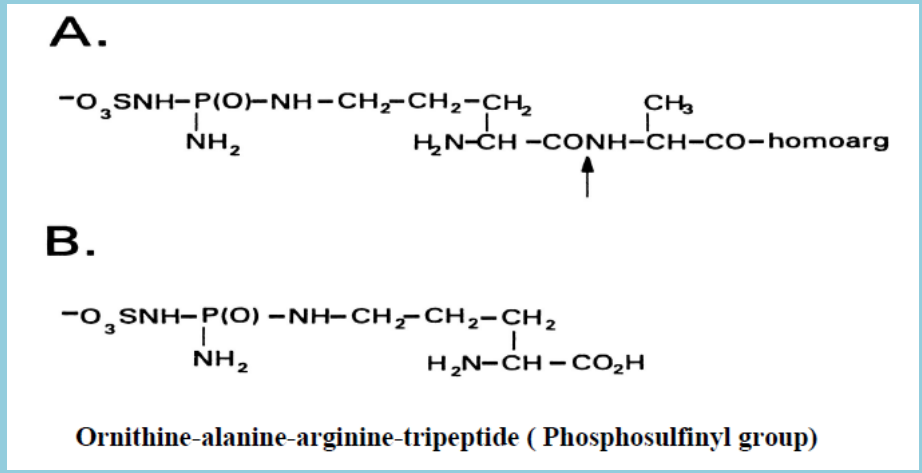


Fig.: Structure of Tabtoxin

Phseolotoxin

Produced by *P. syringae pv. phaseolicola causes halo blight of bean*

- Toxin causes growth reduction.
- Disrupt apical dominance and accumulation of amino acid ornithine
- Phaseolotoxin is a modified ornithine-alanine -arginine tripeptide with phosphosulfinyl group.
- Toxin act by
  - inhibiting pyrimidine nucleotide biosynthesis, reduce activity of ribosomes, interfere with lipid synthesis
  - Changes cell permeability

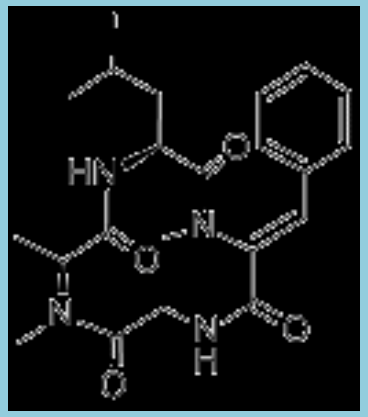


Ornithine-alanine-arginine-tripeptide (Phosphosulfinyl group)

Tentoxin

Produced by *Alternaria alternata (A. tenuis)* causes leaf spots and chlorosis.

- It is cyclic tetrapeptide that bind to and inactivate the protein (chloroplast – coupling factor) involved in energy transfer into chloroplast.
- Also inhibits phosphorylation of ADP to ATP
- Leading to disruption of chlorophyll synthesis



Cercosporin

- Produced by *Cercospora spp. And other fungi* causes leaf spot disease
- This toxin is activated by light and become toxic to plants by generating activated species if oxygen (single O); the activated O destroy the host membrance and provide nutrients to pathogen,
- It is photosensitizing perylenequinone that absorb light energy

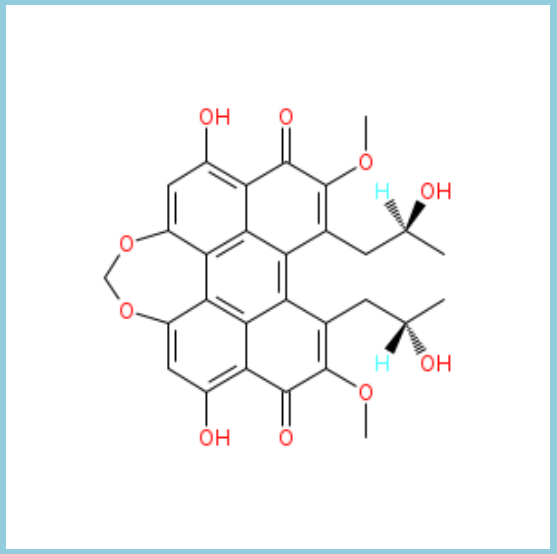


Fig. 12: Structure of Cercosporin

Fusarium toxins

Marticin: pathotoxin produced by *Fusarium oxysporum f sp. pisi – pea wilt; have nepthazarin; red pigmented compounds*

Fusric acid: Vivotoxin, chemically 5-n- butyl-picolinic aacid produced by many spp of Fusairim: *Fusarium oxysporum f sp. Batatis (sweet potato); cubense (banana); lini (Linseed); lycopersici (Tomato); vasinfectum (Cotton)*

Lycomarasmin: *Fusarium oxysporum f sp. lycopersici*

Pyricularin

Pyricularia oryzae – rice blast

Exist in two froms:

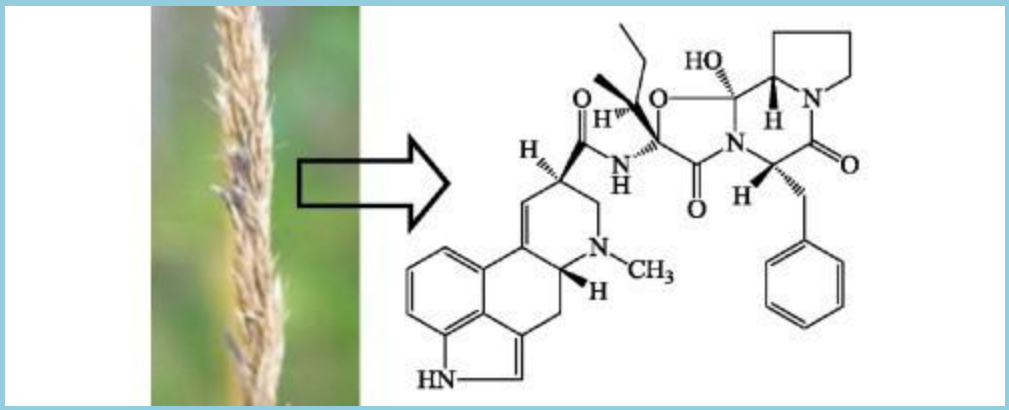
- α-picolinic acid
- pyricularin

Toxic to conidial germination, however, the fungus produces a pyricualrin binding protein (copper oxidase) that binds with pyricularic and destroy the fungitoxicity but not the phytotoxicity.

It affect respiration and growth at low conc. But inhibit at high concentration.

