BIOCHEMISTRY OF THE MICROBES

Fungal biochemistry and applications

P. Usha Sarma
Emeritus Scientist
Indian Agriculture Research Institute
Pusa Campus
New Delhi – 110 012

12-Jun-2006 (Revised 27-Nov – 2006)

CONTENTS

Keywords

Fungi; Biochemical pathways; Secondary metabolites; Allergens; Ergosterol; Antifungal drugs; Genomics; Proteomics; Bioremediation; Fungal diseases; Gibberllin.

General characteristics of fungi

Microbial world comprises four major groups: protozoa, bacteria, viruses and fungi. Fungi are biotechnologically useful organisms synthesizing a wide range of economically important compounds such as antibiotics (penicillin, griseofulvin, etc), organic acids (citric acid, gluconic acid, etc.), enzymes (alpha-amylases, lipases, etc.) and a number of other miscellaneous products, gibberellins, ergot alkaloids, intermediates of steroid bioconversions etc. Fungi facilitate maintenance of ecological balance and play an important role in nutrient recycling. They are the primary agents of decay of organic material. This biodegradation activity can also be deleterious, leading to decomposition of economically useful products, including building timber, man-made materials and food.

Fungi are the main alive agents of numerous diseases in humans and other animals. Incidence of life-threatening fungal diseases is on the rise, along with the increased number of patients suffering from HIV/AIDS, tuberculosis and patients undergoing various transplant surgeries. Fungi are the most important group of plant pathogens that cause devastating economic losses in crop yields worldwide. Various aspects of biology and applications of fungi are depicted in Fig. 1.

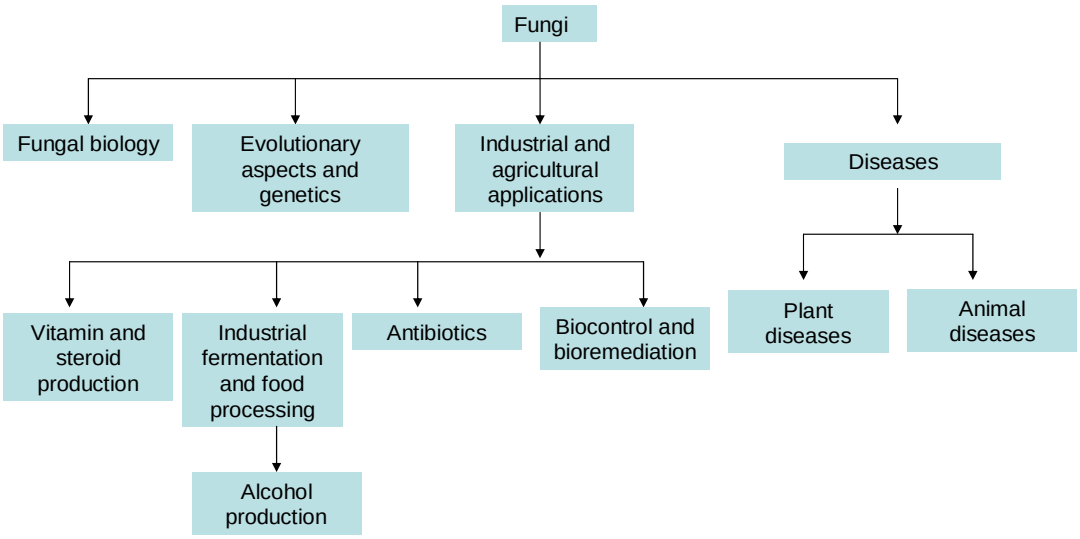


Fig 1: Various aspects of biology and applications of fungi

Knowledge on fungi played a major role in the progress of biochemistry, genetics, and molecular biology. *Beadle* and *Tatum* defined the role of *Neurospora crassa* genes in metabolism, and this resulted in the 20th century revolution in genetics. Their work took advantage of a filamentous fungus, which was first described in 1843 as the causative agents of an orange mold infestation in french bakeries. This fungus was used exclusively as an experimental organism.

Fungi are more closely related to animals than plants. The evolutionary origin of fungi is important in determining the phylogenetic relationships between fungi, animals, and plants.

The key evidence in support of this close relationship is established through analysis of protein sequences and biosynthetic pathways. The hypothesis that fungi evolved from algae or the hypothesis that fungi evolved independently of both plants and animals is not experimentally supported. Cellular components of prokaryotes, primitive eukaryotes and eukaryotes are shown in Table 1.

Table 1: Cellular components of prokaryotes, primitive eukaryotes and eukaryotes

Cellular component	Prokaryotes	Higher Eukaryotes (Plants, Animals)	Primitive Eukaryotes (Fungi)
Nuclear membrane	Absent	Present	Present
Number of Chromosomes	1	>1 (Diploid) 2n	>1 (Haploid) n
Chromosome topology	Circular	Linear	Linear
Histone Proteins	Absent	Present	Present
Ribosomes	70S	80S	80S
Chlorophyll	Absent	Present	Absent
Mode of reproduction	Asexual reproduction (Binary Fission)	Sexual reproduction	Asexual and sexual reproduction (but never by seeds)
Cell wall composition	Lipopolysachharides & Murein (peptidoglycans)	Cellulose, Pectin, Chitin (in Insects)	Glucosamine, and Chitin (major component of cell wall)
Cell membrane composition	Hopanoids (in some prokaryotes)	Cholesterols (for animals) & Phytosterols and Sitosterols for plants	Ergosterols
Amino acid-Lysine biosynthesis	via Diaminopimelic acid (DAP) pathway except some Thermophiles	via Diaminopimelic acid (DAP) pathway	via α-aminoadipic acid (AAA) pathway
Carbohydrates in nutrients	Mainly Glucose	Glucose, Fructose, Starch and other polysaccharides	Soluble carbohydrates (Trehalose, Mannitol & Arabitol) and glycogen as storage carbohydrate

Fungi can survive over a broad range of temperatures. Some fungi can thrive in very high temperatures of 130-150°F whereas some may thrive in very low temperatures below 32°F (below freezing). Humid environment facilitates growth of the fungi. However, some fungi can grow in very dry conditions also. At the other extreme, there are also fungi that can live *under* water.

They depend upon other organisms for their carbon source and are heterotrophs. They are also saprophytic and symbiotic in nature. Their carbon source is basically from the by-products of organisms or dead organisms or decayed products. Fungi require both macro and

micronutrients, which are usually available in excess in their environment. Some fungi may have requirements of growth factors such as thiamin, biotin, sterols, riboflavin, nicotinic acid and folic acid. *The intimate association of two dissimilar organisms in a mutually beneficial relationship is common among fungi such as lichens and mycorrhizae.* This feature is known as Mutualistic symbiosis.

The transport of food from the substrate into the fungal cell walls is by absorption. The mycelium or yeast cells can transport the soluble sugars and amino acids directly through their cell wall. If the available food is insoluble, i.e. a large, complex, organic compound, such as lignin, cellulose and pectin, then the food must first be digested. Digestion is carried out by the production of various enzymes that are substrate specific which break down insoluble food material to soluble compounds and facilitate transport of the food through the cell wall. Higher eukaryotes ingest food and then digest it; while fungi digest their food before ingestion.

Morphology

Most fungi occur in the form of yeasts or molds. Yeast is unicellular microscopic fungus that reproduces, asexually, by budding or fission and is best known microorganism that makes bread rise. Molds are multicellular organisms with nuclei and mitochondria.

Molds

Molds include fungus that grows on bread and oranges in tangled masses of filaments of cells. The collective, filamentous strands make up the mycelia. Strands of mycelium are referred to as hyphae. Two types of mycelium can be seen among fungi, septate and coenocytic. Septate mycelia are divided into cells by cell walls that are laid down at regular intervals along the length of the mycelium. These cell walls are called **septa**. Coenocytic mycelia are **not** divided by septa and form a continuous tubular network. Septa, however, are present occasionally, especially where reproductive structures occur.

Molds are characterized by the development of hyphae, and the colonies are visible under microscope (Fig. 2). Hyphae elongate by a process known as apical elongation, which requires a careful balance between cell wall lysis and new cell wall synthesis. Molds are often differentiated on the basis of conidiophores and conidiogenous cells. Molds produce special sac-like cells called sporangia, which give rise to spores called sporangiospores. Sporangia are typically formed on special hyphae called sporangiophores

Yeasts

Yeasts are fungi that grow as solitary cells and reproduce by budding. Yeast taxa are distinguished on the basis of presence or absence of capsules, size and shape of the yeast cells, mechanism of daughter cell formation (conidiogenesis), formation of pseudohyphae and true hyphae, and presence of sexual spores, in conjunction with physiological data. Morphology is used primarily to distinguish yeasts at the genus level. While the ability to assimilate and ferment various carbon sources and utilize nitrate as a source of nitrogen are used in conjunction with morphology to identify species. Examples of yeasts include *S. cereviseae, C. albicans,* and *Cryptococcus neoformans.*

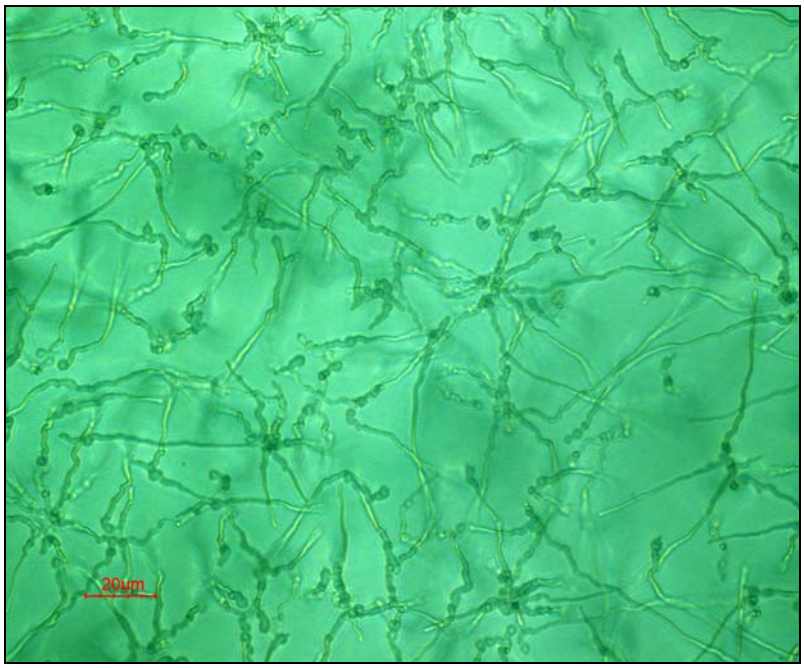


Fig. 2: Spores and Hyphae of *Aspergillus fumigatus*

Dimorphism of fungi

A number of medically important fungi express themselves morphologically in two different forms, which correlate with the saprophytic and parasitic modes of growth. Such fungi are called dimorphic fungi. Dimorphic fungi have both mycelium and yeast. The term "dimorphic" describes fungi that typically grow as mycelium *in vitro* and as yeast cells *in vivo*. Examples of medically important dimorphic fungi include *Blastomyces dermatitidis* (hyphae and yeast cells) and *Coccidioides immitis* (hyphae and spherules).

A number of external factors contribute to the expression of dimorphism. Increased incubation temperature is the single most important factor. Increased carbon dioxide concentration, which probably affects the oxidation-reduction potential, enhances the conversion of the mycelial form to the tissue form in *Coccidioides immitis* and *Sporothrix schenckii*. Development of the yeast form is affected by pH while presence of cysteine and other sulfhydryl-containing compounds affect the growth of other dimorphic fungi. Some fungi require a combination of these factors to induce dimorphism. Other examples of dimorphic fungi include *Histoplasma capsulatum, Paracoccidioides brasiliensis, Penicillium marneffei, and Sporothrix schenckii.*

Fungal cell wall and plasma membrane

Cell wall

Fungal cell walls are rigid and chitinaceous. The rigid cell wall is a stratified structure consisting of chitinous microfibrils embedded in a matrix of small polysaccharides, proteins, lipids, inorganic salts, and pigments that provides skeletal support and shape to the enclosed protoplast. Chitin is a (β-1-4)-linked polymer of *N*-acetyl-D-glucosamine (GlcNAc). It is produced in the cytosol by the transfer of GlcNAc from uridine diphosphate GlcNAc into chains of chitin by chitin synthetase, which is located in the cytosol in organelles called chitosomes. The chitin microfibrils are transported to the plasmalemma and subsequently

integrated into the new cell wall. The major polysaccharides of the cell wall matrix consist of noncellulosic glucans (D-glucose polymers with glycosidic bonds) such as glycogen-like compounds, mannans (polymers of mannose), chitosan (polymers of glucosamine), and galactans (polymers of galactose). Small amounts of fucose, rhamnose, xylose, and uronic acids also may be present. Fig. 3 shows the detailed structure of fungal cell wall and cell membrane.

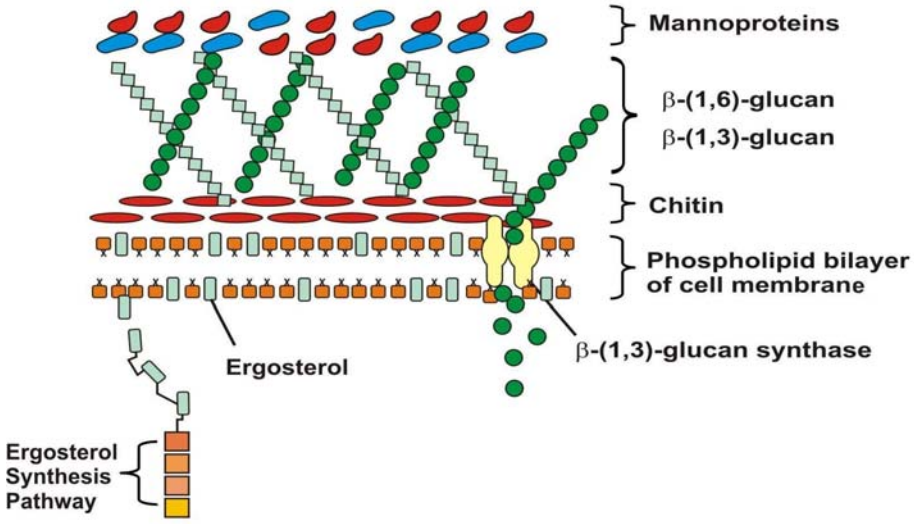


Fig 3: Fungal Cell wall and Cell membrane

Yeasts have soluble peptidomannans as a component of their outer cell wall in a matrix of glucans, Mannans, galactomannans and less frequently, rhamnomannans. *Cryptococcus neoformans* produces a capsular polysaccharide composed of at least three distinct polymers: glucuronoxylomannan, galactoxylomannan, and mannoprotein. The capsule is antiphagocytic, serves as a virulence factor, persists in body fluids, and allows the yeast to avoid detection by the host immune system.

In addition to chitin, glucan, and mannan, cell walls may contain lipid, protein, chitosan, acid phosphatase, β -amylase, protease, melanin, and inorganic ions such as phosphorus, calcium, and magnesium. In the yeast *Candida albicans,* the cell wall contains approximately 30 to 60 % glucan, 25 to 50 % mannan (mannoprotein), 1 to 2 % chitin, 2 to 14 % lipid, and 5 to 15 % protein. The proportions of these components vary greatly from fungus to fungus. The outer cell wall of dermatophytes contains glycopeptides that may evoke both immediate and delayed cutaneous hypersensitivity in human beings.

Sphingolipids, in addition to their crucial role in membrane structure in *Saccharomyces cerevisiae*, play vital roles in various aspects of biology including endocytosis, intracellular protein transport and stress responses. Sphingolipids mediate diverse biological responses such as cell growth, apoptosis, angiogenesis, differentiation, and senescence. Sphingosine-1-phosphate plays a role in Ca^{2+}-mediated guard-cell closure, and a sphingosinetransfer protein is involved in ceramide synthesis.

