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Moulds, Biotechnology and Human Welfare*

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What are moulds or fungi ? And how are they related to our daily life ? Laymen are generally familiar with many fungi such as mushrooms in the forests or fields, bracket fungi on the side of decaying logs or standing trees, puffballs which send-forth clouds of spores when kicked by a playful boy, and perhaps colourful cup fungi on logs or soil. The housewife uses yeast to leaven her bread, and the brewer uses the same organism to form alcohol in his bear, wine or whisky. Perhaps the farmer drowns his sorrows with this alcoholic brew after his crop is destroyed by a devastating fungus disease, such as loose smut of wheat or black stem rust of wheat. The city dweller who may not be familiar with fungi in their natural habitats may be waging a war with ringworm or athletes foot, fungus diseases of humans. Perhaps the same city dweller is involved in the manufacture of antibiotics from fungi, or fungus processed cheeses, or of drugs which are chemically modified, and perfected by chemical activities of fungi. Meanwhile a tribal rite may be taking place in Mexico where hallucinogenic mushrooms are consumed and vivid dreams are experienced. Many fungi rarely come to the attention of the laymen. They include the microscopic inhabitants of almost every habitat, including those fungi which trap nematodes (round worms). Some fungi eject their spores over a precise trajectory and with a precision force, as much as a missile is precisely fired into space. They may become partners with algae, insects or higher plants and enter into complex biological relationships with these other organisms.

Fungi, as agents of decay, play an essential role in maintaining earth's biosphere. They are of ancient lineage and were plantiful during Devonian period (some 400 hundred million years ago) in association with the first land plants as both saprobes and parasites.

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Fungi play such an important role in the slow but constant decomposition taking place around us because of their ubiquity and their astonishingly large numbers. Specifically, fungi are the agents responsible for much of the disintegration of organic matter, and as such they affect us directly by destroying food, fabric, leather and other consumer goods manufactured from raw materials subject to fungal attack. They cause the majority of plant diseases of animals and of man. They are the basis of a number of industrial processes involving fermentation such as the making of the bread, wines, beer, the fermentation of cacao bean, and the preparation of many organic acids, and are responsible for the manufacture of a number of antibiotic drugs, notably penicillin. Fungi are both destructive and beneficial to agriculture. On the one hand they are responsible for millions of dollars worth of damage to crops by causing plant diseases, while on the other hand they increase the fertility of soil by inducing various changes which eventually result in the release of plant nutrients in a form available to green plants.

The activities of these fungi has been ably reveiwed by Christensen (1965), Gray (1959) and Emerson (1973).

Historical aspect of Mycology

According to Alexopoulos and Mims (1979) three and half million years ago, so the legend goes, the Greek hero Perseus in fulfilment of an orac'e accidently killed his grandfather Acrisius. Then according to Pausinias, when Perseus returned to Argos, ashamed of the notoriety of the homicide, he persuaded Megapenthes, son of Proteus to change kingdom with him. So when he received the kingdom of Proteus he founded Myceneae, because the cap of his sabbard has fallen off, and he regarded this as a sign to found a city. It is also said that being thirsty he chanced to take up mushroom (mykes) and the water flowing from it be drank, and thus, one of the Greatest Civilizations ever developed. Mycenean may have been named after the legendary mushroom. Derived from the same Greek word, mycology is the study of mushrooms or fungi.

It is not possible to discuss the development of the subject of Mycology in greater detai's here but it would be worthwhile to mention that the subject developed parallel to the development of printing and microscope.

Fungi are not the private concern of the mycologists, cytologists, geneticists, and biochemists and it has been found that they can be important tools in the study of the fundamental biological processes. Because of the rapidity with which the fungi grow and reproduce, a much shorter time is required to obtain a number of generations of fungi than higher plants or animals. Further more, the fact that fungal spores produced by meiosis will grow into haploid invididuals give geneticists an opportunity for direct and rapid tetrad analysis. They require lesser space in test tubes.

The red bread mold *Neurospora* was discovered by Shear and Dodge in 1927. Dodge step by step laid the foundations of a new branch of science which has come to be known as Haploid Genetics. Slime molds too being widely used in research.