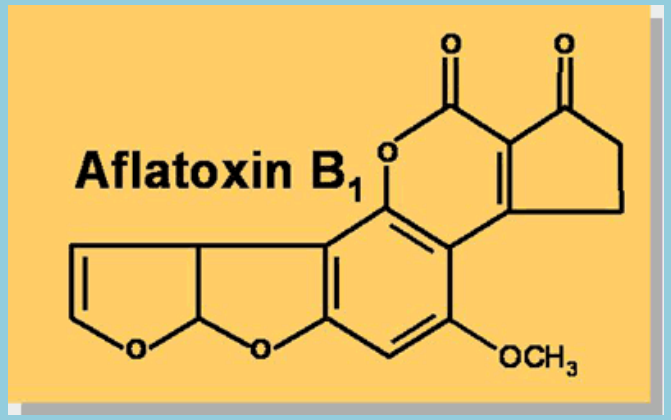
Ergot alkaloid

- The most prominent member of this group is *Claviceps purpurea*.
- This fungus grows on rye and related plants and produces alkaloids.
- Cause ergotisms in humans and other mammals who consume grains contaminated with its fruiting structure called ergot sclerotium



Aflatoxin

- *Aspergillus flavus, Aspergillus nomius and Aspergillus parasiticus.*
- There are four kinds of aflatoxins such as aflatoxin B1, B2, G1 and G2, in which aflatoxin B1 (AFB1) is highly toxic and carcinogenic (Leontopoulos *et al., 2003)*.
- Aflatoxins are known to be potent carcinogenic agents that pose serious hazards to human and animal health (Sidhu *et al., 2009)*.
- In addition, aflatoxin also has an impact on agricultural economy through the loss of crop production (Wu, 2004).
- Food and Agriculture Organization of United Nations (CAST, 2003) shows that 25% feedstuffs is polluted by mycotoxin in the world, and it results in over 1 billion dollars loss for poultry industry annually .



Other non specific toxins include

Fumaric acid : *Rhizopus spp.*
Oxalic acid: *Sclerotinia, Sclerotium spp.*
Alternaric acid: Alternaira spp.
Ophiobolin: *Cochliobolus spp.*
Pyricularin: *Magnaporthe grisea*
Lycomarasmin: *F. oxysporum in tomato*

Non-host selective fungal toxin

Non host selective toxin	Fungus	Diseases
Fumeric acid	*Rhizopous spp*	Almond hull rot
Oxalic acid	*Cryphonectria parasitica*	Chest nut blight
Alternaric acid, alternariol and zinniol	*Alternaria species*	Leaf spot diseases
Ceratoulmin	*Ophistoma ulmi*	Dutch elm diseases
Fusicoccin	*Fusicoccum amygdali*	Twig blight diseases of Almond and peach trees
Ophiobolins	*Cochliobolous sp*	Grain crops
Pyricularin	*Pyricularia grisea*	Blast of rice
Lycomarasmin	*Fusarium oxysporum*	Wilt in tomato

Non host selective bacterial toxin

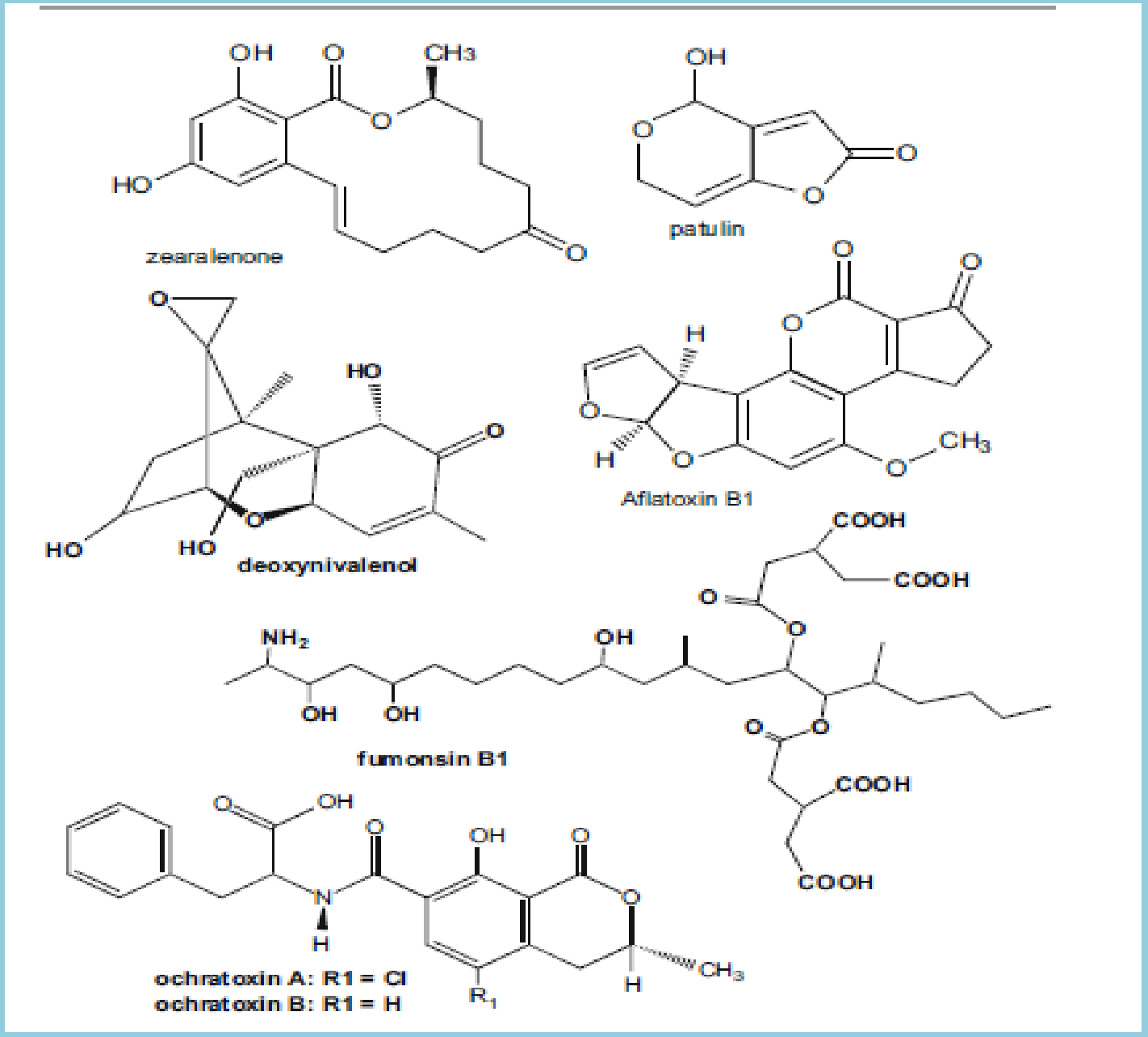
Other non host toxin	Bacteria	Diseases
Coronatine	*Pseudomonas syringae pv atropurpurea*	Grasses and soybean
Syringomysine	*Pseudomonas syringae Pv syringae*	Leaf spot in many plants
Syringotoxin	*Pseudomonas syringae Pv syringae*	Citrus plant
Tagetitoxin	*Pseudomonas syringae Pv tagetis*	Marigold leaf spot
Thaxomins	*Streptomyces sp*	Root an tuber diseases

Toxin produced by fungal plant pathogens

Toxin	Pathogen	Diseases	Target of Function
PC Toxine (Peritoxin A and B)	*Periconia ciriciana*	Milo diseases of sorghum	Plasma membrane
HS toxin A, B and C	*Bipolaris sacchari*	Eye spot of sugarcane	Plasma membrane
PM Toxin A, B, C and D	*Phyllosticta mayadis*	Yellow leaf blight of maize	mitochondria
Ptr- Toxin (Ptr chlorosis toxin)	*Pyrenophora tritici-repentis*	Tan spot of wheat	Chloroplast (Photosynthesis 14 KD Protein)
CC toxin	*Corynespora cassicola*	Tomato	--
AK toxin	*Alternaria alternata*	black spot of japanese pear	Plasma membrane.
AAL toxin	*Alternaria alternata f. sp. Lycopersici*	Leaf spot of many crops	Sphingolipid and ethanolamine metaboloism.
AM Toxin	*Alternaria alternata (A. mali)*	Leaf spot of many crops	Chloroplast of plasma membrane

Contd.