A sphingolipid is essentially composed of three parts: The sphingolipid backbone or long chain base i.e. amide bound to a saturated fatty acid to form ceramide, and a polar head group, linked to ceramide at carbon atom C-1. The head group substitutents in higher eukaryotic cells are glucose, galactose or phosphocholine yielding glucosylceramide, galactosylceramide, and sphingomylein. Yeast, *Saccharomyces cerevisiae*, contains three classes of sphingolipids, inositolphosphorylceramide (InsPCer), mannosylinositol phosphorylceramide (ManInsPCer) and mannosyldinositol phosphorylceramide (ManPIns2Pcer) (Fig. 4). In higher eukaryotic cell and probably also in yeast, this takes place at the cytosolic substance in the endoplasmic reticulum. Yeast sphingolipids more closely resemble mammalian glycolipids in both their head group, structure and having hydroxylated long chain saturated fatty acid. Mammalian cells make dozens to hundreds of sphingolipid species that differ in their precise head group substitutes: inositiolphosphate and mannose. The simplest yeast sphingolipids, inositolphosphorylceramide (IPC) is made by transferring inositolphosphate from the glycerolipid phosphatidyllinositol (PI) onto ceramide, there by generating diacylglycerol (DAG), a potential signaling molecule.

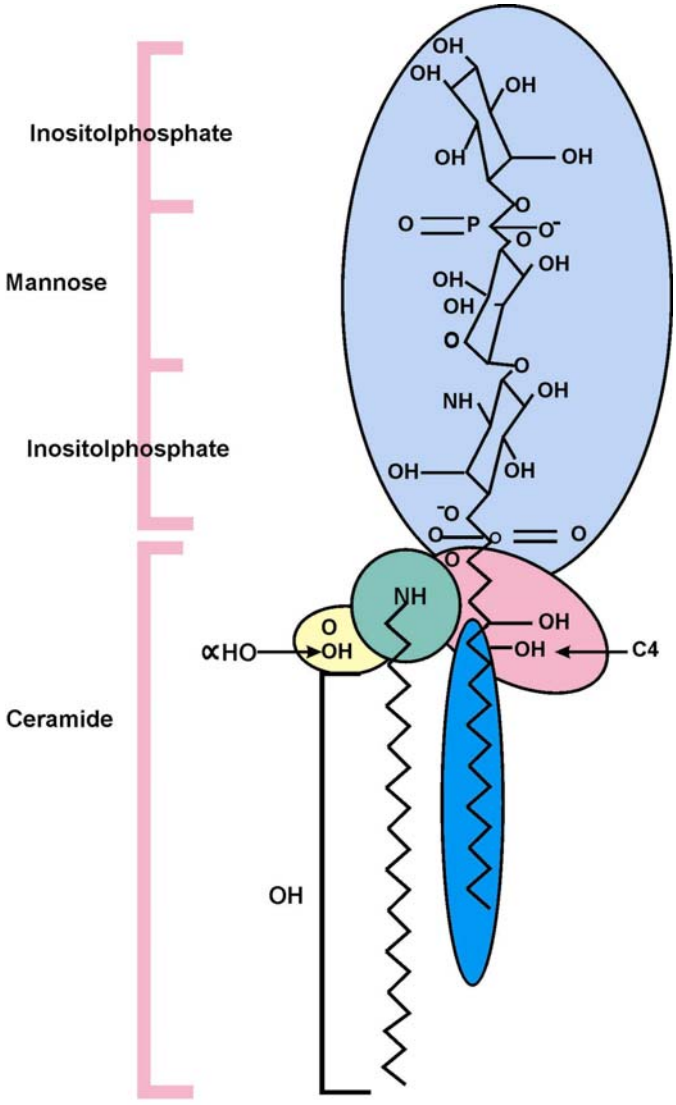


Fig 4. Structure of Sphingolipid in yeast

Plasma membrane

Fungal plasma membranes are similar to mammalian plasma membranes, except in having the nonpolar sterol ergosterol, rather than cholesterol, as the principal sterol. Membrane sterols provide structure, modulation of membrane fluidity, and possibly control some physiological events. The plasma membrane contains primarily lipids and protein, along with small quantities of carbohydrates. The major lipids are amphipathic phospholipids and sphingolipids that form the lipid bilayer. The hydrophilic heads are towards the surface, and the hydrophobic tails are buried in the interior of the membrane. Proteins are interspersed in the bilayer, with peripheral proteins being weakly bound to the membrane. In contrast, integral proteins are tightly bound. The lipoprotein structure of the membrane provides an effective barrier to many types of molecules.

Reproduction in fungi

Many fungi can reproduce both asexually and sexually (Fig. 5). Asexual reproduction is common when nutrients and water are abundant. Sexual reproduction occurs when nutrients or water become scarce.

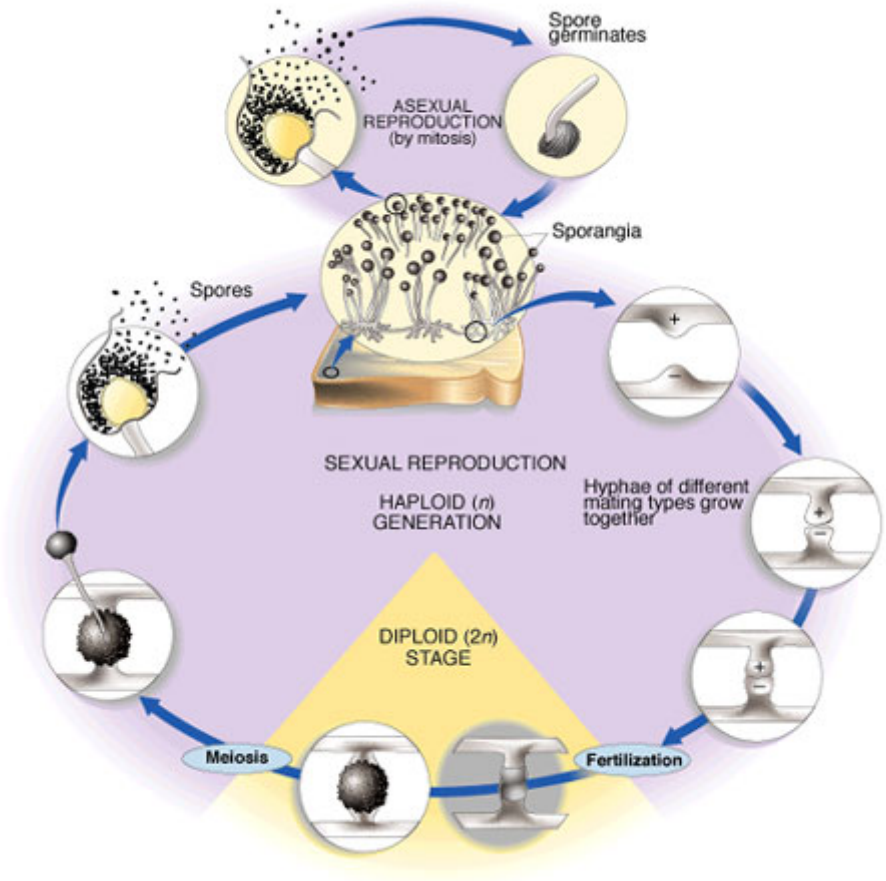


Fig. 5: Reproduction in fungi

Asexual reproduction is by spores. The spores are dispersed and spread the growth of the fungi. Each spore is haploid in nature and contains a nucleus and dehydrated cytoplasm

surrounded by a protected coat. A spore is usually one-celled that is capable of developing into a new individual. Asexually, fungi produce thousands of genetically identical haploid spores, on hyphae. A fruiting body consists typically of a stalk and a sac in which spores are produced. When a spore lands on a moist surface (favourable environmental conditions) where nutrients are available, the cytoplasm absorbs water and forms hyphae. The hyphae will then transform into new mycelium.

Penicillium, which produces penicillin, is a fungus that reproduces asexually by means of conidia. Conidia, which are formed without the protection of an enclosed sac, are formed on top of a stalk-like structure called a conidiophore. When fungi are ready to reproduce, fruiting bodies form. Fruiting bodies are formed at the reproduction stage, asexual or sexual process. Fungal spores cannot move themselves, but spores are small and light, can be dispersed by wind, animals, insects, or water. Fungal spores are ubiquitous in occurrence and universal in the world.

Spores and Conidia

Spores and Conidia are the propagating tools of fungi. Spores can be produced either asexually or sexually. Asexual spores are always formed in a sporangium following mitosis and cytoplasmic cleavage. Sporangiophores are specialized hyphae that look like upright stalks. On top of the sporangiophore is an enclosed sac called a sporangium. Inside each sporangium, spores called sporangiospore**s** are formed. *Rizopus*, a bread mold, is an example of sporangiospore forming fungus.

The number of sporangiospores and their arrangement in the sporangium are used to differentiate various zygomycetes. Ascospores are formed in a saclike cell (called an ascus) by free-cell formation, basidiospores form on basidia, and zygospores form within zygosporangia. Oospores are sexual spores. Sexual spores are rarely seen in clinical isolates because most fungi are heterothallic (i.e., sexually self-sterile).

Conidia are always asexual in origin and develop in any manner that does not involve cytoplasmic cleavage. The conidial formation in conidiogenesis and their arrangement, colour, and septation are used to differentiate various genera of molds. Some fungi have melanin in the cell wall of the conidia, the hyphae, or both.

Sexual reproduction

There are no male or female fungi. The two mating types are called PLUS (+) Mating Type and the Minus (-) Mating Type. Fertilization occurs when the Hyphae from a Plus Mating Type and a Minus Mating Type fuse. Under favorable environment, rapid asexual reproduction takes place and increase spread of species occurs. During environmental **stress**, sexual reproduction ensures genetic recombination, increasing the likelihood that offspring will be better adapted to the new environment.

Classification

True fungi with walls typically containing chitin, and with many other characteristic cellular and biochemical features are divided in five sub-groups. Annexure 1 gives classification and relevance of fungi to human health, environment and industry.

Biochemical pathways in fungi

It is necessary to understand various biochemical pathways operating in fungi for different useful applications. Biochemical pathways and metabolic networks are well worked out in *Saccharomyces cerevisiae*. Understanding of genomes of *Neurospora Crassa*, *A. nidulans*, *A. flavus* facilitated a deeper insight into the biochemical pathways, variations and homologies among various fungi.

Insight into the functional organization of fungi is gained mainly through the information on metabolic networks in *Saccharomyces cerevisiae*. Several biochemical pathways are identified in *S. cerevisiae*, which are common in other organisms and are discussed in briefly.

Salient metabolic pathways include carbohydrate, fatty acid, amino acid and nucleotide metabolisms. Energy formation in the cell is an important outcome of metabolic pathways.

Carbohydrate metabolism

Soluble carbohydrates enter hyphae by diffusion, followed by active uptake across the fungal membrane. For the saprophytic fungi most of the available carbon in the environment is present as complex polymers like cellulose, chitin or lignin. These materials need to be broken down enzymatically before they can be utilized. Therefore fungi release degradative enzymes such as cellulases, chitinases, proteases and multi-component lignin degrading enzymes, depending on the type of substrate the fungus is growing on. Regulation of these enzymes is by substrate-induction and end product inhibition. Carbohydrate metabolism in fungi is via glycolysis (also called Embden-Myerhof-Parnas pathway) and the tricarboxylic acid cycle. In some fermentative fungi glycolytic pathway leads to the reduction of pyruvate to lactic acid to produce alcohol. Examples include *Saccharomyces cervesiae, S. carlsbergensis, S. uvarum, S. sake*, *Aspergillus oryzae*.

Glycolysis: Two important ways a cell can harvest energy from food are by fermentation and cellular respiration. Both start with the first step: the process of glycolysis, which is the breakdown of glucose (6 carbons) into two molecules of pyruvic acid (3-carbon). Detailed glycolysis pathway has been shown in Fig. 6. The energy from other sugars, such as fructose, is also harvested using this process. The process, glycolysis does not need oxygen as part of any of its chemical reactions. It serves as a first step for both aerobic and anaerobic energy-harvesting reactions.

Kreb's cycle: The end product of glycolysis, i.e. pyruvate, undergoes series of reactions and is converted into oxaloacetate by means of various enzymes. Relevant enzymes for catalyzing these reactions are present in the mitochondria where oxidative decarboxylation takes place. On complete combustion of CO_2 and H_2O, one mole of glucose yields about 700 kcal. In presence of oxygen the metabolism of glucose via glycolysis and TCA cycle (Fig. 7) coupled to electron transport chain yields a total of 36 ATP molecules (about 252 k.cal).

Glyoxylate cycle: The glyoxylate cycle is the variation of the citric acid cycle and occurs in plants, fungi, bacteria, but not in animals. In this cycle, acetyl Co A condenses with oxaloacetate to form citrate exactly as in the citric acid cycle. The breakdown of isocitrate does not occur via the isocitrate dehydrogenase reaction, but occurs through a cleavage catalysed by the enzyme isocitrate lyase, to form succinate and glyoxylate (Fig. 8). As glyoxylate cycle does not occur in eukaryotes, this cycle may have a potential role in fungal pathogenesis

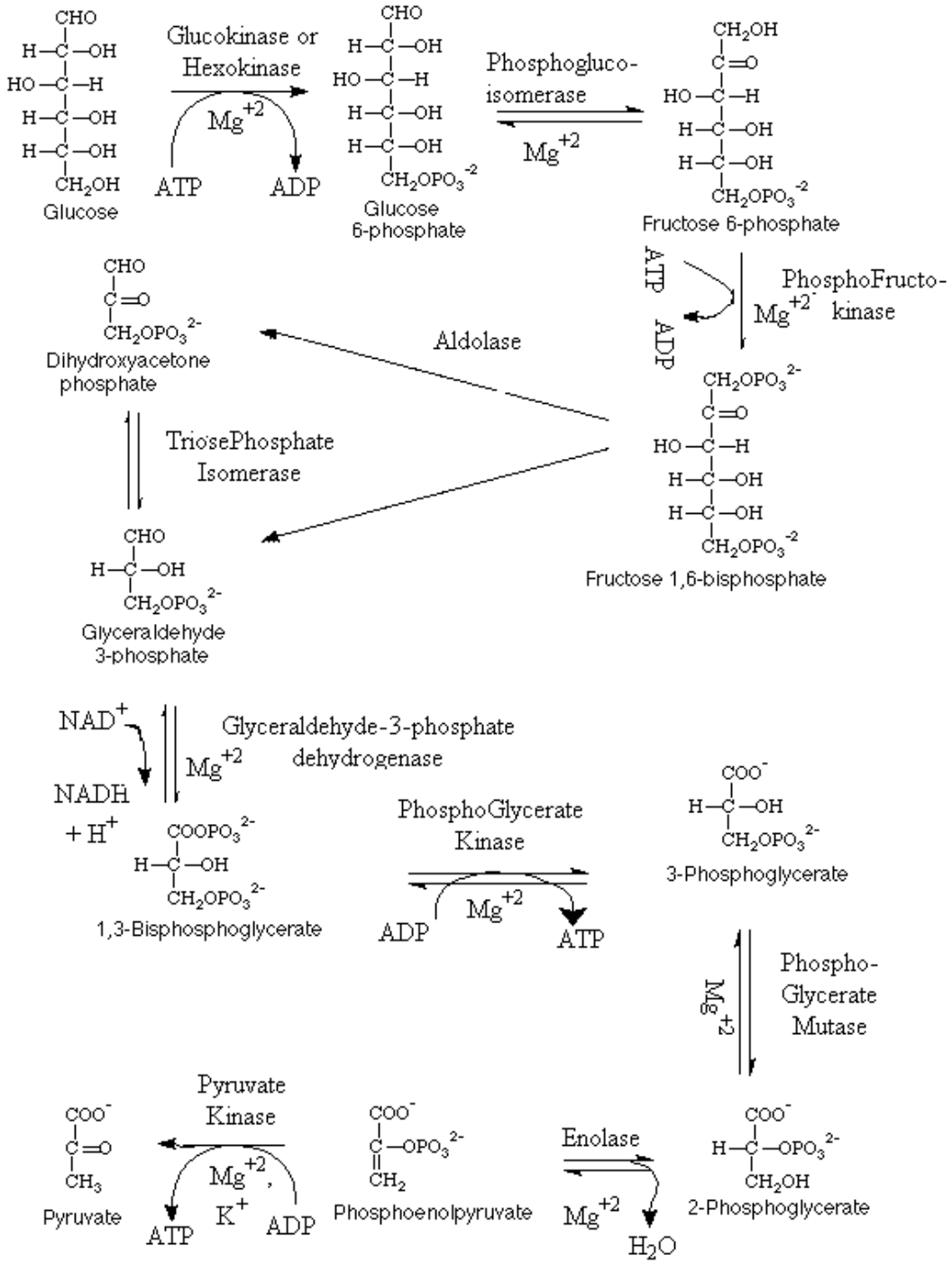


Fig 6: Glycolysis in Saccharomyces cerevesiae

Lipid metabolism

Fatty acid synthesis (Lipogenesis) takes place in the cytosol and is distinct from the oxidation of fatty acids (Lipolysis) in the mitochondria. Fatty acids are converted in to an active form fatty acyl CoA by acyl CoA synthetase enzyme. This enzyme is present in the endoplasmic reticulum, inside and on the outer membrane of mitochondria. The reaction is catalyzed on the outer membrane and thus the conversion of fatty acid to its activated form (fatty acyl CoA) occurs, involving one high-energy phosphate. Fatty acyl CoA is formed outside the

mitochondria, whereas the fatty acid oxidizing machinery is present inside the inner membrane of mitochondria, which is impermeable to CoA and its derivatives. Fatty acyl CoA is transferred into mitochondrial matrix via carnitine shuttle, where β-oxidation takes place.