Although fungi are a diverse group of organisms, a feature which they have in common with each other is their mode of nutrition. Like animals fungi are heterotrophic organisms which must consume preformed organic matter. They may live as saprophytes which digest and consume dead plants or animals, their parts or their wastes. Alternatively they may live as parasites and assimilate tissues of living plants and animals. In all cases digestive enzymes are liberated from the fungal cell as a watery solution.

According to Christensen (1965) there are 2 million different kinds of organisms. And these are small and inconspicuous, but they make up for this in other ways that allow them to roll with blows of environment or fate more adaptly than man or many other higher animals are able to do. They have not exactly inherited the earth, they eventually occupy a goodly portion of living things on earth, as well as the goods products made from these living things. They affect us and other living things in many ways. Some of them are discussed here.

Edible and Poisonous Mushrooms

With a warm rain they spring up everywhere. They are a delicacy, but beware, some mushrooms are deadly poisonous as the bite of a poisonous snake. The early man knew their special properties. The Aztecs used them as hallucinogens and for divination. They called them as "God's flesh". According to the American scientist Gordon Wasson (1968), the intoxicant "Soma" mentioned in the Vedas was nothing but the extract of a fly agaric, *Amanita muscaria*. Sudher Ghatnekar (1973) has written a beautiful popular article on mushrooms.

The most deadly are death caps i.e. *Amanita phalloides*, Destroying Angele or *Amanita virosa*, Fools Cap e.g. *A. verna*. The toxic principle is a aminotoxin and β-aminotoxin and phalloidin. Cooking does not destroy them not it is effected by human digestive system. Symptoms of toxin injury appear only 8 to 24 hours after ingestion. By then the toxin is absorbed by the body and neither vomitting nor a stomach pump can help. And you can commit this mistake of consuming a mushroom only once in your life. *A. phalloides*, in older days, has been used to help the political career of certain ambitious type throughout history. The Roman Emperor Claudius (41 A. D.) was poisoned by his second wife Agrippina. Agrippina wanted her son Nero to become the Emperor of Rome after Claudius and she decided that Claudius should die. For this she poisoned the dish of Emperor Claudius of edible *A. caesarea* with the poisonous *A. phalloides*. Claudius as the story goes became a God because of a poisoned dish of mushroom and Nero became the Emperor of Rome and who was later responsible for a great political mess. Thus the entire history of Rome was changed because of a poisonous mushroom.

Panther cap (*Amanita pantherina*) contains atropine which affects the central nervous system causing violent convulsions, and dialation of the pupils and palpitations. A study of poisonous fungi may ultimately help in the field of medicine. Non-edible mushrooms have been eaten in religious rites or orgiastic ceremonies. For example certain Siberian tribes consume *Amanita muscaria*, because of its extremely stimulatory effects. The drug like stimulant is not decomposed in the body and passes unchanged in the urine. Several authors have referred to the common practice of drinking urine of some one who had earlier eaten the mushroom.

In Scandanavian countries the act of going berserk (fighting with frenzy) came from eating the fungus which seems to have led to intoxication, hallucination, and often superhuman feats. In schizophrenia (split personality) a disease of humans, such drugs have been used to find causes and cure of it. It has been suggested that schizophrenia is associated with abnormal indole synthesis in the bodies of individuals. In view of the known hallucinogenic power of certain fungi and also in light of isolation of triforine (an hallucinogenic indole compound) from *A. muscaria*, the suggestion needs careful consideration.

Some common edible mushrooms are *Agaricus brunnescens*, paddy stray mushroom (*Volavariella volvacea*). *Lentinus edodes* is the " Shiitake " of Japan and China. *Pleroutus* species is cultivated in Jammu. It is popularly called as "Dhingri."

Cultivation of the mushroom : A small hut (about 3m × 6m × 6m) with walls and roofs made of straw and sticks of Arhar (pigeon pea) and bricklined floor has been used and nearly as good as a glass house with temperature around 30 40 C, and relative humidity around 57%. Gram or Urd powder is used to increase the production of the crop in our country.

Cooked mushrooms are equal to any vegetable with appreciable amount of vitamins. Vitamins D is found in several mushroom, which are low in calories but rich in minerals and good sources of protein. In U. K. 27,000 to 32,000 kg of dry protein per acre per year is obtained by mushroom cultivation. Compare this with 32 kg/per acre per year and from 270 kg of fish farming. You will well realize the importance of mushroom cultivation.