Toxin	Pathogen	Target of function
Rhizobitoxine	*Pseudomonas andropogonis*	B-Cystathionase, inactivating homocysteine synthesis
corpeptin	*Pseudomonas corrugate*	Membrane active
Fuscopeptine	*Pseudomonas fuscovaginae*	Membrane active
viscosin	*Pseudomonas marginalis*	Membrane active
coronatine	*Pseudomonas syringae*	Molecular mimic of jasmonicacid, a plant signal molecule
phaseolotoxin	*Pseudomonas syringae Pv phaeseoli*	Inhibitor of ornithine carbamoyl transferase, inhibiting amino acid synthesis
Syringomysine	*Pseudomonas syringae Pv syringae*	Increase host membrane permeablity
Tabtotoxin	*Pseudomonas syringae Pv tabaci and other*	Glutamine synthetase
Tagetitoxin	*Pseudomonas syringae Pv tagetis*	Inhibitor of chloroplast RNA polymerase
Albicidin	*Xanthomonas albilineans*	Inhibitor of Plastid DNA replication



Structures of selected mycotoxins

5. Fungi as food and beverage and food processing

Alcoholic beverage, mushrooms and other macro fungi, edible biomass from yeast and moulds, single cell proteins (SCP) Bread, soybean products, cheese and fermented milk, other fermented foods

.

Production of Foods and Beverages:

- Among the tombs, stone and wooden sculptures and other artifacts of the ancient Egyptian civilizations are numerous depictions of life there almost 6000 years ago. Among these depictions are scenes of bakeries, breweries, vineyards, and wine presses. This is perhaps some of the first solid evidence of the use of fungi in large-scale food and beverage production. Archaeological data, however, are still unraveling the mysteries of "how long has man been doing this?" Current discovery of the **ice man** in the foothills of the Alps with cereal grain and mushrooms in his pouch suggest that bread making and mushroom use may go back 10,000 years. And, the discovery of wine residues in clay urns in Iran date back to 7,000 BC. Yet the discovery of the organism responsible of fermentation that goes on in bread and wine making was not until 1680 when Antoine van Leeuwenhoek first discovered yeast cells under his microscope. It was more than 200 years later before Louis Pasteur concluded that through anaerobic respiration (fermentation) sugar was converted into carbon dioxide and alcohol by such tiny yeast. While yeast are central to the baking and brewing industries, many filamentous fungi are utilized in the production of cheeses and a wide array of exotic foods. We will examine first the use of fungi in brewing and baking; then in the production of cheeses and other exotic fermented foods and beverages.

- There are several types of bakers yeast (*Saccharomyces cerevisiae)*. **Compressed yeasts:** What we commonly refer to as "yeast cakes" or wet yeast. It contains about 70% moisture and has a shelf life of about 3-4 weeks. Such yeast is perishable and should be stored under refrigeration (4-5º C). **Crumbled yeast** forms are similar to yeast cakes but with less water and in a crumbled condition. This type is often sold in 50 lb bags. **Active Dry Yeast**: Dry yeast is prepared similar to compressed yeast but is dried under specially controlled conditions. The moisture content is 6-8%, which enables them to be stored for a few months. Dry yeast should be revived in water at 105-110º F. **Instant Dry Yeast**: Through a quick drying process under controlled conditions, more porous yeast can be produced that become active immediately on rehydration. This is probably the most popular yeast used in home baking.

Fermented Beverages

- **Ethanol Production by Yeasts:** For large-scale production of ethanol by yeast, an organism that is tolerant to high concentrations of ethanol and highly osmotic substrates is essential. Species of *Saccharomyces* are the most tolerant to ethanol. Wine yeasts can tolerate levels up to 20%, whereas, bakers yeast used in beer making and bakeries can tolerate levels at from 4 to 6%. Most organisms are inactivated in concentrations of ethanol above 15%. Extensive research has been done to produce strains that will be effective in utilizing various substrates and tolerate high levels of glucose or sucrose. They must also be resistant to certain metabolites that may be toxic. Ethanol tolerance is genetically controlled by a large number of genes (Ismail & Ali, 1971. Fol. Microbiol. 16:300-359).

- **Distilled Alcohol: Distillation** involves the conversion of a substance into a vapor that is subsequently condensed to the liquid state. The process goes back to the time of Aristotle (around 350-380 BC). Distillation is used in particular to separate mixtures of liquids in which the boiling points of the liquids differ. In the production of distilled alcoholic beverages, the fermented substrates contain, water, alcohol, oils and other liquids. The **boiling point of alcohol is 79.3° C and that of water is 100° C; thus the alcohol will vaporize at a much lower temperature**. Its vapor can be condensed in a cooling coil from the still and thus separated from the other liquids. Species of *Saccharomyces* and *Schizosaccharomyces* are used to produce distilled alcoholic beverages.
- **Industrial alcohol** is produced in the U.S. with corn as the primary substrate. In Brazil, sugarcane is used in large-scale alcohol production for automobiles. With the ever-increasing depletion of fossil fuel reserves, production of ethanol from plant products is more attractive. With industrial ethanol, flavor and other qualities are not as important as consumable products. Therefore, the yeast strains used are selected for their efficiency to digest substrates and in their tolerance to high levels of ethanol.

Alcoholic beverages

- **Alcoholic beverages** are made from a large variety of starchy or sugary products. **Whiskey** is made largely from barley, but sometimes from other grains. They are essentially aged grain alcohol that start out as clear but becomes colored when aged inside of charred barrels. **Bourbon** is a form of whiskey made from fermented corn, or occasionally other grains. **Vodka** is produced largely from potatoes in Russia and other east European countries because they are cheap. They often use grains as well. **Rum** is a distilled spirit made from sugar-containing substrates like molasses and cane juice. It is initially clear, but like bourbon, it is darkened by storage in wooden barrels; or in recent years adding caramel after distillation. It is popular in tropical and subtropical countries where sugarcane is grown. **Cognac** is made from distilled grape wine and gets its name from Conacais, France where it was first made. British and Dutch merchants began to distill wine to prevent spoilage in shipment. **Brandy** is made in a similar way as cognac, initially from grape wine but later in North America from fermented juices of pears and peaches. **Tequila** is a briny liquor made exclusively in Mexico from fermented juice of the *Agave* cactus. The Mexicans were making a fermented drink 1000 years before they were invaded by Spain. The Spaniards brought to Mexico with them the art of distillation; hence, tequila came into existence.