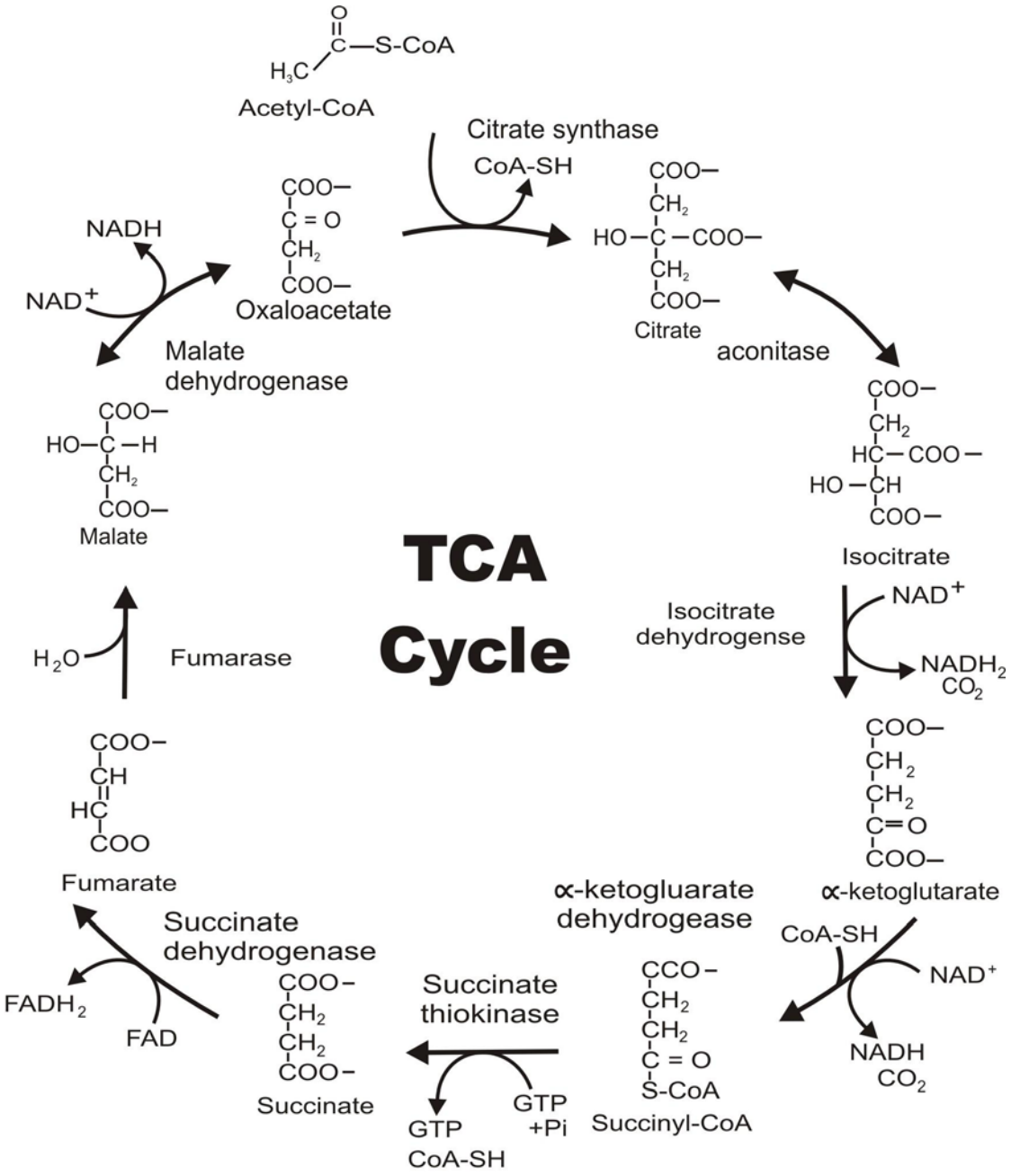


Fig 7: TCA cycle in *Saccharomyces cerevesiae*

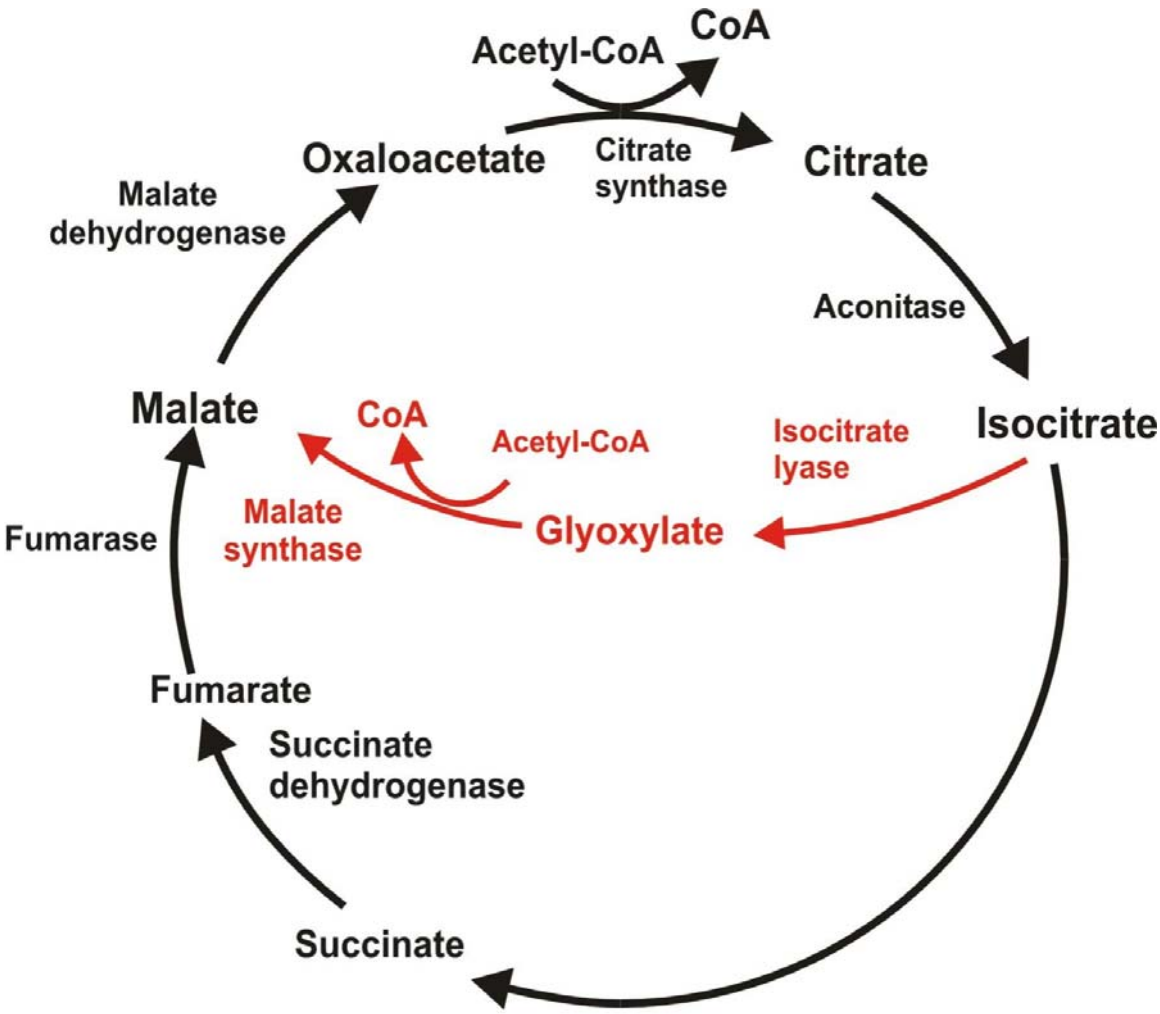


Fig 8: Glyoxylate cycle in *S. cereviseae*

Amino acid metabolism

Fungi utilize nitrate, ammonia and some amino acids by direct uptake across the hyphal membrane, however they cannot fix gaseous nitrogen. More complex nitrogen sources, such as proteins and peptides, can be utilized, once extra cellular proteases have degraded them into amino acids.

Transamination reaction i.e. transfer of the amino group from α-amino to keto acid is predominant in the amino acid metabolism. Nitrogen of all the amino acids can enter into glutamate. Ammonia of L-glutamate is released by an oxidative deamination catalysed by L-glutamate dehydrogenase. In fungi, the ammonia released from the amino acids is detoxicated by conversion to urea by urea cycle as described in the Fig. 9.

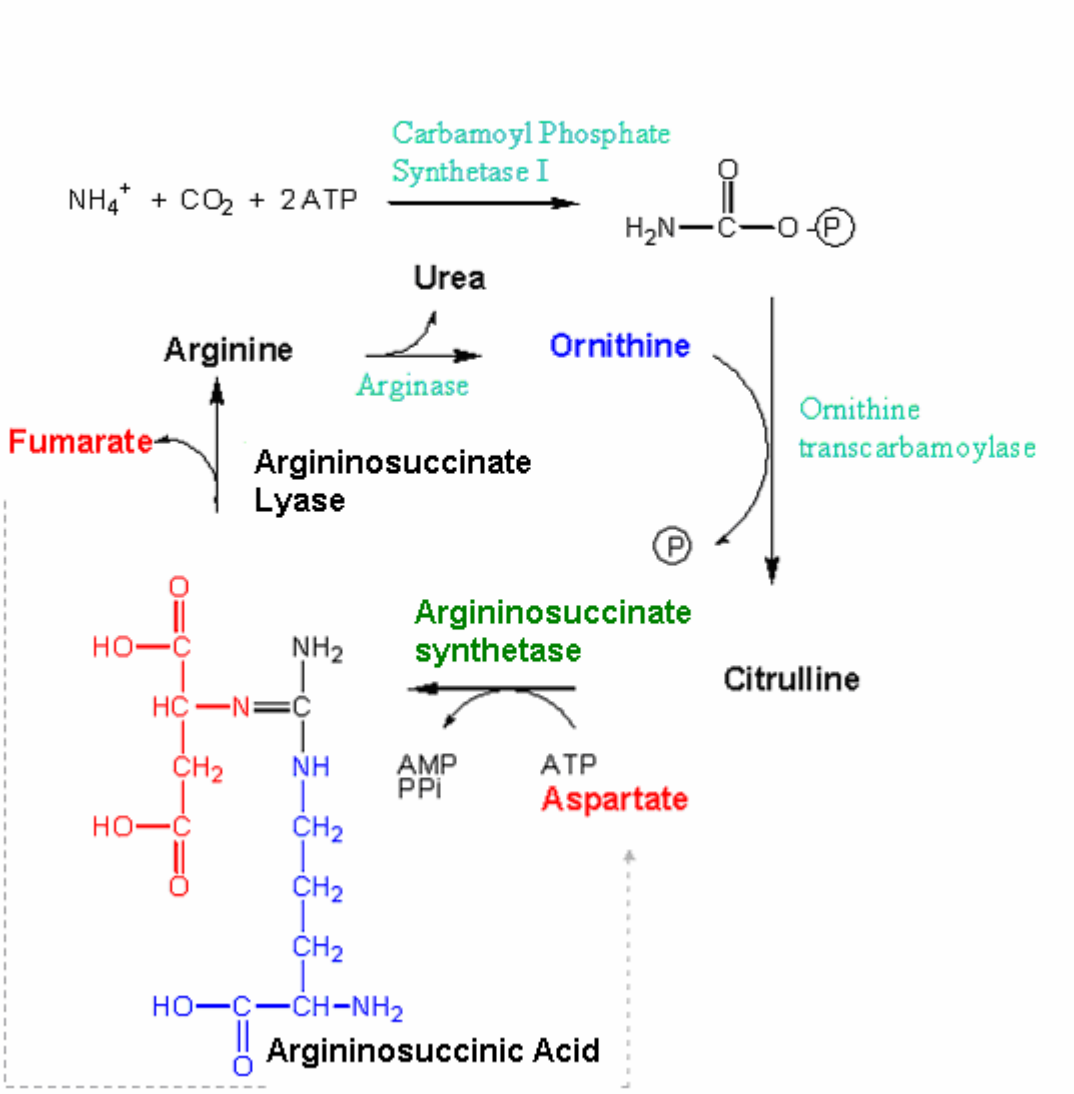


Fig 9: Urea cycle in *Saccharomyces cerevesiae*

Nucleotide metabolism

Nucleotides are essential for making genetic machinery of the organism. Purines and pyrimidines are the basic elements forming DNA and RNA in nucleus. The *de novo* (*de novo* means newly-synthesized) synthesis of purine nucleotides is from simple precursors like CO_2, amino acid, one carbon unit etc. The precursors required for the biosynthesis of pyrimidine nucleotides are aspartate, CO_2, and glutamine. These molecules by enzymatic reaction form uridine monophosphate (UMP) and other pyrimidines like cytosine and thymine are formed from UMP. In fungi, purine nucleotides are degraded via ureides, allantoin and allantoate to NH_3 and CO_2 by the purine catabolic pathway while pyrimidine bases uracil and thymine, are mainly catabolised by the sequential reactions catalyzed by different enzymes. The end products of this pathway are either ß-alanine or ß-aminoisobutyrate. In both cases, NH_3 and CO_2 are released as byproducts.

Energy

Energy is derived by the organelles from carbohydrates, fats and proteins in foodstuffs. In most of the fungi, carbon and hydrogen skeleton of compounds are completely oxidized by

aerobic fragmentation to CO_2, and H_2O. In some fermentative fungi carbohydrates are converted into alcohol via anaerobic pathway, where no energy is required. Fermentation process does not yield more energy but serves to regenerate NAD+ necessary for continuation of glycolysis.

Carbohydrates, fats and proteins are metabolized via different biological pathways and enter into TCA cycle to produce CO_2, H_2O, NADH, FADH, and small number of ATP (through substrate level phosphorylation). NADH and FADH act as a starting molecule for oxidative phosphorylation to generate ATP via electron transport chain (while most of the ATP is formed by passage through the electron transport chain and oxidative phosporylation, a certain numbers of ATP is obtained by oxidation at the substrate level). This process requires several enzymatic complexes present on the mitochondrial membrane. The complete oxidation of one glucose molecule yields 36 ATP molecules (700kcal).