Wine

- Wine in the strict sense is the fermented juice of grapes. However, wines are now made of many juices. The making of wine is known from the earliest history of man; one of the earliest written is the Biblical account of Noah (perhaps 5000 BC). To quote a recent report (Gainesville Sun, June 5, 1996), "Talk about a vintage! Scientists say they have found the oldest evidence of wine residue at the bottom of a squat, **7,000-year old pottery jar**. Traces of two chemicals in the jar, found in the Zagros Mountains of **Iran,** extend the known history of wine-making 2,000 years." The jar came from a time when people were first establishing permanent settlements and were likely already cultivating the grapes from which the wine was made. For centuries wine was stored in flasks of animal skin or in clay vessels. During the rise of the Roman Empire, wooden vessels as well as clay urns were common. The use of bottles and corks became common towards the end of the 17th century.
- While wine making has gone on for centuries, the process was brought to this country by the early settlers. Perhaps the earliest wine making in the U.S. was done in Florida by early Spanish explorers. Yet today, only a few wineries occur in Florida where they use native **muscadine** grapes.

- Wines are named after the type of grapes or the geographic area or specific village where they were first produced. For example, **Burgandy, Bordeaux, Champagne**, and **Alsacs** are important wines of France. During the 17th century, wine makers in the Rhine valley found that grapes allowed to rot on the vine gave the wine a sweeter taste. There are **three basic types of wines**, (1) table wines, (2) fortified wines, and (3) sparkling wines. **Table wines** are made from pressed grapes fermented in vats with the addition of sugar, yeasts, sulfur dioxide. *Saccharomyces ellipsoideus* is the common yeast used in the fermentation process. The alcohol content is around 12-15%. **Sulfur dioxide** is used to keep down the vinegar producing bacterium *Acetobacter*. Wine may be chilled in vats to cause sedimentation and the "free run" wine is decanted. **Fortified wines** receive an addition of alcohol, brandy or other alcoholic beverages, and the final alcohol level is from 16-23%. A common fortified wine is **port wine** which gets it name because sailors who used to come into port would purchase wine spiked with brandy or liquors to give a greater alcohol level. **Sparkling wines** such as champagne go through a double fermentation in which the alcoholic content reaches 20%. Some sparkling wines have a natural effervescence, others are made effervescent by bubbling them with carbon dioxide. All natural wines are below 20% alcohol because beyond that level, the fermenting yeast would be killed.

- There are red, white, and pink (rosé) wines, **Red wines** are made from black grapes in which the husks (mush or skins) are left in contact with the juice throughout fermentation. Most **white wines** are made from green colored grapes or from black grapes in which the husks are removed soon after pressing. Leaving the husks of black grapes in the fermenter for a short period of time produces a **pink wine**.
- European wine production was almost devastated when in the 19th century imported American rootstock infested with a **louse**, *Phylloxera*, which feeds on grape roots, destroyed more than 2,500,000 acres throughout France and surrounding countries. Many growers never recovered. Others imported resistant rootstock and the industry was soon reestablished.

BEER

- Beer is a beverage obtained by the alcoholic fermentation by yeasts of a malted cereal, usually **barley malt**, with or without other starchy materials, and to which hops have been added . (For more information on beer-making, see Hardwick, 1983. Biotech.5:166-229). Brewing seems to have originated in the Babylonian Empire (Mesopotamia) before 6000 BC. By 2000 BC, many types of beer existed in Babylon. There is evidence that the brewing of beer developed independently in Egypt. Some have suggested that the Hebrews learned to use hops to flavor beer while they were in captivity in Babylon during the 8th and 9th century BC. The Greeks learned to make beer from the Egyptians and the Romans in turn learned from the Greeks.
- There are two types of beers, **lager** and **ales**. Lager beer employs bottom fermentation in which the "spent yeast" settles to the bottom and the green beer is aged. With aging, it mellows and is carbonated with C02. In the brewing of ales, the yeast selected is a top fermenter, forming a foam that can be removed. Enzymes are often used in the early stages of brewing, i.e. the "mashing" stage, and later in the brewing process. Amylase produced by other fungi increases starch digestion and results in low carbohydrate, or "lite" beers. Several other carefully monitored enzymes are added during the brewing process. Without protease, for example, beers would become hazy, and glucoamylases produced by certain species of *Aspergillus* are used to sweeten beers.

- Four things are vital to brewing beer, **barley malt, hops, water,** and **yeast.** In the making of **barley malt**, barley is allowed to go into dormancy after harvest. During malting certain enzymes are formed within the grain. Before malting the barley is soaked in water to initiate germination, after which grains are dried to about 2% moisture level. Timing of germination, temperature and duration of drying, and other factors affect the kind of malt that is produced. Corn, rice, and other grains may be added as adjuncts to add texture and flavor. These are closely guarded industry secrets. **Hops** are not essential for the manufacture of beer. The flowers of the female hops plant are used, and only from unfertilized flowers. Pollinated hops will give the beer a bitter flavor. Hop growing in the U.S. is chiefly in Oregon, California, Idaho, and New York. The best hops for brewing come from Slovakia and the Czech Repuplic (formerly Czechoslovakia). Hops are added to mashed malt solution (**wort**) during boiling. **Water** quality is vital to beer production, especially pH. Alkaline or "hard" water gives poor results and water with sulphur, iron, or bacteria should be avoided. Apparently, there must be ideal water in the mountains around Golden, Colorado! The **yeast** used in beer brewing is *Saccharomyces cerevisiae.*

- The main stages of brewing are mashing, boiling, and fermenting. Prior to mashing, malt is crushed between rollers to form flour. Mashing involves adding water (100-150F), solubilizing the starch by enzyme action, and separating out the husk. After mashing, the “wort” is boiled in large copper tanks or kettles, stopping enzyme action. The wort is filtered and passed into a high-speed centrifuge to achieve clarity. It then passes into large coolers. Fermentation begins when yeast is added to the cooled wort at 1 lb/barrel. After about 8 days most all fermentable substrate has been converted into alcohol. The beer is then bottled, canned, or stored in large kegs for marketing. The following images show the various types of cookers, fermentation tanks, and sediment tanks utilized in the manufacture of beer. The manufacture, distribution, and sale of beer are major industries in the U.S. and in Florida.

OTHER TYPES OF FERMENTED PRODUCTS

- **Rice Beer**: Rice beer is called **sake** in Japan, **murcha** or **pachwai** in India. It is made with rice plus species of *Mucor* or *Aspergillus oryzea* and sugar.
- **Kanji**: A beerlike beverage made with carrots and beets fermented with *Hansenula*. Taste much like cherry wine.
- **Toddy:** Sweet fermented sap from tropical palms using *Saccharomyces cervisiae*. Many substitute organisms can be used.
- Others fermented products include: **Shoyu** (=soy sauce): This is one fermented product well recognized throughout the US and Europe. Shoyu, referred to as **Ketjap** in Indonesia, has been produced in Japan for over 1000 years. The average Japanese consumes more than 3 gallons of soy sauce per year. Shoyu is prepared by soaking soybeans for 15 hr and autoclaving them for 1 hr. Clean, roasted wheat powder is added. After cooling, a starter, tane koji composed of *Aspergillus oryzae* or *A. soyae* is used as the inoculum. After fermentation for 3-4 months, or up a year, the material is combined with equal parts of brine and placed in tanks. The resulting mash is compressed and the liquid supernatant is retrieved, sterilized and bottled.

- **Tea Fungus**: Perhaps one of the strangest fermentations encountered is the so called “tea fungus” of eastern Europe and Asia sometimes referred to as **Hongo**. This drink is made from dry tea leaves steeped in a liter of water, and the leaves removed and 100 gm of sugar added. The solution is autoclaved and a “starter” culture is added and the material is allowed to ferment. Two yeasts and *Acetobacter* have been isolated from the starter.
- **Anchu** is an alcoholic drink in Taiwan made from red rice. **Awamori** is an alcoholic drink prepared from sweet potatoes in Japan. **Braga** is a fermented drink made from millet in Romania.

Cheese Making:

- We do not know when and where cheese was first made; its discovery was probably an accident.
- In the Judeo-Christian Bible, II Samuels 17:27-29 says that cheese of the herd was given to King David (approx. 1000 BC). So, cheese has been around for a while! The production of cheese is a multimillion dollar industry in the US and is also a dominant industry in other countries. It involves the fermentation of milk from different animals, using various organisms, and processing them under rigidly controlled conditions. Milk is coagulated, the liquid **whey** removed, and the solid **curd** is preserved. Curd may be made in three ways: **acid coagulation, heat coagulation,** and **rennet coagulation**. There are block cheeses, powdered cheeses, soft cheeses, and various processed cheeses. **Ricotta** and similar cheeses are made from heat coagulated curd .
- Two of the most common cheeses in which filamentous fungi are used in fermentation are Camembert and Roquefort.

- **Camembert Cheese** originated in the town of Camembert in the Normandy district of France. Legend attributes its discovery to a milkmaid, **Maria Harel**, who first shared it locally with neighbors. This **blue cheese** is now marketed worldwide and is manufactured in a number of countries. Camembert cheese, also called **Brie**, obtains its flavor and texture from lipolytic and proteolytic activity of molds belonging to *Penicillium camemberti* and *P. casicolum***.**
- The production of camembert cheese requires rigidly controlled conditions or else other fungi, particularly *Scopulariopsis brevicaulis*, will ruin the cheese. For this reason, it has been very difficult to establish a successful industry in other countries. Camembert cheese has not gained wide popularity in the US.
- **Roquefort Cheese** is made from the fermentation of milk curd by *Penicillium roqueforti*, a species first isolated in 1904 and described by Thom (1906, USDA, Bur. Animal Ind. Bull. 82).

- There are apparently many strains of this fungus, some of them being described as distinct species. Thom and Raper (ibid.) discuss a dozen such strains. The different enzymes produced by *P. roqueforti* results in various cheese flavors. *P. roqueforti* is a common fungus on the surface layer of silage. This is a likely source of the original clones that were discovered in the production of cheese. Charles Thom, USDA, Peoria, IL, introduced the production of roquefort cheese in the US in 1906. Thom and co-workers discovered the lipolytic and proteolytic enzymes of the mold released **caproic, caprylic**, and **capric** acids which imparted flavor to roquefort cheese. Unlike Camembert which has only surface growth of the fungus, the veins one sees in blue cheeses are zones of *Penicillium* growth within. Other blue-veined cheeses similar to roquefort are **Dolce Verdi** produced by *Penicillium expansum* and **Ellischour**, with a red pigment, produced by *P. nalgiovensis*.
- Other blue cheeses include **fromage blue cheese** in central France (from sheep and cow milk); **gorgonzola** in northern Italy, **stilton** in England, **cammelost** in Norway, and **neufchatal** in Germany.

Fungi used in Food & Beverage Production

Product Type	Fungal Species	Raw Material	Process	Commercial Product
Miscellaneous Industrial Products	*Saccharomyces cereveciae*		synthesis of enzyme, invertase	used in preparation of soft-center candies, eg cordial cherries
	Aspergillis niger	starches	aerobic metabolism	citric acid
	Aspergillus niger		produce alpha-d-galactosidase enzyme	enzyme suppresses methane production in humans =Beano©
	Aspergillus sp.	starches	synthesis of amylase enzymes	used for bread making & textile fibers
	Aspergillus sp.		synthesis of pectinase enzymes	clarification of fruit juices
	Penicillium notatum	corn starch solution	aerobic metabolism	penicillin
	Penecillium notatum		synthesis of enzyme, glucose oxidase	used to remove oxygen from canned fruits, dried milk and other products
	Fusarium moniliforme	corn starch solution	aerobic synthesis	plant hormone-gibberellin
Cheeses	*Penicillium roqueforti*	milk curd	production of blue pigment	Roquefort cheese
	Penicillium candidum & P. camemberti	milk curd	aerobic metabolism	Bri, Camembert and Limburger cheeses

Fungi used in Food & Beverage Production

Alcoholic Beverages	*Saccharomyces* sp.	germinated grain (malt)	natural fermentation	beer
		fruit juice	natural fermentation	wine
		rice	natural fermentation	sake
		fruit juice	fermentation & distillation	brandy
		grain mash	fermentation & distillation	whiskey
		molasses	fermentation & distillation	rum
		potatoes	fermentation & distillation	vodka
		agave	fermentation	tequila
Asian Foods	*Aspergillus oryzae, A. soyae, Saccharomyces rouxii, Candida etchellsii*	soybeans	fermentation	Miso
	Aspergillus soyae, A. oryzae, Saccharomyces rouxii, Candida versatilis	soybeans	fermentation	soy sauce
Coffee	*Saccharomyces* sp.	coffee beans	fermentation	used to help remove berry skin and flavor the bean
Bakery Products	*Saccharomyces* sp.	dough	fermentation	CO_2 production to cause dough to rise

*some processes also use bacterial species

Single Cell Protein

- *During World War II, when there were stortages in proteins and vitamins in the diet, the Germans produced yeasts and a mould (Geotrichum candidum) in some quantity for food; this led to the idea to produce edible proteins on a large scale by means of microorganisms during 1970s.*
- *Several industrial giants investigated the possibility of converting cheap organic materials into protein using microorganism.*
- *Single-Cell Protein (SCP) is a term coined at Massachusetts Institute of Technology by Prof C.L. Wilson (1966) and represents microbial cells (primary) grown in mass culture and harvested for use as protein sources in foods or animal feeds.*

Single Cell Protein (SCP) is not a pure protein but refers to whole cells of bacteria, yeasts, filamentous fungi or algae.

- *Contains carbohydrates, lipids, nucleic acids, mineral salts and vitamins.*
- *Carbon substrates: major substrates used in commercial SCP production is alcohols, n-alkanes, molasses, sulphite liquor and whey.*

Yeasts Fermentation in Bread-making.