Interlinks of metabolic pathways

Metabolic network is formed through the interaction between different metabolic pathways like carbohydrate, fat and protein, as depicted in Fig. 10. Fats are broken down to fatty acids and glycerol and then glycerol enters the glycolytic pathway via glyceraldehyde-3-phosphate. Fatty acids are oxidized to acetyl CoA and then enter the TCA cycle. Glucogenic amino acids, amino acids whose carbon skeleton give rise to citric acid cycle intermediates, are either oxidized to CO_2, and H_2O by the citric acid cycle or may be converted to other compounds e.g. glucose, ketone bodies or fats. While ketogenic amino acids, amino acids whose carbon skeletons give rise to acetyl CoA or crotonyl CoA, give rise to fatty acids as they all can form acetyl CoA. *Signaling* networks comprise cellular responses to inter-cellular signals. *Gene expression* networks describe regulatory interactions between genes and gene-products.

Secondary metabolites

Many fungi express secondary metabolites, which are low-molecular-weight compounds. These metabolites often have potent physiological activities. Morphine and quinine are plant secondary metabolites, whereas penicillin, cephalosporin, ergotrate and the statins are well known fungal secondary metabolites. Many of these have been found to be useful in industry and medicine. Although chemically diverse, all secondary metabolites are produced by a few common biosynthetic pathways, often in conjunction with morphological development. Recent advances in molecular biology, bioinformatics and comparative genomics have revealed that the genes encoding specific fungal secondary metabolites are clustered and often located near telomeres. The most important secondary metabolites seem to be synthesised from one or a combination of three biosynthetic pathways: polyketide pathway, mevalonate pathway and other pathways involved in amino acid such as shikimic acid pathway. Various secondary metabolites include mycotoxins, antibiotics, alkaloids and plant growth regulators.

Mycotoxins

Mycotoxins are secondary metabolites produced by fungi and are capable of causing disease and death in humans and other animals. While all mycotoxins are of fungal origin, not all toxic compounds produced by fungi are called mycotoxins. The target and the concentration

of the metabolite are important for their toxicity. Due to their pharmacological activity, some mycotoxins or mycotoxin derivatives are in use as antibiotics (fungal products that are mainly toxic to bacteria), growth promotants, and other kinds of drugs; some have been implicated as chemical warfare agents. Some of the mycotoxins are associated with human and veterinary diseases (Mycotoxicoses). The toxins associated with the diseases include aflatoxins, citrinin, ergot akaloids, fumonisins, ochratoxin A, patulin, trichothecenes, and zearalenone. Neither, the role of mycotoxins in fungal biology or the mechanism of action of aflatoxins in humans is well understood.

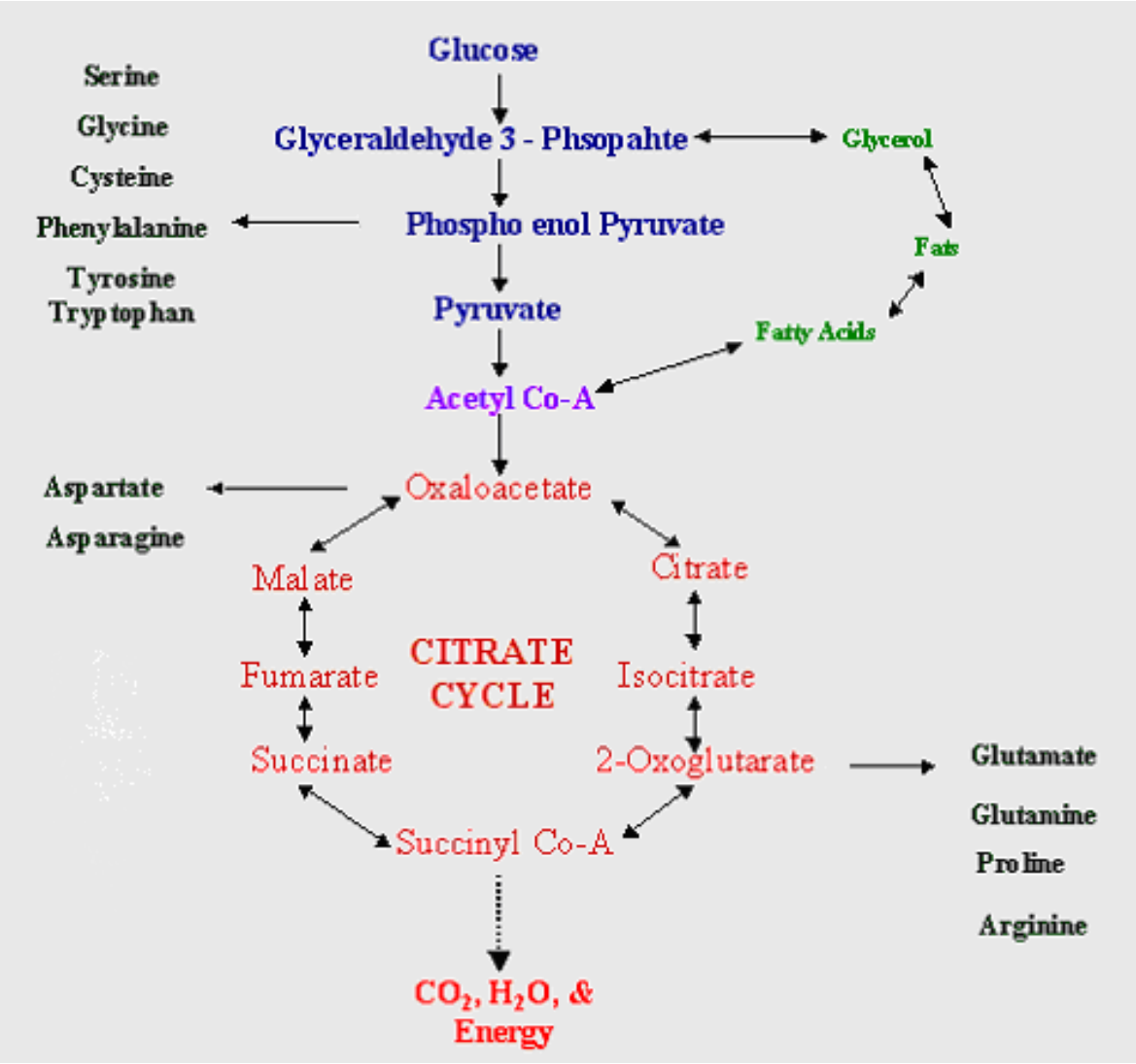


Fig 10: Interlinks of metabolic pathways

Aflatoxins are natural secondary metabolites produced primarily by the fungus *A. flavus* and *A. parasiticus*. Aflatoxins were discovered about 40 years ago after the devastating loss of poultry in England (Turkey X disease). They are a group of polyketide-derived furanocoumarins with at least 16 structurally related toxins that have been characterized. There are four major Aflatoxins – B1, B2, G1, and G2 (Fig. 11). *A flavus, A. pseudotamarii* and *A. ochraceoroseus* produce only the B aflatoxins and *A. nomius, A. bombycis, A. parasiticus* and an unnamed taxon from West Africa produce both B and G toxins. Aflatoxins

were originally isolated from *A. flavus* and hence the name A-fla-toxin. Other significant members of the aflatoxin family, M1 and M2 are oxidative forms of aflatoxin B1 modified in the digestive tract of some animals and isolated from milk, urine and feces. The aflatoxin molecule contains a coumarin nucleus linked to a bifuran and either a pentanone as in aflatoxin B1 and the dihydro-derivative aflatoxin B2, or a six membered lactone as in Aflatoxin G1 and its corresponding derivative aflatoxin G2. These four compounds are separated by the colour of their fluorescence under long wave UV illumination (B=Blue, G=Green). The subscripts relate to their relative chromatographic mobility. Of the four, B1 is found in highest concentrations followed by G1 and G2. The mode of action involves the chemical binding of the liver Cytochrome P450 activated aflatoxin forms adducts with guanine residues in DNA (p53 gene), which results in a G to T change ultimately causing liver cancer in animals.

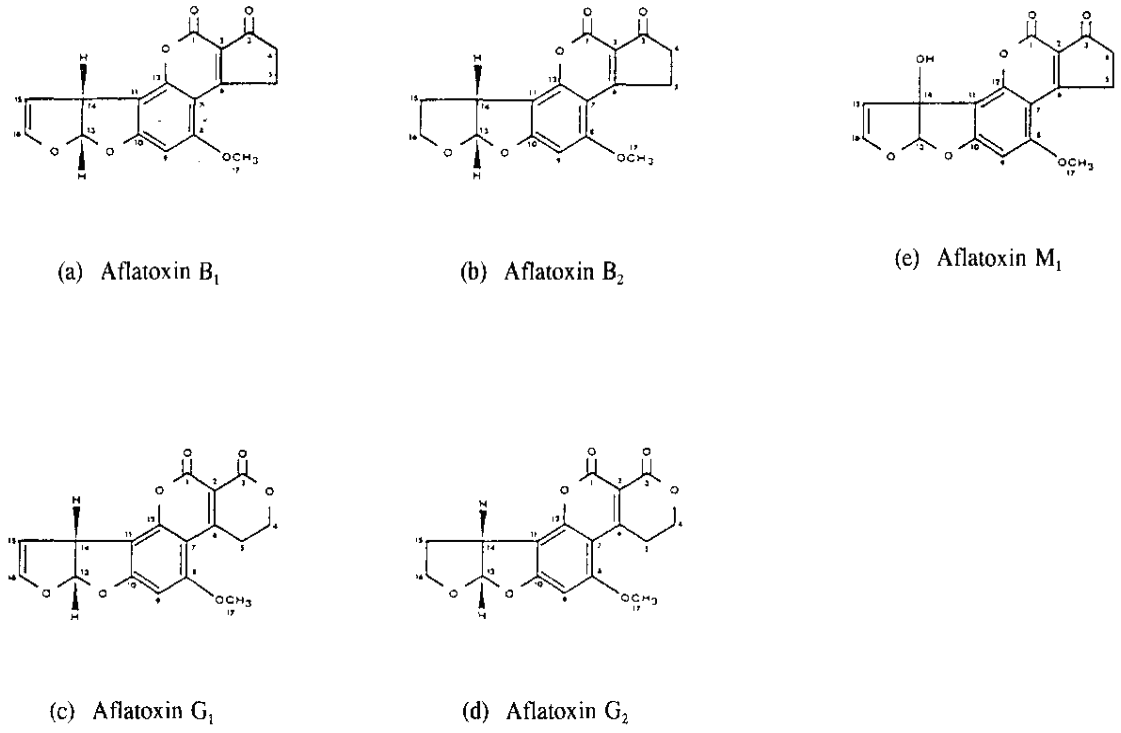


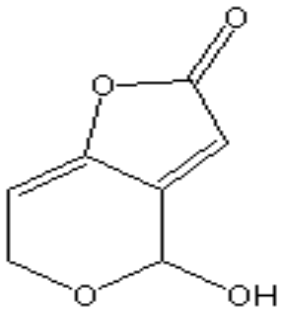
Fig 11: Chemical structures of aflatoxins

Aspergillus flavus and *A. Parasiticus,* are saprophytic in nature, with worldwide distribution. They can colonize several important crops like corn, cottonseed, peanuts and tree nuts, produce aflatoxins, which are highly toxic, mutagenic and carcinogenic to animals and have been implicated in hepatocellular carcinoma, acute hepatitis, Reye's syndrome, cirrhosis in malnourished children, and Kwashiorkor. Diseases produced by aflatoxins are called Aflatoxicoses.

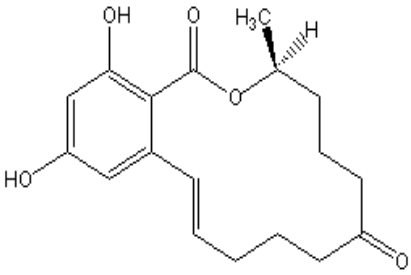
Other toxins

Patulin is a polyketide lactone produced by *Penicillin* and *Aspergillus* growing on apples, pears, grapes and other fruits. Its biosynthetic pathway is still unclear, but it appears that several alternate pathways may result in the same end product. Patulin has an effect on carbohydrate metabolism and causes breaks in DNA, inhibits aminoacyl-tRNA synthetase. Patulin has an influence on intracellular ion flux and interaction with cellular membranes. It was first thought to be a potential antibiotic.

Zearalenones (aromatic and cyclic compounds) are also synthesised via the polyketide biosynthetic pathway._Zearalenone is one interesting example from this group regulating perithecium formation in the fungus. It has an estrogenic effect in mammals.



Patulin

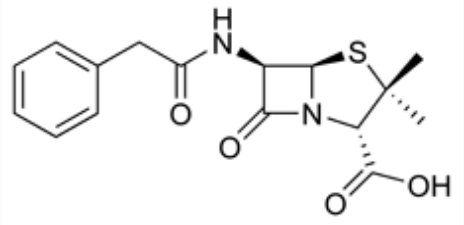


Zearalenone

Consumption of high doses of zearalenone is commonly detrimental in pigs, but the compound has also been used deliberately at low doses as a growth supplement in sheep and cattle. The reproductive behaviour of pigs consuming feed contaminated with *Fusarium graminearum* may be modified. *F. graminearum* is a common hyphomycete found in soil. Some isolates are widely recognised as important plant pathogens in temperate and warm temperate climates. Thus contamination of feeds is likely to be widespread where there is poor control over humidity during storage. The main function of zeralenone appears to be its role in perithecium formation.

Antibiotics and alkaloids

Penicillin and cephalosporin are β- lactam antibiotics produced by a few Ascomycota (e.g. *Penicillium chrysogenum*, *Aspergillus nidulans* and *Penicillium nalgiovense*). Several bacteria also produce penicillin. The precursors of these antibiotics are amino acids. Synthesis of active antibiotics is directed by the inclusion of different organic fatty acids in the growth medium. This results in the formation of side chains on the compound.

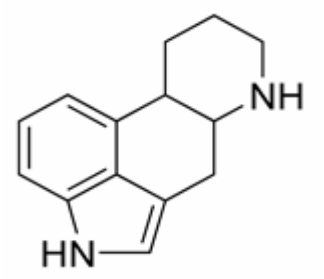


Penicillin -G

Defensins, another group of antibiotics derived from the amino acid pathway, are peptides that act against bacteria. They are found in animals and function to protect organs such as the gingiva where bacterial densities are very high. The first defensin found in fungi has been called plectasin.

Toxins derived from amino acid synthesis include psilocybin (Psilocybe) and Bufotenine (Amanita). These compounds act on nerve impulses, resulting in hallucinations. Amatoxins (Amanita) are cyclic peptides that act on RNA synthesis in all eukaryotic organisms. They are extremely toxic and are particularly dangerous because their effect is evident after 24 to 48 hours after ingestion.

Ergot is synthesized by a *Claviceps purpurea*, a parasitic fungus on grasses and cereals, *C. fusiformis, C. paspali,* and *C. africana*, *C. purpurea* can affect a number of cereals including rye (its most common host), triticale, wheat and barley. It affects oats rarely.

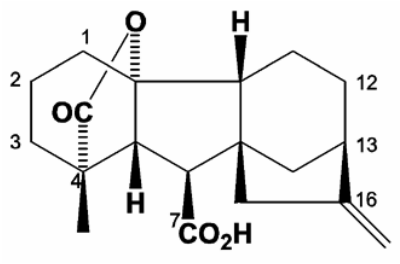


Ergoline

Ergot alkaloids are synthesized from several pathways. Trypotophan from the shikimic acid pathway is attached to an isoprenoid moiety derived from the mevalonate pathway. Several amino acids from primary metabolism are added depending on the final ergot product. Ergot alkaloids are produced as a complex mixture of related compounds. These compounds may function as vasodilators, hormone regulators, and feeding deterrents active in mammals and insects. Their function in the fungi is unclear. Given the importance of amino acids in the synthesis of alkaloids, it follows that nitrogen nutrition of the fungi is important. Carbon/nitrogen balance, carbon source, availability of precursors, and repression by excess of regulators such as ammonium are important. Regulation of other secondary metabolites is as varied as their structures.