- Bread is a baked product made of dough that has been raised by yeasts or other gas forming organisms. Why or when man first ate seeds or grasses, learned to grind them into flour, mix the flour with water or milk and bake it into bread is not known. Remains in the sediment of a Swiss Lake dweller show that humans were baking bread about 10,000 years ago. Archeological data reveal that bread making has been a central part of almost all subsequent cultures of mankind. The Egyptians appear to be the first to discover that letting dough ferment would result in light, flavorsome bread. Until the last two centuries, however, **light bread** was more or less restricted to nobility. Today, we all enjoy the noble use of light bread. At the peak of the Greek Empire, several types of bread were produced and baking later became a vital business in most medieval towns (Sanderson *et al.* 1983. Yeast fermentation in bread making.
- **How to make a lot of dough**? In the early years, bread making relied on natural fermentation. During the late 1700's, commercially available yeasts were used in large-scale production of bread. **Charles Fleischman** of the U.S. introduced in 1886 a new type of compressed yeast for distillers and bakers. An average baking formula for white bread requires 100 lbs of white flour, 2 ½ lb of yeast, 2 lbs of salt, 6-7 lbs of sugar, 4 lbs of dry milk, and enzymes when sometimes needed. Modern mixers can handle more than 1600 lbs of dough. Larger US bakers have continuous mixers. By the mid 1960's, more than 40% of bread making was done in continuous mixers. Close to 50,000,000 loaves of white bread are sold in the U.S. annually; it is a $5 billion dollar industry!

- Yeast is the essential ingredient in many bakery products, giving it lightness and a delicious flavor. The common baker's yeast is *Saccharomyces cerevisiae*, a cellular, budding yeast. The use of fermentation in producing bakery products dates back to beyond 2000 BC where Egyptian hieroglyphics clearly depicts the practice. It was only in the 19th century that a scientific basis for the process was understood when scientists such as Charles Hansen of the Carlsburg Labs in Denmark developed methods of isolating and culturing pure strains of the yeast. The commercial production was a tremendous boost to the baking and brewing industries. Today, large tanks or fermentors are used to culture yeast. Water and molasses are commonly used and a supply of oxygen is admitted. It is estimated that approximately 1.4 million tons of compressed yeast is produced each year.

Mushroom

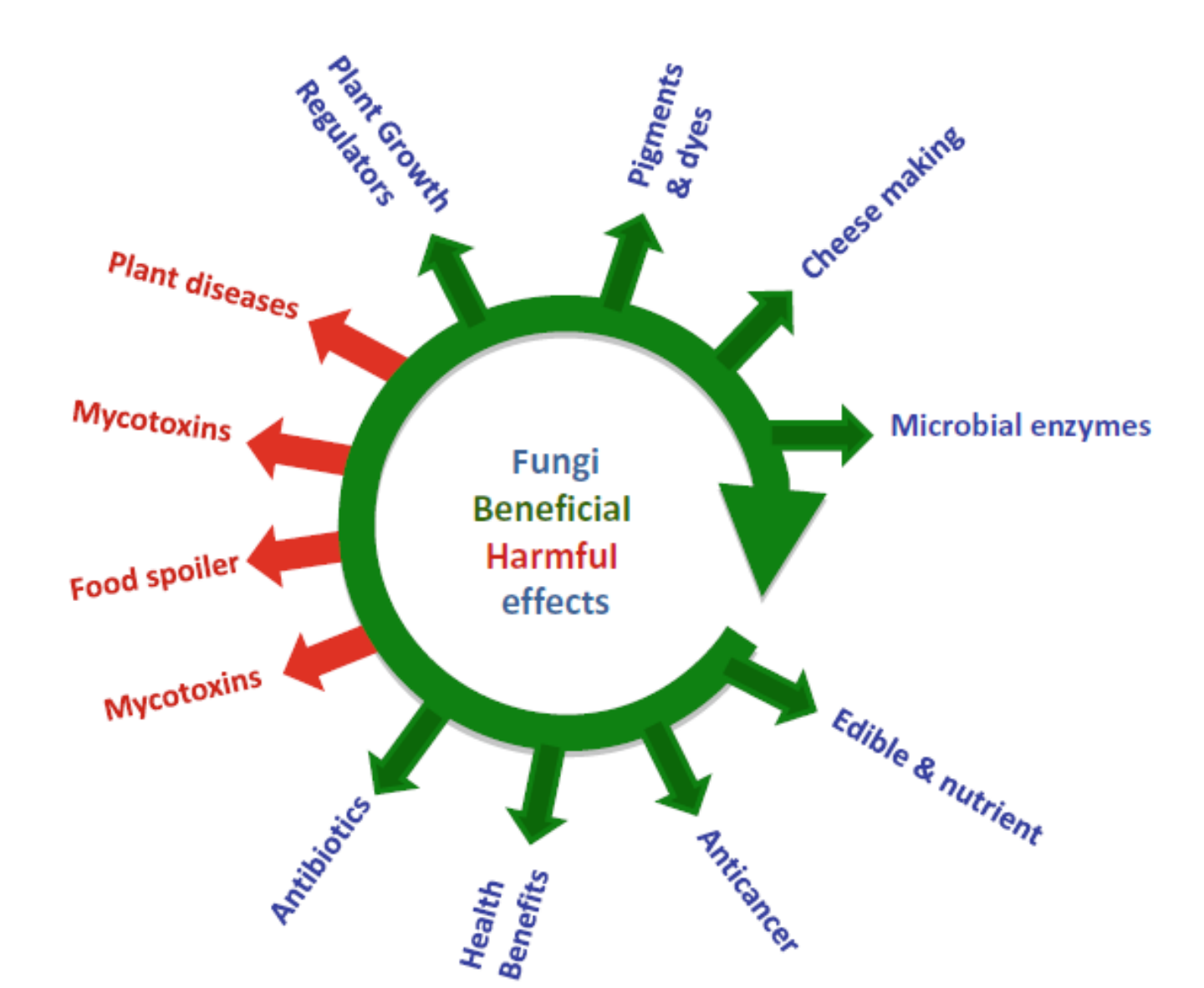
- **Edible mushrooms** are the fleshy and edible fruit bodies of several species of macrofungi (fungi which bear fruiting structures that are large enough to be seen with the naked eye). They can appear either below ground (hypogeous) or above ground (epigeous) where they may be picked by hand. Edibility may be defined by criteria that include absence of poisonous effects on humans and desirable taste and aroma.
- Edible mushrooms are consumed for their nutritional value and for their culinary value. Mushrooms consumed by those practicing folk medicine are known as medicinal mushrooms. While psychedelic mushrooms are occasionally consumed for recreational or entheogenic purposes, they can produce psychological effects, and are therefore not commonly used as food. There is no evidence from high-quality clinical research that 'medicinal' mushrooms have any effect on human diseases.
- Edible mushrooms include many fungal species that are either harvested wild or cultivated. Easily cultivated and common wild mushrooms are often available in markets, and those that are more difficult to obtain (such as the prized truffle, matsutake and morel) may be collected on a smaller scale by private gatherers. Some preparations may render certain poisonous mushrooms fit for consumption.