Plant Growth Regulator: Gibberellins

Many pathogenic and non-pathogenic fungi produce auxin, cytokinins, gibberellins and abscisic acid. In fact the gibberellins were first found in the fungus *Gibberella fujikuroi,* a pathogen that causes tall, straggly growth of rice. The gibberellins are diterpenes produced by the mevalonate/isoprenoid pathway. The functions of these compounds in fungi that colonise plants seem clear. Modification of host plant tissue would enhance colonisation, release facilitates nutrients and regulate host reproduction. The function of plant growth regulater in fungi is unclear.



Gibberellin

Fungal diseases

Fungi are parasitic on almost all groups of eukaryotic organisms, ranging from cellular amoebae, protozoa, and algae to larger plant groups such as liverworts, mosses, ferns, and seed plants, and animal groups from the smallest inconspicuous ones to the larger animals and humans.

Plant diseases

Fungi, bacteria and viruses mostly cause plant diseases. Serious damage occurs to crops each year by fungal infections of plants such as smuts, rusts, and ergots. Damage to the crops also leads to feed spoilage and timber destruction commonly. Table 2 shows the fungal pathogens causing diseases in plants. Agricultural crops such as groundnut, cotton, and soybeans are affected by contamination of toxins such as aflatoxins, citrinin, ergot akaloids, fumonisins, ochratoxin A, patulin, trichothecenes, and zearalenone etc. World health organization has recommended 20 ppm level of toxins in different agricultural products for human consumption.

Pathogen attack on plants results in numerous host-specific biochemical responses, activation of some of them being critical for the ability of the plant to withstand disease. Among the proteins induced in reponse to infection are proteins involved in protein synthesis, or in protein folding and stabilization, as well as proteins involved in oxidative stress tolerance.

Areas of special interest include (i) specificity and regulation of the host by fungus, (ii) entrance of the fungus into the plant, (iii) mechanism of action of fungal toxins during disease development, (iv) changes in the physiology and biochemistry of the host during diseases, (v) plant resistance mechanisms, (vi) gene expression during host-pathogen interactions.

Animal diseases

Mycotic diseases are caused by fungi, which can affect skin, nails, body hair, internal organs such as the lungs, and body systems such as kidney, liver, the nervous system. Important mycotic diseases include: Aspergillosis, Blastomycosis, Candidiasis, Coccidioidomycosis, Cryptococcosis, Histoplasmosis, Sporotrichosis.

In fact, the first reported animal disease was by the fungus *Beauvera bassiana* that nearly destroyed the silk industry in China by attacking silkworms. Some fungi are restricted to particular animals. The zoophilic (animal loving) dermatophyte *Microsporum gallinae* causes disease on chickens, but not humans. In contrast, the dermatophyte *Microsporum canis* may cause ringworm on both animals and humans. By understanding the epidemiology of the

disease and the disease-causing fungus, epidemics caused by dermatophytes can be predicted and prevented. In domesticated animals, antifungal agents that are used to treat fungal disease in humans are used to treat their infections.

Table 2: Fungal Pathogen causing diseases in plants

Fungal pathogen	**Plant disease**
Phytophthora infestans	Late blight of potato
Bremia lactucae	Downy mildew of lettuce
Aphanomyces cochlioides	Aphanomyces root rot of sugar beet
Synchytrium endobioticum	Black wart of potato
Alternaria solani	Early blight of potato and tomato
Didymella bryoniae (sexual state)	Black rot of pumpkin
Cryphonectria parasitica	Chestnut blight
Claviceps purpurea	Ergot contamination of cream and milk
Colletotrichum lindemuthianum	Bean anthracnose
Gibberella zeae	Head blight of wheat
Fusarium graminearum	Head blight of wheat
Monilinia oxycocci	Cottonball disease/tip blight of cranberry
Erysiphe graminis	Powdery mildew of cereal grasses
Sclerotinia sclerotiorum	Sclerotinia stem rot of Soybean, affecting Tofurkey production
Aspergillus flavus	Aflatoxin contamination cream and milk
Aspergillus parasiticus	Aflatoxin contamination cream and milk
Penicillium digitatum and many other species	Rot of apples and oranges
Trichoderma viride	Green mold disease of mushrooms
Hemileia vastatrix	Coffee rust
Puccinia graminis	Black stem rust of wheat
Ustilago zeae-maydis	Corn smut
Rhizopus nigricans, *Rhizopus tritici*	Soft Rot of sweet potato

Human diseases

Fungal diseases are classified into four types depending on the degree of tissue involvement and mode of entry into the host.

1. **Superficial infection** is localised to the skin, the hair, and the nails e.g. ringworm or tinea, infection of the skin by a dermatophyte caused by *Trycophyton, Microsporum* and *Epidermophyton spp*. Candidiasis typically infects the mouth or vagina. It is part of the normal flora of the vagina and gastrointestinal tract.

2. **Subcutaneous infection** is confined to the dermis, subcutaneous tissue or adjacent structures e.g. sporotrichosis caused by *Sporothrix schenckii*

3. **Systemic infection** is deep infections of the internal organs including respiratory disorders such as histoplasmosis, blastomycosis, coccidiomycosis, paracoccidiodomycosis and allergic aspergillosis.

4. **Opportunistic infection** occurs only in the immunocompromised patients. The diseases include aspergillosis, systemic candidiasis and cryptococcosis.

Table 3 shows the list of pathogenic fungi causing various diseases in humans.

Table 3: Human diseases caused by fungi

Pathogenic Fungi	**Disease in organs**
Absidia corymbifera	Pulmonary, rhinocerebral, cutaneous types of infection
Arthroderma benhamiae	Skin, hair, and nails
Aspergillus flavus	Sinuses, lungs, ear and eye, CNS, dissemination in case of immunosuppression
Aspergillus fumigatus	Sinuses, lungs, CNS, Urinary tract, pre-existing lung cavity, dissemination in case of immunosuppression
Aspergillus niger	Secondary invader following bacterial otitis, lungs in case of immunosuppression
Blastomyces dermatitidis	Lungs, skin, long bones, ribs and vertebrae, central nervous system, thyroid, pericardium, adrenal glands and gastrointestinal tract
Cladophialophora carrionii	Skin, subcutaneous tissues, and nail
Exophiala dermatitidis	Brain infections
Epidermophyton floccosum	Keratinized areas of the body like hair, skin and nails
Histoplasma capsulatum	Lungs are the main site of infection but dissemination to the liver, heart and central nervous system
Paecilomyces variotii	Peritoneal dialysate (after using continuous ambulatory peritoneal dialysis)
Paracoccidioides brasiliensis	Lungs, mucosa of the mouth and nose, and neighboring teguments, with frequent spread to the lymph nodes, adrenal glands, and other viscera
Penicillium marneffei	Lungs, lymphatic system, liver, spleen and bones are usually involved.
Pneumocystis carinii	lung infection individuals with weakened immune systems
Pseudallescheria boydii	Lung and brain
Scedosporium apiospermum	External ear, lung (in patients with poorly draining bronchi or paranasal sinuses) and "fungus ball" formation in pre-formed cavities.

Sporothrix schenckii	Skin, hair, and nails.
Candida albicans	Skin, surfaces, buccal mucosa, intestinal tract and vagina mucosa.
Candida krusei	Skin, nails and gastrointestinal, respiratory and urinary tracts (patients with granulocytopenia)
Candida parapsilosis	Blood stream (patients with malignancy)
Candida pelliculos	Pancreas
Geotrichum candidum	Lung, oral, vaginal, cutaneous and alimentary infections
Cryptococcus neoformans	Lung and brain
Schizophyllum commune	Lungs and sinus
Trichosporon	Lungs, kidneys, and spleen (in immunocompromised patients) and superficial infections
Rhodotorula rubra	Skin, lungs, urine and peritoneum (patients on continuous ambulatory peritoneal dialysis (CAPD)
Mucor circinelloides	Mucocutaneous and rhinocerebral infections, septic arthritis, dialysis-associated peritonitis, renal infections, gastritis and pulmonary infections (immunocompromised hosts)
Rhizopus oryzae	Rhinocerebral forms of infection.

Fungal hypersensitivity

First case of fungal sensitivity was reported as early as 1726 by Floyer. In 1925, Van Leeuwen reported the spores of some saprophytic fungi like *Mucor*, *Penicillium* and *Aspergillus* as the cause of the most common allergic disorder called asthma.It is estimated that over 20% of the world population suffers from IgE-mediated allergic diseases, such as allergic asthma, allergic rhinitis and allergic conjunctivitis, atopic eczema/atopic dermatitis, and anaphylaxis. The prevalence of respiratory allergies due to fungi is imprecisely known but is estimated at 20 to 30% of atopic (allergy- predisposed) individuals or up to 6% of the general population. Complex immunological interactions and responses in fungal allergies cause morbidity and mortality in immunocompetent as well as in immunocompromised hosts. Allergic disease is an inflammatory disease with eosinophilia. The primary characteristic feature of asthmatic individuals is episodic reversible airway obstruction that manifests as periodic cough, and tightness in chest.

Fungal allergens

In recent years, considerable attention has been paid in obtaining purified relevant allergens from fungi associated with allergy. Using molecular biology techniques, a number of mold allergens have been obtained by cloning the genes encoding the allergens. Currently, about 89 out of total 502 allergens approved by the International Allergen Nomenclature Committee are fungal allergens (Table 4). However, not many of these allergens have been analysed for their diagnostic specificity and sensitivity.

Diagnosis of allergic disease is mainly based on clinical symptoms of the patients, mycologic culture and direct microscopic examination, skin test reaction, detection of total IgE in serum, allergen-specific serum IgE antibodies (radioallergosorbent test-RAST, ELISA), allergen

specific serum IgG antibodies and in some cases provocative inhalation challenge test. The process of reaching the diagnosis of allergic fungal rhinosinusitis and allergic bronchopulmonary aspergillosis may require the CT and histopathologic examination.

Table 4: Important Fungal allergens

Class	**Genus**	**Allergen**	**Protein name**	**Mol wt**
Ascomycota Dothideales	**Alternaria alternata**	Alt a 1		28
		Alt a 3	heat shock prot. 70	
		Alt a 4	prot. disulfideisomerase	57
		Alt a 5	acid ribosomal prot. P2	11
		Alt a 6	enolase	45
		Alt a 7	YCP4 protein	22
		Alt a 8	mannitol dehydrogenase	29
		Alt a 10	aldehyde dehydrogenase	53
		Alt a 12	acid ribosomal prot. P1	11
		Alt a 13	glutathione-S-transferase	26
	Cladosporium herbarum	Cla h 2	Ag54	23
		Cla h 5	acid ribosomal prot. P2	11
		Cla h 6	enolase	46
		Cla h 7	YCP4 protein	22
		Cla h 8	mannitol dehydrogenase	28
		Cla h 9	vacuolar serine protease	55
		Cla h 10	aldehyde dehydrogenase	53
		Cla h 12	acid ribosomal prot. P1	11
Ascomycota Eurotiales	Aspergillus fumigatus	Asp f 1		18
		Asp f 2		37
		Asp f 3	peroxisomal protein	19
		Asp f 4		30
		Asp f 5	metalloprotease	40
		Asp f 6	Mn superoxide dismut.	26.5
		Asp f 7		12
		Asp f 8	ribosomal prot. P2	11
		Asp f 9		34
		Asp f 10	aspartic protease	34
		Asp f 11	peptidyl-prolyl isomeras	24
		Asp f 12	heat shock prot. P90	90
		Asp f 13	alkaline serine protease	34
		Asp f 15		16
		Asp f 16		43
	Aspergillus niger	Asp n 14	beta-xylosidase	105

		Asp n 18	vacuolar serine protease	34
		Asp n 25	3-phytase B	66-100
		Asp n ?		85
	Penicillium chrysogenum (formerly *P. notatum*)	Pen ch 13	alkaline serine protease	34
		Pen ch 18	vacuolar serine protease	32
		Pen ch 20	N-acetyl glucosaminidas	68
	Penicillium citrinum	Pen c 3	peroxisomal mem. prot.	
		Pen c 13	alkaline serine protease	
		Pen c 19	heat shock prot. P70	
		Pen c 22w	enolase	
		Pen c 24	elongation factor 1 beta	
Basidiomycotina Urediniomycetes	Malassezia furfur	Mala f 2	MF1, peroxisomal	21
			membrane protein	
		Mala f 3	MF2, peroxisomal	20
			membrane protein	
		Mala f 4	mitochondrial malate dehydrogenase	35
	Malassezia sympodialis	Mala s 1		
		Mala s 5		18
		Mala s 10	heat shock prot. 70	86
		Mala s 11	Mn superoxide dismut.	23
		Mala s 12	glucose-methanol-choline (GMC) oxidoreductase	67
		Mala s 13	thioredoxin	12

The characterization of specific immunoglobulin E (IgE) inducing allergens is fundamental for clinical diagnosis and also for immunotherapy. Hyposensitization is a form of immunotherapy where the patient is gradually vaccinated against progressively larger doses of the allergen to reduce severity or eliminate hypersensitivity. While current treatment strategies are limited for allergic diseases (in controlling allergic response with pharmacological management), recent technological advances in immunotherapy may soon provide better therapeutics for allergic diseases. Immunotherapy may permanently modify underlying inflammatory allergic immune responses with protective allergen with long-term alleviation of symptoms and minimal side–effects.

Some of the important developments in immunotherapy of IgE-mediated allergy include peptide immunotherapy, immunotherapy using immunostimulatory sequences (ISS-ODN), and naked DNA vaccination.

Systemic infections and invasive disease

Fungal infections have recently emerged as a growing threat to human health, especially in persons whose immune systems are compromised in some way. For example, fungi are associated with complex disease entities in complex medical patients, e.g. cryptococcosis in AIDS patients, or aspergillosis in bone marrow or organ transplant patients. In the absence of adequate host immunity, the opportunistic fungi disseminate to other organs causing a systemic and fatal form of disease which is a serious concern for immuno-suppressed patients undergoing organ transplants, chemotherapy or suffering from AIDS. Despite advances in early diagnosis and new antifungal agents, the majority of cases of these patients remain undiagnosed and untreated with associated mortality rates as high as 90%.

The drugs available for invasive aspergillosis (polyenes, azoles, echinocandins, polyoxins and nikkomycins) target cell wall molecules (ergosterol, β-1, 3 glucan, chitin) or molecules involved in their synthesis but have serious limitations of resistance development in pathogen and toxicity in host.

Antifungal agents

Significant antifungal chemotherapy began in 1903, with the successful use of potassium iodide (KI) for the treatment of sporotrichosis. There was little progress for the next 50 years until nystatin, the first useful polyene, was introduced in 1951. This was soon followed by amphotericin B in 1956, still the standard against which new systemic antifungals are compared. Since the 1950s, antifungal drug discovery has identified three classes of natural products (griseofulvin, polyenes and echinocandins) and four classes of synthetic chemicals (allylamines, azoles, flucytosine and phenylmorpholines), which are effective against fungal infections (Table 5). .

Amphotericin B, a polyene, is used as a first line of drug for the treatment of systemic fungal diseases. It binds to ergosterol in the cell wall leading to pore formation resulting in leakage of potassium from the cell and possibly initiation of a lipid peroxidation cascade, which irreversibly damages the cell wall of fungi.

Infectious fungi, being eukaryotic (primitive) pose problems in identification of specific drug targets against them. There is a relative shortage of antifungal agents for treating life-threatening mycoses as there are limitations with the antifungal agents. Discovery of new antifungal targets and the development of effective antifungal drugs form an important area of fungal research .Amphotericin B has been nearly the unique available therapy for invasive mycoses for more than four decades

Recent advances in yeast functional genomics and proteomic studies are changing the field of fungal research. Many of these new technologies are readily applicable to drug target identification and various aspects of drug discovery. Most successful drugs or drug targets probably will come from programs that integrate various aspects of genomics and proteomics.

Table 5: Anti-fungal drugs and their mode of action

Antifungal drugs	Spectrum of activity	Mode of action
Natural		
Polyenes: amphotericin B, nystatin	Both yeast and filamentous forms	Membrane disrupting agents
Griseofulvin	Scalp ringworms	Anti-mitotic (spindle disruption)
Echinocandins : Caspofungin, anidulafungin, micafungin	Candidiasis, Aspergillosis and other less common fungal infections	Glucan synthesis inhibitors
Synthetic		
Allylamines : Terbinafine, butenafine	Aspergillus spp., Fusarium spp. and other filamentous fungi	Ergosterol synthesis inhibitors
Azoles Imidazoles: Ketoconazole, Triazoles: Fluconazole, Itraconazole, Voriconazole, Posaconazole, Ravuconazole	Ketoconazole and itraconazole are effective in patients with the chronic, indolent forms of the endemic mycoses, including blastomycosis, coccidioidomycosis, and histoplasmosis. Itraconazole is effective in patients with sporotrichosis. Fluconazole is useful in the common forms of fungal meningitis, namely, coccidioidal and cryptococcal meningitis.	Ergosterol synthesis inhibitors
Flucytosine	Candidiasis, Cryptococcosis and Chromomycosis, and is affected by native resistance, and the development of resistance on therapy	Nucleic acid inhibitor
Phenylmorpholines : Amorolfine	Used in agriculture	Ergosterol synthesis inhibitors

Genomics and proteomics applications

Genomics

21st century has seen advancement of science in terms of knowledge on human and microbial genomics. Fungi are known to have numerous secondary metabolic pathways with biotechnological applications and pharmacological properties. Genome sequencing of *Saccharomyces cerevisiae*, *Neurospora crassa*, *Magnaporthe grisea*, *Fusarium graminearum, Ashbya gossypii*, *Aspergillus nidulans, Aspergillus oryzae, Ustilago maydis, Candida tropicalis* and *Aspergillus fumigatus* have been completed whereas genome sequencing for *Coccidiodes, Cryptococcus neoformans, Histoplasma, Paracoccidiodes, Chaetomium globosum, Trichophyton, Rhizopus oryzae, Aspergillus flavus* etc. are under various stages (Table 6). Comparison of sequences from one genome to another and correlating the differences with physiological and functional properties of microbes such as

pathogenicity or production of drugs may enable the identification of relevant genes of interest. Genomic information of *S. cerevisiae* predicted 5,916 open reading frames (ORFs), which express protein sequences. These ORFs revealed that a total of 1,105 (18.7%) genes were essential for the growth of the fungus. About half of these were previously known to be essential. 17% of non-essential ORFs and 9% of the essential ORFs encode new proteins. Essential genes were observed to have homologues in other organisms such as *N. crassa, A. nidulans* etc (82% of the essential genes and 67% of the non-essential genes and their gene products are similar to proteins in organisms). Species of *Aspergilli* are important due to its medical and industrial significance. *A. terreus* is a major source of lovastatin used in the treatment of hypercholesterolemia and secondary metabolites such as patulin, citrinin, isocitrinin, asteroquinone and a commercially important enzyme xylanase. *A. niger* is used for the production of citric acid, enzymes, and for heterologous expression of various proteins. Enzymes such as xylanases and mannanases are of commercial value as agents in the pulp and paper industry. A metabolic network of *A. niger* covering 284 metabolites and 335 reactions has been reconstructed with the available genomic and biochemical data. Genome information of Aspergillus *flavus* has revealed genes involved in aflatoxin production, signal transduction and pathogenicity etc.

Table 6: Fungal genomes

Species	**Strain**	**Estimated genome size (Mb/ no of chromosomes/appro. no of genes)**	**GenBank Accession**
S. cerevisiae	S288C	**13/16/5406**	NC_001143
Aspergillus nidulans	FGSC #A4	31/8/11,000	AACD01000000
Fusarium graminearum	PH-1	40	AACM01000000
Magnaporthe grisea	70-15	40	AACU01000000
Neurospora crassa	74-OR23-IVA	40/7/11,000	AABX01000000
Ustilago maydis	521	20	AACP01000000
Aspergillus fumigatus	Af293	30/8/9926	
Candida guilliermondii	ATCC 6260	12	AAFM01000000
Candida lusitaniae	ATCC 42720	16	AAFT01000000
Candida tropicalis	MYA-3404	30	AAFN01000000
Chaetomium globosum	CBS 148.51	36	AAFU01000000
Coccidiodes immitis	KS	29	AAEC01000000
Coprinus cinereus	Okayama 7	38	AACS01000000
Cryptococcus neoformans, serotype A	H99	20/12-13/6000	AACO01000000
Cryptococcus neoformans, serotype B	R265 (outbreak strain	20	AAFP01000000
Rhizopus oryzae	RA99-880	40	AACW01000000
Saccharomyces cerevisiae	RM11-1a	12.2	AAEG01000000
Stagonospora nodorum	SN15	37.1	AAGI00000000
Aspergillus orzyae		37/8/12,074	

Comparative genomics of Aspergillus species and evolution

Differences in the genome sequences among various species of *Aspergili*, provide a wealth of information about the evolution of this fascinating group of organisms. The beneficial and detrimental characteristics of fungi are revealed through comparative genomics of *Aspergillus* species. The genome of *Aspergillus oryzae* has seven to nine megabases more DNA than *A. fumigatus* and *A. nidulans*. It was proposed that some genes were transferred to *A. oryzae* from other species during evolution. The extra DNA stretches are dispersed throughout the genome and are enriched in genes involved in the synthesis and the transport of numerous secondary metabolites. Species closely related to *A. oryzae*, such as *A. flavus* and *A.niger*, have similar gene acquisitions. For instance, the toxic *A. flavus* has 25 genes encoding proteins involved in the pathway that produces the poisonous 'aflatoxin'. These genes are present in *A. oryzae* but are not expressed. It is likely that an ancestor of *A. flavus* passed these genes to *A. oryzae*, and these genes were then inactivated during the subsequent evolution of *A. oryzae*.

A. nidulans genome was crucial for the comparative analysis of the three aspergilli, with the identification of putative binding sites for gene regulatory factors and control elements, as well as many ORFs that lie upstream of genes. These short sequences may stall the expression of neighboring genes. In addition, the sequence disclosed many previously unknown genes involved in metabolic (fatty-acid oxidation), developmental (polarized growth) and DNA-repair pathways.

The sequencing of genomes of other *Aspergilli* is under way and will provide an even broader perspective on the biology and evolution of these fungi. The most keenly anticipated *Aspergillus* sequence is that of *A. niger*, which has long been used in the industrial production of citric acid.

Microarray technology applications and advancements

Use of microarray technology approach has led to identification of molecular and biochemical mechanisms of pathogenic fungi such as *Candida albicans*, in an environment of nutrients, oxygen and challenges to host defense. Efficient comparison of the genomes of unrelated strains of yeast and examination of gene expression of the pathogens grown under a variety of different conditions became possible through this technology. Identification of genes involved in the cell cycle regulation is also a contribution of microarray technology. Up-regulation of stress response (heat shock protein, pH responsive glycosyl transferase II), antioxidative response (thioredoxine reductase I, catalase, copper magnesium super-oxide dismutase), glyoxylate cycle (isocitrate lyase, malate synthase, malate dehydrogenase, acetyl-coenzyme-A-synthetase) and putative virulence related genes (hyphal wall protein 1, cell elongation protein 1, hyphal-regulated protein 1, secretory aspartic proteinase gene, phospholipase B family) have been observed during stage specific gene expression of *C. albicans* in human blood.

The powerful analytical micro array technique involves preparation of artificially constructed grids of DNA, such that each element of the grid probes for a specific mRNA sequence. That is, each holds a DNA sequence that is a reverse complement to the target mRNA sequence. The basic technique involves extraction of RNA from biological samples either in normal or interventional states. The mRNA is then copied, while incorporating either fluorescent nucleotides or a tag, which is later probed with a fluorescent dye. The labeled RNA is then hybridized to a microarray for a period of time, after which the excess is washed off and the

microarray is scanned under laser light. With oligonucleotide microarray, probes have been designed to be theoretically similar with regard to hybridization temperature for a single sample and provide an absolute measurement level for each RNA molecule. With cDNA microarray, for which each probe has its own hybridization characteristic, each microarray measures two samples, and provides a relative measurement level for each RNA molecule. Scanned image of Microarray slide of *Aspergillus fumigatus* is shown in the Fig. 12. Regardless of the technique, the end results are 4,000-50,000 genes, which are differentially expressed per biological sample.

Currently, genome sequences for more than 800 organisms are available in international repositories. However, the biological function of most of these genes remains unknown, or has been predicted only through homology to genes with known functions. The functions of these genes can be identified through repeated measurement of their RNA transcript.

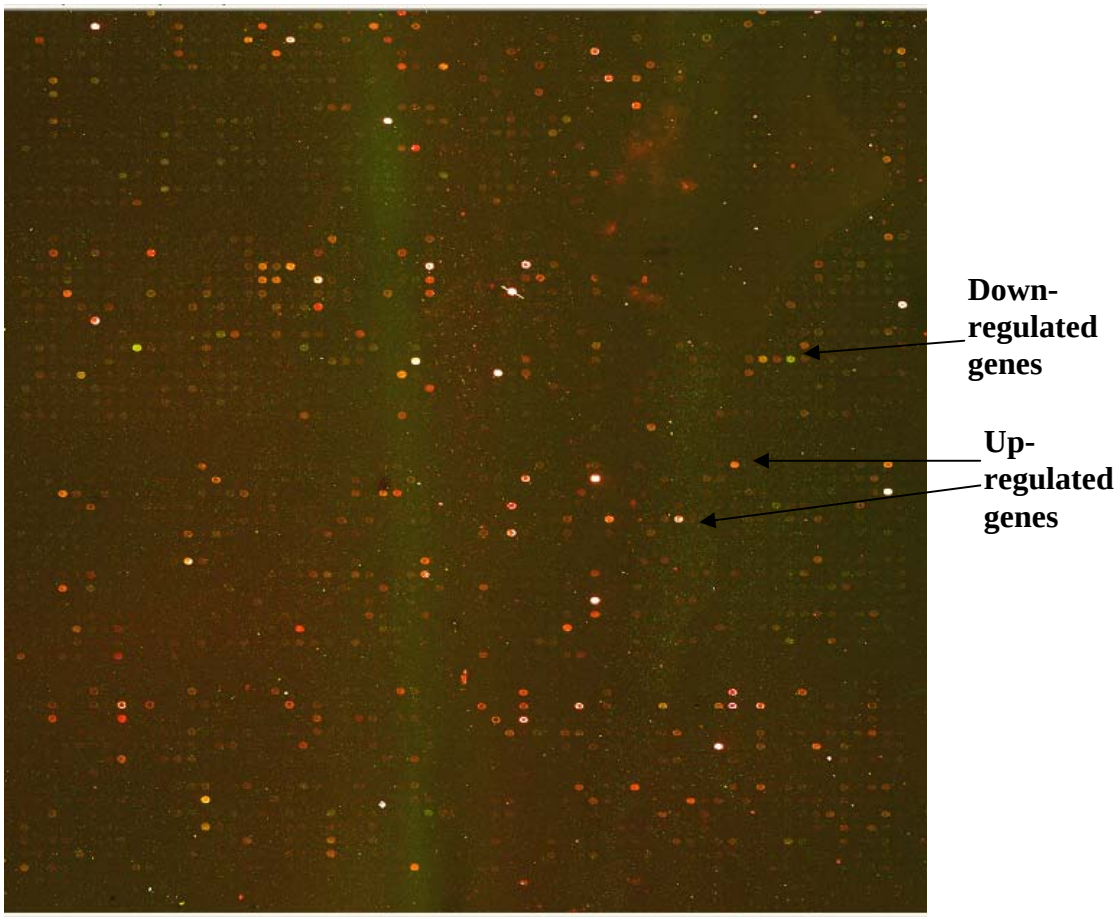
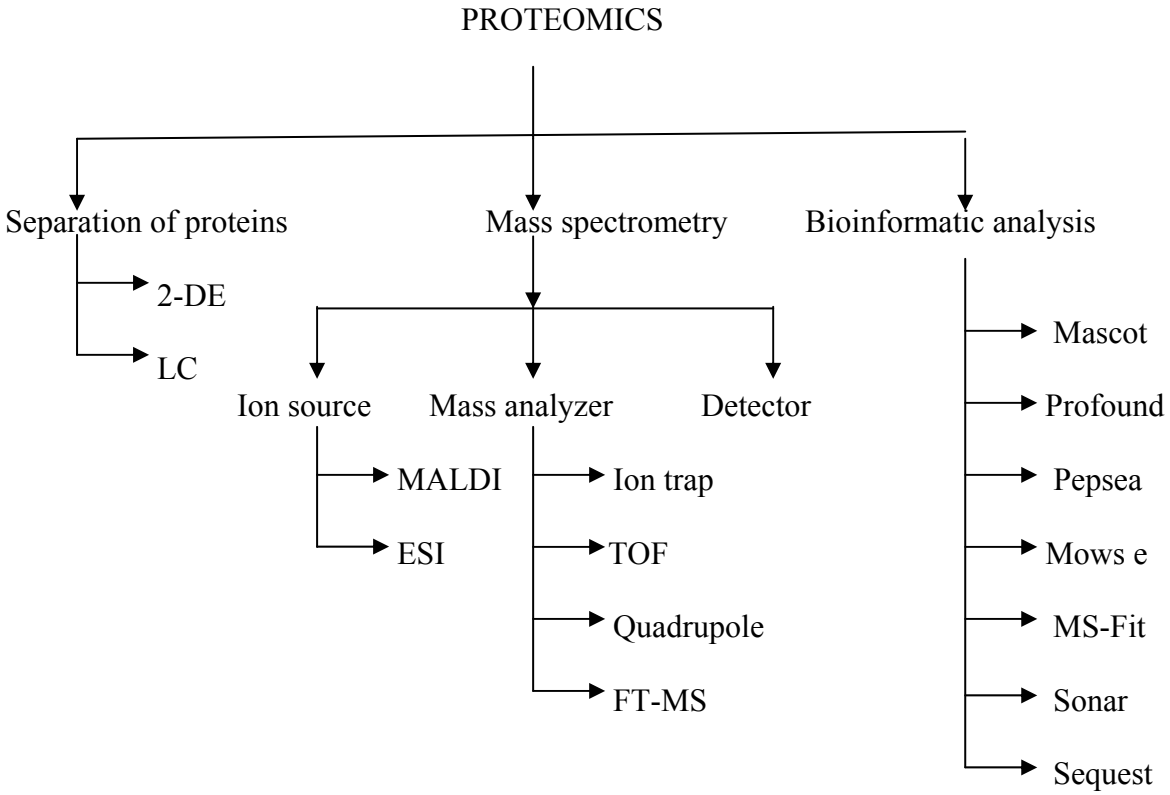


Fig 12: Image scan of differentially expressed genes of *A. fumigatus* after Amphotericin B treatment. [Slide represents the hybridization of samples (Conidia treated with and without antifungal drug Amphotericin B. Treated sample was labeled with biotin (RED) and untreated sample was labeled with fluorescein (GREEN). Spots appeared in red color are up regulated, spots in green are down-regulated and yellow spots indicate the co-expression in both samples. White spots in the microarray slide indicate that the probe is saturated with cDNA labeled with biotin (RED). Intensity of red spots decides level of up-regulation of genes].

Proteomic studies in fungi

Proteomics has been used for studying fungal biology, evolutionary aspects, use in industrial processing and understanding of fungal pathogenesis in plants as well as animals including humans. The results of proteome studies on few fungi, both non-pathogenic and pathogenic such as *Saccharomyces cereviseae* (non-pathogenic), *Candida albicans* (human pathogen) are available on SWISSPROT database. Comparative analysis of various pathogenic and non-pathogenic fungi is being carried out to understand the mechanism of pathogenesis. Most of such studies are on *S. cereviseae* (non-pathogenic) and *Candida albicans* (pathogenic). Based on such studies it is possible to formulate a variety of exciting new strategies to address biological questions. This may lead to creation of a predictive and personalized approach to medicine.

Marc Wilkins coined the term proteomics in 1994. He defined it as "the study of proteins, how they are modified, when and where they are expressed, how they are involved in metabolic pathways and how they interact with one another can be identified by the qualitative and quantitative comparisons". The technique involves separation of proteins, mass spectrometry and bioinformatics analysis (Fig. 13). Proteome is an entirety of proteins in existence in an organism throughout its life cycle, or on a smaller scale the entirety of proteins found in a particular cell type under a particular type of stimulation.