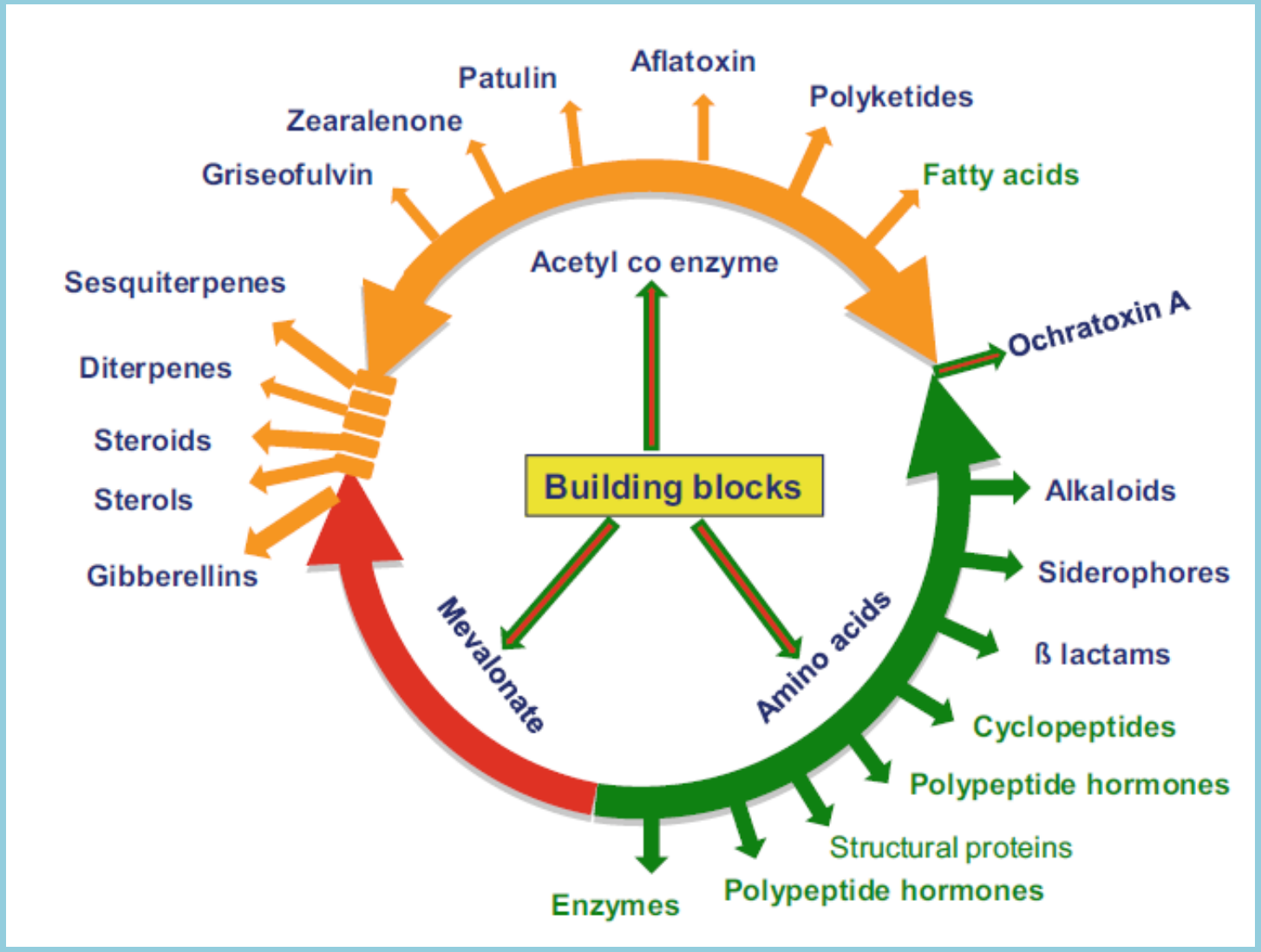
- Before assuming that any wild mushroom is edible, it should be identified. Accurate determination and proper identification of a species is the only safe way to ensure edibility, and the only safeguard against possible accident. Some mushrooms that are edible for most people can cause allergic reactions in some individuals, and old or improperly stored specimens can cause food poisoning. Great care should therefore be taken when eating any fungus for the first time, and only small quantities should be consumed in case of individual allergies. Deadly poisonous mushrooms that are frequently confused with edible mushrooms and responsible for many fatal poisonings include several species of the genus *Amanita*, in particular, *Amanita phalloides*, the *death cap*. It is therefore better to eat only a few, easily recognizable species, than to experiment indiscriminately. Moreover, even normally edible species of mushrooms may be dangerous, as mushrooms growing in polluted locations can accumulate pollutants such as heavy metals.

7. Fungal metabolites

Primary metabolites of economic importance, secondary metabolites in medicine and agriculture

Beneficial and harmful effects of fungi





Biosynthesis of different metabolites by fungi using building blocks. Building blocks are photosynthates; **green fonts-primary metabolites**, **blue fonts-secondary metabolites**

Metabolic pathways

The pathways are usually named after enzymes or intermediates involved and are also commonly used to classify secondary metabolites. Three most common pathways studied are

- (1) the mevalonic acid pathway (synthesize terpenoids, steroids, etc).
- (2) the shikimic acid pathway (synthesize aromatic amino acids, alkaloids, etc).
- (3) the acetate pathway (synthesize polyketides, fatty acids, etc).
  - The enzymes associated with these pathways are nonribosomal peptide synthetases (NRPSs), polyketide synthases (PKSs), terpene cyclases (TCs), dimethylallyl tryptophan synthetases (DMATs), and geranylgeranyl diphosphate synthases (GGPPs), etc.
  - These enzymes utilize building blocks like acetyl-coA, amino acids, mevalonate, and their different counterparts for the production of different fungal metabolites

Difference Between Primary Metabolites and Secondary Metabolites

- The metabolites which are required for the growth and maintenance of cellular function are called **primary metabolites**, while such metabolites which are not required for the growth and maintenance of the cellular functions and are the end products of the primary metabolism are called as **secondary metabolites**.
- The primary metabolites consist of the vitamins, amino acids, nucleosides and organic acids, which are necessary at the time of logarithmic phase of microbial growth. But the products like alkaloids, steroids, antibiotics, gibberellins, toxins are the secondary metabolite compound produced during the stationary phase of the cell growth.
- The microorganisms possess the tremendous capacity to synthesize the variety of products that are commercially used.

Primary Metabolites

- The metabolism products that are produced during the growth phase of an organisms in order to perform the physiological functions and supports in overall development of the cell are called primary metabolites.
- It occurs at the Growth phase
- These are produced in large quantities, and their extraction is easy.
- Same in every species, which means they produce the same products.
- These products are used in industries for various purpose.
- Primary products play the significant role in the cell growth, reproduction and development.
- E.g. Vitamins, carbohydrates, proteins and lipids are some of the examples.

Secondary Metabolite

- The end products of primary metabolism that are synthesized after the growth phase has been completed and are important in ecological and other activities of the cell are known as secondary metabolites.
- It occurs at the Stationary phase.
- These are produced in small quantities, and their extraction is difficult.
- Varies in different species.
- Secondary metabolites such as antibiotics, gibberellins are also important.
- They also indirectly support the cell, in sustaining their life for long duration.
- E.g. Phenolics, steroids, essential oils, alkaloids, steroids are few examples.