2-DE	Two-dimensional gel electrophoresis
LC	Liquid chromatography
MALDI	Matrix assisted laser desorption/ionization
ESI	Electrospray ionization
TOF	Time of flight
FT-MS	Fourier transform-ion cyclotron

Fig. 13: Various steps in proteomic analysis

First step in Proteomic analysis is separation of proteins, followed by mass spectrometry and bioinformatic analysis. Two-dimensional electrophoresis (2-DE) separates proteins based on their sizes, and their individual unique isoelectric points (Fig. 14). The most useful application of 2-DE is to resolve protein isoforms of the same size, or proteins that have undergone various post-translational modifications such as phosohorylation and glycosylation.

The proteins resolved by 2-DE are detected by silver staining and Coomasie blue staining. After 2-D electrophoresis and protein visulization by staining, images of gels are subjected to analysis by special image analysis software (e.g. Image Master from Amersham Biosciences or PDQUEST from BioRad).

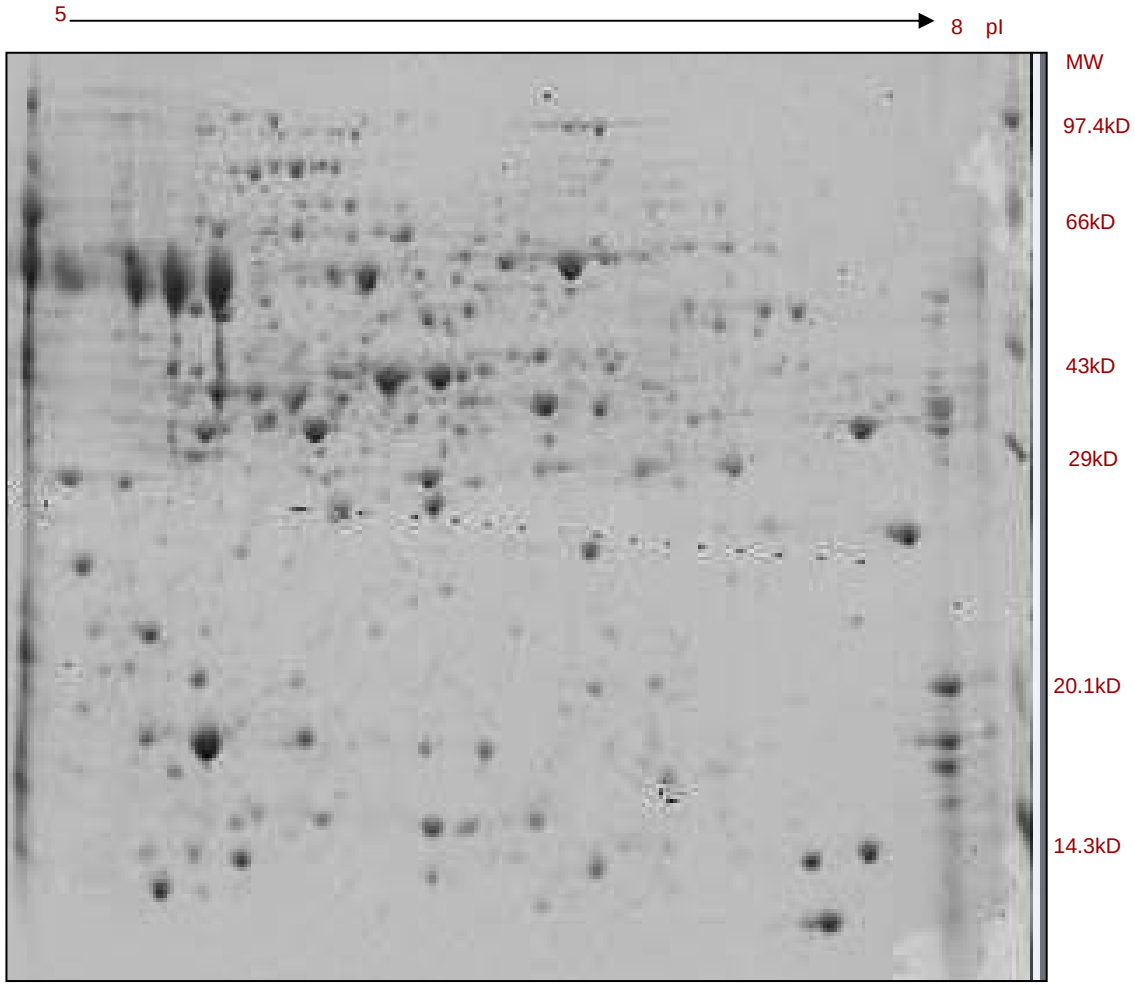


Fig 14: 2-D gel of third week cultural filtrate Antigens of *Aspergillus fumigatus*

The next step involves protein identification. The protein spots are excised and in-gel digested with an enzyme (e.g. trypsin or chymotrypsin) and the digest is then applied onto a sample plate and coated with matrix. The plate is then put into the MALDI-TOF mass spectrometer to obtain mass spectra.

Proteins are usually identified by peptide mass fingerprinting (PMF) and database searching. In PMF, the peptide masses of unknown proteins are compared to the predicted masses of

peptides from the theoretical digestion of proteins from a database. Several software packages are available on Internet to perform database matching such as MASCOT, ProFound and Protein Prospector.

Biotechnological application of fungi

White biotechnology /Industrial biotechnology

The beginning of the 21st century brought the use of about 20 products of fungi in biotechnology. These products are useful in industrial fermentation, food processing, papermaking etc. Single cell protein source of food for cattle feed, drugs or medicine such as ergot alkaloids (ergotamine; ergomatrine; ergocornine), antimicrobials (penicillin, cephalosporins antifungals) and vitamin, and steroids are few examples. With the advent of molecular biology techniques, a number of genes of industrially important enzymes have been cloned and expressed in order to improve the production of enzymes with desired qualities.

Fungal enzymes

Fungal enzymes play an important role in the breakdown of organic materials; many of these enzymes are now produced commercially and are used in food processing (Table 7). **Cellulase** enzymes have applications in textile industry and biopolishing, the removal of fuzz from the surface of cellulosic fibres to eliminate pilling making the fabrics smoother and cleaner-looking.

Table 7: Industrially important enzymes and applications

Enzyme	Main Source	Application
CARBOHYDRATES		
Alpha-Amylase	*Aspergillus niger,*	Textiles, starch syrup, laundry, dishwashing detergents, paper desizing, fermentation ethanol, animal feed
	Aspergillus oryzae	
ß-Glucanase	*Aspergillus niger*	Brewing industry
	Penicillium emersonii	
	T. reesei, T. viride	
Dextranase	*Penicillium spp.*	Hydrolysis of dextran
Glucoamylase	*Aspergillus niger,*	Manufacture of dextrose syrup and high fructose syrup
	Aspergillus oryzae	
	Rhizopus	
	Endomyces	
Cellulase and Hemicellulase	*Aspergillus niger, Aspergillus oryzae*	Baking, fruit juice manufacture, wood pulp processing
	T. reesei, T. viride	
	P. emersonii	
Xylanase	*Aspergillus niger*	Baking,fruit juice manufacture,

	T. reesei	wood pulp processing
Lactase	*Kleyveromyces lactis*	Lactose elimination from dairy product
	Aspergillus oryzae	
Pectinase	*Aspergillus niger*	Fruit processing
	Rhizopus oryzae	
PROTEASES		
Acid proteinase	*Aspergillus niger, Aspergillus oryzae*	Improvement of dough handling
Alkaline protease	*Aspergillus niger*	Detergents, leather, fur
LIPASES		
Lipases	*Aspergillus niger, Aspergillus oryzae*	
Lysozyme	*Saccharomyces cereviseae*	Antibacterial , germicidal activity
	Pichia pastoris	
OXIDOREDUCTASE		
Alcohol dehydragenase	*Saccharomyces cereviseae*	
Catalase	*Aspergillus niger,*	
	Penicillium spp.	

(Source: Report by Biotechnology Industry Organization's (BIO's) Industrial and Environmental Section on New Biotech Tools for Cleaner Environment)

Fungi are excellent source of several antibiotics. Cyclosporin was first isolated from *Tolypocladium inflatum* in 1976 as antifungal compound and later shown to possess immunosuppressive activity. Cyclosporin A is currently the most widely used drug for preventing rejection of human organ transplants.

The polysaccharides chitin and its derivative chitosan have a wide range of applications(e.g. for cosmetic, medical and pharmaceutical applications). Filamentous fungi grown under controlled conditions are an attractive source of chitin and chitosan where a high-quality product is required.

Use of fungi in food application

Brewing and baking have been carried out for centuries and both are dependant on the conversion of sugar into alcohol and carbon dioxide by yeasts. In bread making carbon dioxide causes the dough to rise giving lightness to the bread whilst the alcohol is driven off during baking. Cheese production also has ancient origins. The growth of the mould *Penicillium roqueforti* in the body of blue-veined cheeses and the surface growth of the moulds *Penicillium candidum, Penicillium caseicolum*, or *Penicillium camemberti* on Camembert, Brie and related types of cheeses play an important role in the development of the characteristic flavours of these cheeses. Traditional fermentations could take several months but this has been reduced to 2-3 days in a modern plant. The principal fungus

involved, *Aspergillus oryzae*, is now also used to produce a range of commercially important enzymes.

A recent innovation in food technology has been the development of Quorn myco-protein from a filamentous fungus *Fusarium graminearum*. Quorn is produced from mycelia grown in large fermenters. The filamentous nature of the biomass is responsible for the meat-like texture and appearance of the final product.

Biocontrol and bioremediation by fungi

Biocontrol

The use of fungi as biocontrol agents to kill insects (mycoinsecticides) and weeds (mycoherbicides) has the potential to replace many of the toxic chemicals currently in use. Several species of fungi have now been commercially formulated as mycoinsecticides. Spores of *Metarhizium anisopliae* were first used in Russia in the late 1800s as a mycoinsecticide.

Main areas in which parasitic fungi are beneficial to man are in the use of fungi to control plant diseases, insects, nematodes and weeds.

a. Fungi to Control Plant Diseases
The yeast, *Pichia inositovora* and *P. acaciae*, have been shown to produce antifungal cytotoxic proteins that will inhibit the growth of a number of other yeasts. Table 8 comprises plant diseases, infectitivity and antifungal compounds used to combat them.

b. Biocontrol of Insects
Louis Pasteur was one of the first to suggest that microorganisms could be used to control insect pests. Extensive research on the use of *Beauvaria* to control **chinch bugs** had mixed success. Several major groups of fungi have entomogenous species, which grow on insects. Many of them have been used as mycoinsecticides e.g. are Beauveria bassiana, B. brongniartii, Metarhizium anisopliae, Verticillium lecanii and Hirsutella thompsoni. Beauveria bassiana is marketed under the name "BotaniGard ES." It is effective on whiteflies, thrips, aphids, and other soft-bodied sucking insects.

c. Biocontrol of Nematodes
Species of *Dactyella* and *Arthrobotrys* are well known as nematode trapping fungi. They have peculiar nets, constricting rings, knobs that trap nematodes. Once the hyphae trap a nematode, it will invade the body cavity, resulting in death.

d. Biocontrol of Weeds
There are about 30,000 species of plants that are considered to be weeds and are directly or indirectly harmful to humans and animals. Mycoherbicides have advantages over chemical herbicides in that they can be more host-specific, preparation costs will be less expensive, and human health hazards can be eliminated. There have been some exciting breakthroughs in recent years. A number of mycoherbicides including *Alternaria cassiae* to control sicklepod and *Fusarium* to control the velvetleaf weed, both major weed problems in soybean fields.

Table 8: Fungi as Biocontrol agents for plant diseases

Plant disease	**Plant pathogen**	**Fungi used as Biocontrol agent**	**Antifungal compound used / mode of action**
Spot blotch of Wheat, Ascochyta blight (chickpea)	*Drechslera sorokiniana* and *Bipolaris sorokiniana*	*Chaetomium globosum, Trichoderma viride, Acremonium implicatum*	fungal antagonists
Rice blast and wheat leaf rust	*Magnaporthe grisea* (wheat), *Puccinia recondite* (rice)	*Chaetomium globosum*	fungal antagonists
Powder mildew of wheat, tomato, grape, tree fruits, strawberry etc.	*Blumeria graminis* (wheat), *Erysiphe necator* (grape)	*Ampelomyces quisqualis*	fungal antagonists
Root rot, seedling rot, damping off of cotton, chicken pea, pigeon pea, Bengal gram, groundt nut, soyabean, tobacco and other veg.	*Colletotrichum acutatum and Botrytis cinerea* etc.	*Trichoderma viride, T. harzianum, Trichoderma atroviride*	fungal antagonists
Diseases of strawberry and other citrus fruits	*Botrytis cinerea*.	*Candida fructus, Candida glabrata* and *Candida oleophila*	fungal antagonists
Chestnut blight	*Cryphonectria parasitica*.	*Cryphonectria parasitica*	Vegetative competitive strain (Hypovirulent strains)
Root rot of Conifers (pine)	*Heterobasidium annosum*	*Phlebia gigantean*	Competes for food base
Post-harvest rot of citrus fruit	*Penicillium*, commonly *P. chrysogenum*	*Pichia guillermondii*	Fungal Antagonist
Grey and blue mould of apple	Botrytis cinerea and Penicillium expansum	Cryptococcus albidus	Antagonist
Damping off of cotton	Pythium ultimum and Rhizoctonia solani	Gliocladium virens and Gliocladium catenulatum	Gliovirin, Gliotoxin

A close-up of a thistle leaf shows yellowing and death caused by the rust and recently inoculated leaves bear numerous rust lesions. The mycoherbicide “Divine” by Abbott Labs incorporates *P. palmivora* in the biocontrol of this weed pest.

"Collego" is a new mycoherbicide that utilizes *Colletotrichum gloeosporoides* to control jointvetch where in rice fields, chemical control presents a real problem. A new fungus *Phomopsis amaranthicola* is used to control pigweed; *Dactylaria higginsii* to control purple sedge; *Dreschlera/Exerohilum* cocktail to control various grasses; *Bipolaris saccharii* to control cogon grass; and *Uromycicola tepperianium* to control invading locust trees.

Fusarium oxysporum, specific to marijuana is being tested to control this drug plant. *Trichoderma atroviride* has a natural ability to parasitise phytopathogenic fungi such as *Rhizoctonia solani* and *Botrytis cinerea*, therefore providing an environmentally sound alternative to chemical fungicides in the management of these pathogens. Identified up-regulated proteins include known fungal cell wall-degrading enzymes such as N-acetyl-beta-d -glucosaminidase and 42-kDa endochitinase. Three novel proteases of *T. atroviride* were identified, containing sequence similarity to vacuolar serine protease, vacuolar protease A and a trypsin-like protease. Several cell wall-degrading enzymes were identified from the *T. atroviride* culture supernatant, providing further evidence that a cellular response indicative of biological control had occurred.

Bioremediation

Fungi have been very useful to human beings and environment. Transformation of various toxic chemicals to less harmful forms by fungi leads to control of environmental pollutants. General approaches to bioremediation are to enhance natural biodegradation by native organisms (intrinsic bioremediation), to carry out environmental modification by applying nutrients or aeration (biostimulation) or through addition of microorganisms (bioaugmentation). Bioremediation is a cost effective process and can be carried out on-site and is limited in the number of toxic materials it can handle. Fungi are used in the accumulation of heavy metals such as cadmium, copper, mercury, lead and zinc. Systems using *Rhizopus arrhizus* have been developed for treating uranium and thorium.

A variety of extracellular enzymes of fungi like basidiomycetous fungi can decompose lignocellulose, the most important degradative step in the carbon cycle of earth. The role of fungi in the degradation of complex carbon compounds such as starch, cellulose, pectin, lignin, lignocellulose, inulin, xylan, araban etc. is well established. *Trichoderma reesei* is known to possess the complete set of enzymes required to breakdown cellulose to glucose. Degradation of lignocellulose is the characteristic of several basidiomycetous fungi.

The ability of fungi to transform a wide variety of hazardous chemicals arouses interest in using them in bioremediation. The white rot fungi are unique among eukaryotes for having evolved for the degradation of lignin; curiously they do not use lignin as a carbon source for their growth. *Phanerochaete chrysosporium, P. sordida* and *Tramates hirsute* are three fungi that degrade a number of toxic xenobiotics such as aromatic hydrocarbons (benzo alpha pyrene, phenanthrene, pyrene) chlorinated organics (alkyl halide insecticides, chloroanilines, DDT, pentachlorophenols, trichlorophenol, polychlorinated biphenyls, trichlorophenoxyacetic acid), nitrogen aromatics (2,4-dinitrotoluene, 2,4,6-trinitrotoluene-TNT) and several miscellaneous compounds such as sulfonated azodyes. Several enzymes, which are released such as laccases, polyphenol oxidases, lignin peroxidases etc. play a role in the degradative process. In addition, a variety of intracellular enzymes such as reductases, methyl transferases and cytochrome oxygenases are known to play a role in xenobiotic degradation.

Phanerochaete chrysosporium has been shown to affect the biobleaching of organic dyes, which decolorizes azo-triphenyl methane dyes. Lignin peroxidase and manganese peroxidase from *P. chrysosporium* decolorizes olive mill waste water. *Phanerochaete chrysosporium* and microbial consortia were also shown effective in color removal from textile dye effluent.

The basidiomycetous fungus *Pleurotus ostreatus* has been shown to produce an extracellular hydrogen peroxide dependent lignolytic enzyme, removing the color due to remozol brilliant blue. Oxidative enzymes play a very major role in biodegradation. Other fungi, which can be used in bioremediation, are members of Zygomycetes. Aquatic fungi and anaerobic fungi are the other candidates for bioremediation.

Among other fungi used in bioremediation, the yeasts, e.g., *Candida tropicalis*, *Saccharomyces cerevisiae, S. carlbergensis* and *Candida utilis* are important in clearing industrial effluents of unwanted chemicals. *Agaricus bisporus* and *Lentinus oloides* are important in lignocellulose decomposition. *Corius versicolor* is important in cleaning up pulp and paper mill wastes. Consortia of fungi and bacteria are used in composting, the most useful waste disposal practice. Phenolic azo dye has been shown to be oxidized by the enzyme laccase produced by *Pyricularia oryzae*. Table 9 shows various fungi, which may be used for bioremediation.

The greater potential of fungi by virtue of their aggressive growth, greater biomass production and extensive hyphal reach in soil may facillate usage of the diverse fungal flora for bioremediation in future. There are several promising fungi that can degrade zinc, cyanide and chromium in the effluents.

The high surface-to-cell ratio of filamentous fungi makes them better degraders under certain niches like contaminated soils. Fungi have been shown to even solubilize partially coal, a highly polymeric substance more complex than lignin. There is no doubt, therefore, regarding fungi being harnessed more and more in environmental bioremediation work in future.

Other applications

Several applications of fungi are still under exploration. For example, textile dyes could be produced by fermentation.. Many fungi produce pigments during their growth which are substantive as indicated by the permanent staining that is often associated with mildew growth on textiles and plastics. Some fungal pigments have been shown to be anthraquinone derivatives, resembling the important group of vat dyes. The production and evaluation of microbial pigments as textile colorants is still under investigations. Recent studies have suggested that lignin-degrading or white-rot fungi such as *Phanerochaete chrysosporium* and *Trametes versicolor* could replace some of the chemical steps used in paper making. An industrial biopulping/biobleaching process would eliminate the pollution problems associated with the use of chemicals. Lignin-degrading fungi or their enzymes also have the ability to degrade highly toxic organic compounds such as dioxins and polychlorinated biphenyls (PCB), and could have an important role to play in the remediation of contaminated soils and the disposal of chemical wastes. A recent report also indicates that lignin-degrading fungi can even degrade synthetic textile polymers such as nylon previously thought to be non-biodegradable.

Table 9: Fungal enzymes in bioremediation

Fungal species	Enzymes	Functions
Phanerochaete chrysosporium	Laccases, polyphenol oxidases, lignin peroxidases etc. play a role in the degradative process. A variety of intracellular enzymes such as reductases, methyl transferases and cytochrome oxygenases are known to play a role in xenobiotic degradation	Degrade a number of toxic xenobiotics such as aromatic hydrocarbons (benzo alpha pyrene, phenanthrene, pyrene) chlorinated organics (Alkyl halide insecticides, chloroanilines, DDT, pentachlorophenols, trichlorophenol, polychlorinated biphenyls, trichlorophenoxyacetic acid), nitrogen aromatics (2,4-Dinitrotoluene, 2,4,6-Trinitrotoluene-TNT) and several miscellaneous compounds such as sulfonated azodyes
	lignin peroxidase and manganese peroxidase	Bioleaching of organic dyes (Nigam *et al.*, 1995). Decolorization of azo-triphenyl methane dyes by lignin peroxidase (Pauli Ollikka *et al.* (1993) decolourization of olive mill waste water (Sami and Radhaune) (1995)
Fusarium sp.	-	Decolourization ranging from 35 to 85 %.
The basidiomycetous fungus *Pleurotus ostreatus*	An extracellular hydrogen peroxide dependent lignolytic enzyme Oxidative enzymes	Removes the colour due to remozol brilliant blue Play a very major role in biodegradation
Zygomycetes	-	Aquatic fungi and anaerobic fungi are the other candidates for bioremediation
Candida tropicalis	-	Clearing industrial effluents of unwanted chemicals
S. cerevisiae	-	Clearing industrial effluents of unwanted chemicals
S. carlbergensis	-	Clearing industrial effluents of unwanted chemicals
Pyricularia oryzae	Enzyme laccase	Phenolic azo dyes have been shown to be oxidized (Chivukula and Renganathan, 1995)
Rhizopus arrhizus	-	Developed for treating uranium and thorium (Teen-Seers *et al.*, 1984)

Conclusions

Questions to be addressed

The biology of pathogenic fungus needs to be understood especially with reference to host pathogen interactions using biological machinery. Other factors include the means by which the fungus reaches the host, such as wind, insect vectors like beetles (they carry the conidia of

Ophiostoma ulmi which causes Dutch elm disease), or inappropriate storage conditions of the seeds where the fungus can remain until a new crop is stored.Areas of special interest are the specificity of the fungus to the plant, interaction, how the fungus recognizes the host plant, how the fungus gains entry into the plant, the role of fungal toxins during disease development, how plant metabolism is modified, plant resistance mechanisms develop, and gene expression during host-pathogen interactions. Comparative microbial genome analysis can establish the virulence factors, pathogenecity etc.

Glossary

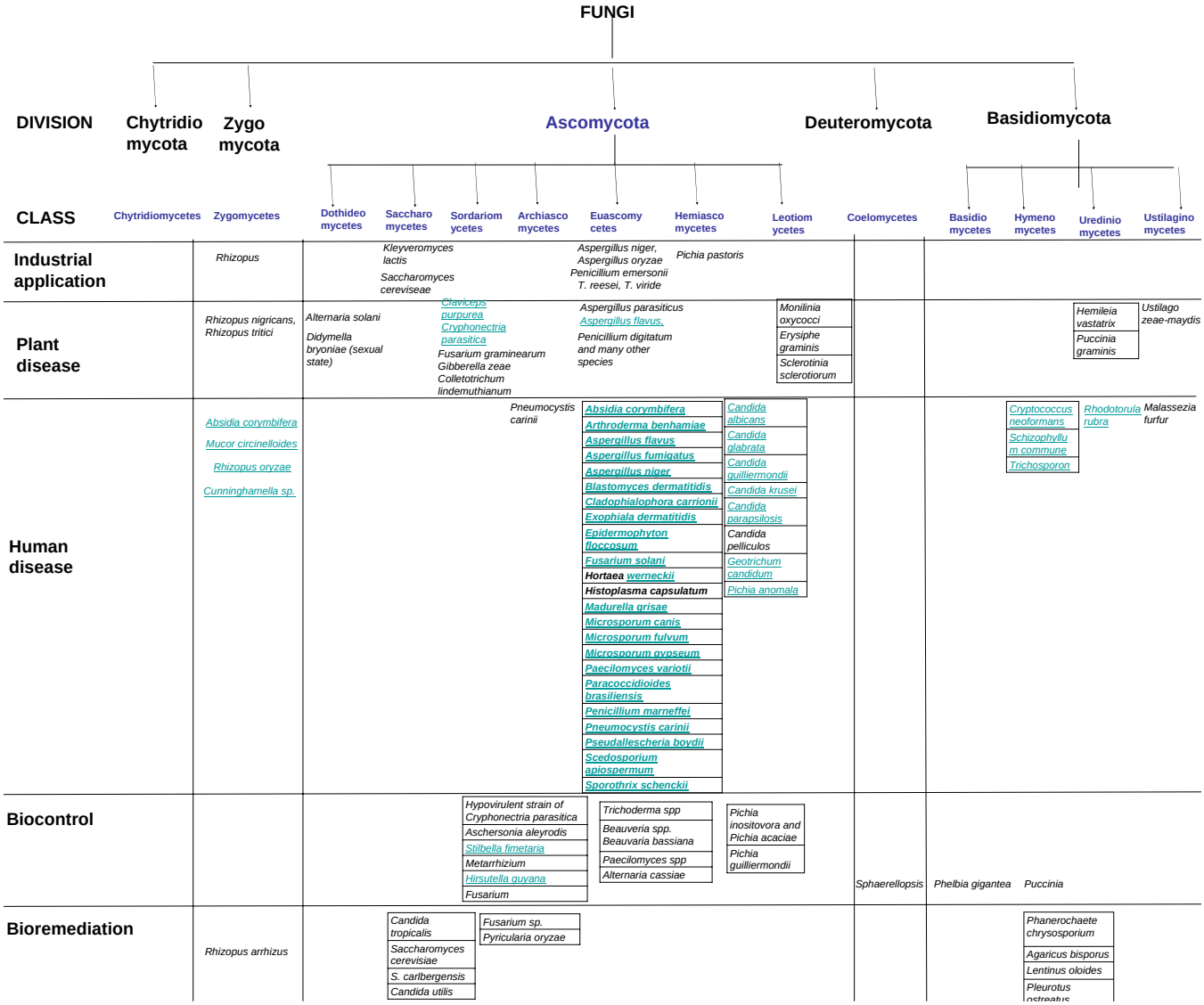
Biodegradation: Biodegradation is the decomposition of organic material by microorganisms. It is often used in relation to sewage treatment, environmental remediation (bioremediation) and to plastic materials.
Bioleaching: Bioleaching is the extraction of specific metals from their ores through the use of bacteria.
Bioremediation: The use of biological agents, such as bacteria or plants, to remove or neutralize contaminants, as in polluted soil or water.
Cell-mediated immunity: It is an immune response that does not involve antibodies but rather involves the activation of macrophages and natural killer cells, the production of antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen.
Delayed hypersensitivity: Type IV hypersensitivity is often called delayed type as the reaction takes two to three days to develop. Unlike the other types, it is not antibody mediated but rather is a type of cell-mediated response.
EST (Expressed Sequence Tag): - An expressed sequence tag or EST is a short sub-sequence of a transcribed protein-coding or non-protein coding DNA sequence. It was originally intended as a way to identify gene transcripts, but has since been instrumental in gene discovery and sequence determination.
Glycosylation: It is the process or result of addition of saccharide to proteins and lipids. The process is one of four principal co-translational and post-translational modification steps in the synthesis of membrane and secreted proteins and the majority of proteins synthesized in the rough ER undergo glycosylation.
Immunocompromised patient: Patient who is incapable of developing a normal immune response, usually as a result of disease like AIDS, malnutrition, or immunosuppressive therapy.
Immediate hypersensitivity: Type I hypersensitivity is an allergy reaction provoked by reexposure to a specific antigen. Exposure may be by ingestion, inhalation, injection, or direct contact. The reaction is mediated by IgE antibodies and produced by the immediate release of histamine, arachidonate and derivatives by basophils and mast cells. This causes an inflammatory response leading to an immediate (within seconds to minutes) reaction.
Mass spectrometry: Mass spectrometry is an analytical technique which determines the mass-to-charge (m/z) ratio of ions. It is most generally used to find the composition of a physical sample by generating a mass spectrum representing the masses of the components of a sample
Matrix-assisted laser desorption/ionization (MALDI): It is a soft ionization technique used in mass spectrometry, allowing, to ionize bimolecules (like proteins, peptides and sugars) which tend to be more fragile and quickly lose structure when ionized by more conventional ionization methods. A laser beam (normally a nitrogen-laser) triggers the ionization. A matrix is used to protect the biomolecule from being destroyed by direct laser beam.

Phylogenetics: It is the study of evolutionary relatedness among various groups of organisms. (e.g., Species, populations)

Suggested Reading

1. The Fungi. Carlile, M.J. & Watkinson, S.C. (1994). Academic Press, London.
2. Handbook of Applied Mycology – Fungal Biotechnology by Dilip K. Arora, Ricahrd p. Elander, K. G. Mukerji. (1992). Marcel Dekker, New York
3. Fungi: Biology and Applications by Kevin Kavanagh. . John Wiley and Sons. (Jun 17, 2005)
4. Handbook of applied Mycology : Mycotoxins in Ecological systems. Vol. 5. by Bhatanagar, D.,Lillehoi, E.B.,Arora, D.K. Marcel Dekker, New York.
5. Fungi as Biocontrol Agents: Progress, Problems and Potential By Tariq M. Butt, Chris Jackson, Naresh Magan. CABI Publishing. (1 Dec 2001).
6. Fungal Physiology of fungi by Arora D.K, Gupta, Seema. (1996) 1st edition (Advances in plant physiology series 5). Anmol publication, New Delhi.
7. Molecular fungal Biology by Ricahrad P, Michael Schweizer Cambridge University Press. (Aug 5, 1999).
8. Biochemistry & Molecular Biology of Antimicrobial Drug action by Trevor j. Franklin. & G A Snow, Mar 3, 2005, Springer
9. An Introduction to Fungal Biotechnology by Wainwright, M. (1992). Wiley, Chichester.
10. Biochemistry of the cell walls & membranes in Fungi by Paul j. Kuhn, Anthony P. J. Trinci, Springer-Verlag, 1990.
11. Beadle, G. W., and E. L. Tatum. 1941. Genetic control of chemical reactions in *Neurospora*. Proc. Natl. Acad. Sci. USA 27:499-506.
12. Lorenz MC, Fink GR The glyoxylate cycle is required for fungal virulence. Nature. 2001 Jul 5;412(6842):83-6.
13. WHO/WAO Meeting on the Prevention of Allergy and Allergic Asthma Geneva,
14. http://www.doctorfungus.org
15. http://bugs.bio.usyd.edu.
16. http://www.botany.hawaii.edu
17. http://gsbs.utmb.edu
18. http://www.bio.org
19. http://fungus.org.
20. http://scarab.msu.montana.edu
21. http://TomVolkFungi.net
22. http://en.wikipedia.org/wiki/Hyposensitization
23. http://www.ncbi.nlm.nih.gov

Annexure I - Classification and relevance of fungi to human health, environment and industry.



(Source:

1. New Biotech Tools for a Cleaner Environment Industrial Biotechnology for Pollution Prevention, Resource Conservation, and Cost Reduction Brent Erickson Christopher J. Hessler. Report by the Biotechnology Industry Organization's (BIO's) Industrial and Environmental Section.
2. Fungal Biotechnology by Paul F HamlynMushroomer, Snohomish County Mycological Society, August/September 1997. British Mycological Society Newsletter, May 1998.
3. Carlile, M.J. & Watkinson, S.C. (1994). The Fungi. Academic Press, London.
4. http://www.angelfire.com/wizard/kimbrough/Textbook/FungiAsBiocontrolAgents_blue.htm
5. www.ncbi.nlm.nic.org)