Key Differences Between Primary and Secondary Metabolites

- The **primary metabolites** are considered as the products that are produced during the growth phase of organisms and are primarily involved in the growth and development of an organism. On the other hand, **secondary metabolites** are said to be as the end products of the primary metabolites, involved in the stationary phase during the growth of a microorganism and play a role in ecological functions.
- Primary metabolism pathway occurs at the **growth phase** and is also known as **trophophase** while the secondary metabolism pathway occurs at the **stationary phase** and is also known as **idiophase**.
- Primary metabolites are produced in **large quantities**, and their extraction is easy, in fact, the products are same in every species, whereas secondary metabolites are produced in **small quantities**, and their extraction is difficult, even their products are different for different species.
- The products manufactured during the primary metabolism pathway are useful in industries for various purpose and also plays very important role in the cell growth, reproduction and development.Whereas secondary metabolites such as antibiotics, gibberellins are also important and they also indirectly support the cell, in their survival for a long time.
- **Examples** of primary metabolites are vitamins, carbohydrates, proteins, and lipids while secondary metabolites examples are phenolics, steroids, essential oils, alkaloids, steroids are few examples.

Recent examples of bioactive molecules of fungi and their biological activities

Metabolites	Source	Bioactivity
Ascomycone B and 6-deoxyfusarubin	*Biatriospora* sp. CCF 4378	Cytotoxicity
Asperterpenoid A; asperlones A and B, mitorubrin	*Aspergillus* sp. 16-5c	Inhibitor of Mycobacterium tuberculosis protein tyrosine phosphatase B
Aspiketolactonol, aspyronol, epiaspinonediol	*Aspergillus* sp. 6-02-1	Cytotoxic: human cancer cell lines K562, HL-60, HeLa, and BGC-823
Apicidin F	*Fusarium fujikuroi*	Antimalarial
Beauvericin	*Fusarium* sp.	Trypanocidal activity
Citrinin	Sponge associated *Penicillium* sp.	Antibacterial and cytotoxic
Cladosin C	*Cladosporium sphaerospermum* 2005-01-E3	Antiviral activity: influenza A H1N1 virus
Cercosporenes F	*Cercospora* sp.	Cytotoxic: human cancer cell lines HeLa, A549, MCF-7, HCT116, T24 and induces autophagy in HCT116 cells
1-(2,6-dihydroxyphenyl) pentan-1-one	*Cryptosporiopsis* sp.	Antibacterial
6,8-di-O-methylaverufin	*Aspergillus versicolor*	Antibacterial
Dihydronaphthalenone 2	*Nodulisporium* sp.	Antimycobacterial activity

Recent examples of bioactive molecules of fungi and their biological activities

Dinapinone AB2	*Talaromyces pinophilus* FKI-3864	Inhibition of triacylglycerol synthesis in mammalian cells
Fumiquinazoline Q and Protuboxepin E	*Penicillium expansum* Y32	Mitigative effect on bradycardia and vasculogenetic activity
Gliotoxin	*Aspergillus* sp. YL-06	Cytotoxic: human cancer cell lines HeLa
Ganoleucoins A and C	*Ganoderma leucocontextum*	Inhibitory activity against HMG-CoA reductase
4-Hydroxymellein	*Phoma sp.*	Inhibitory activity against P388 murine leukemia cells
Herqueidiketal	*Penicillium* sp.	Significant activity against *Staphylococcus aureus* sortase A.
Hispidin	*Phaeolus schweinitzii*	Antioxidant activity
Isosclerone	*Aspergillus fumigatus*	Antiproliferative:MCF-7 human breast cancer cells
Nodulisporiviridin G	*Nodulisporium* sp. (No. 65-17-2-1)	Amyloid β42 aggregation inhibitory activities
Neoechinulin A	*Eurotium* sp. SF-5989	Anti-inflammatory effect
Pestalotiopsone A	*Pestalotiopsis* sp.	Antibacterial

Recent examples of bioactive molecules of fungi and their biological activities

Metabolites	Source	Bioactivity
Polyporusterone B	*Polyporus umbellatus*	Antitumor activity: HepG2 cells
Phenylpyropenes E and F	*Penicillium concentricum* ZLQ-69	Cytotoxic: MGC-803 cell line
Pinazaphilones B and (±)-penifupyrone	*Penicillium* sp. HN29-3B	Inhibits α-glucosidase
Reduced gliotoxin , 6-acetylbis(methylthio) gliotoxin	*Neosartorya pseudofischeri*	Cytotoxic and antibacterial
Solaninaphthoquione	*Fusarium solani* PSU-RSPG227	Cytotoxic: MCF-7 human breast cancer cells
Sorbicatechols A and B	*Penicillium chrysogenum* PJX-17	Antiviral:influenza virus A (H1N1)
Stemphyperylenol	*Botryosphaeria dothidea* KJ-1	Antifungal and cytotoxicity against HCT116 cancer cell line
Verrucosidin	*Penicillium* sp. TPU1271	Antimycobacterial activity.

Secondary metabolites (Pharmaceutical preparations)

Fungal source	Active Ingredient	Medicinal properties
Ganoderma lucidum	**Ganoderic acid, Beta-glucan**	Liver protection, Antibiotic properties, Inhibits cholesterol synthesis
Lentinula edodes	**Eritadenine, Lentinan**	Lower cholesterol, Anti-cancer agent
A. bisporous	**Lectins**	Enhance insulin secretion
P. sajor-caju	**Lovastatin**	Lower cholesterol
G. frondosa	**Polysaccharide, Lectins**	Increases insulin secretion, Decrease blood glucose
Auricularia auricula	**Acidic, polysaccharides**	Decrease blood glucose
Flammulina velutipes	**Ergothioneine, Proflamin**	Antioxidant, Anti cancer activity
Trametes versicolor	**Polysaccharide-K (Kresin)**	Decrease immune system depression
Cordyceps sinensis	**Cordycepin**	Cure lung infections, Hypoglycemic activity, Cellular health properties, Anti-depressant activity

Secondary metabolites (Pharmaceutical preparations)

PHARMACODYNAMIC	COMPONENT	SPECIES
Antibiotics	Beta Methoxy Acrelate	Oudemanasilla radicata
Antiviral	Protein Polysaccharide	Lentinula edodes
Cardio tonic	Volvatoxin Flammutoxin	Polyporaceae volvariella
Decrease Cholesterol	Eritadinine	Collubia vellutipes
Reduce Blood Pressure	Triterpene	Ganoderma Lucidum
Anti Thrombus	5-GMP	Psolliata hartensis
Increase Bile secretion	Armillarisia A	Armillariella Tobescens
Analgesic/Sedative	Marasmic Acid	Maramius androsaceus

8. Future of fungal biotechnology

Production of mammalian proteins by fungi, other applications of gene cloning in fungi and their importance

Recombinant DNA technology:

Manipulation of industrially important fungi, edible mushroom and bio-control agents