Regional Research Lab., Jammu, National Botanical Gardens, Lucknow, I. A. R. I. New-Delhi, Fruit Research Station at Bangalore and certain private farms in various hill stations and in Delhi are growing mushrooms in a large scale. Certain farmers in Sonepat region of Haryana are growing mushrooms very successfully. We have a National Mushroom Research and Training Centre at Chambaghat at Solan. Helvellas are also eaten (helvellic acid is posonous to some). *Clavaria* are also eaten. There are several other fungi which are eaten and the most important is *Morchella esculenta. M. esculenta* forms stalked apothecia, 10-15 cm high. Efforts are being made to cultivate Morchellas in the laboratory. The morels season is short and does not exceed one month in the spring, this is why, morels are occasionally found in the market fresh. The mycelium grows on a variety of media but does not form fruit bodies. Although growing morels outdoor as a horticultural crop presents no problem in western countries. It is exceptionally challenging problem for mycologistis to cultivate them commercially like other mushrooms. Perhaps biotechnology may help us here in getting Morchellas in the laboratory.

Members of Tuberales are edible. Truffles are underground fruit bodies or spore producing bodies in the order Tuberales. There are numerous kinds but only a few of them are edible. They were known and highly prized by upper crust Romans two thousand years ago, and pigs both wild and tame have been relishing them every since there were both pigs and truffles. The procedure of hunting truffles is interesting. There are some mushrooms which are luminiscent. *Mycena* spp. are biolumenescent in the dark. There are some others. Quite a good number of members of Agaricales have mycorrhizal forms.

Fungi cause a large number of diseases in plants. There are the rusts, the powdery mildews, the downy mildews, the smuts and bunts, the anthracnoses, the foot rots and root rots, the leaf spots, the blights, the die backs, and the white rots and brown rots of trees and so on. They cause collosal losses to our crops plants, forest trees and plantation crops. Some of the important genera involved in white rots and brown rots are the members of the Polyporales in which Prof. S. R. Bose showed considerable interest. Mycologists and plant pathologists are making every effort to control these diseases by means of latest available chemicals and other means.

I will discuss a few important diseases which have made history, changed the entire economics of the countries involved and added many dark pages in our history.

Horsfall and Cowiing (1987) have discussed some diseases which have made much impact on man. Ergot is a disease of rye, the fungus invades the grain and forms sclerotia like structure. The English call it purple cocks spur and French call it as roosters spur. The sclerotia or ergot contains a large amount of alkaloids of many types (Mclaghlin *et. al.*, 1964). The alkaloids are ergometrine, ergometrinine, ergotamine and ergotaminine. If the sclerotia are eaten they can cause severe illness and sometimes death. One effect of the toxin is that it constricts blood vessels, and the impaired circulation may result in gangrene or loss of limbs. Another effect is on the nervous system, resulting in convulsions and hallucinations. The alkaloids produce a fantastic array of symptoms. If small amounts of contaminated bread is eaten by pregnant cattle, the alkaloids induce abortions. If larger amount are eaten, there is first a tingling in the fingers and toes. This is followed by a raging high fever. The fever can lead to mental derrangement and may be fatal. Sometimes gangrene sets in and toes fall from the foot, feet from the ankle, fingers from the hands, hands from the arm. The disease cause hallucinations and victims may die of convulsions. In the middle ages the disease was called as St Anthony's fire and there are numerous outbreaks of the disease. Ergotism had its influence on war. According to Carefoot and Sprott (1967), Peter the Great in Russia lost the battle at Astrakhan from the Turks because his army's horses and soldiers were afflicted by ergotism. Peter's dream turned sour and his army turned back in 1772.

Late blight of potato and Irish Famine of 1845

In 1845-46, epidemic of late blight caused the complete failure of the potato crop in Ireland. Since potato was grown exclusively and Irish lived on potato alone, it is estimated that hundred of thousand of people died of starvation and hunger and even more were forced to migrate to the United States of America and Canada resulting in the reduction of population of Ireland by one fourth. Carefoot aud Sprott (1967) in their book entitled "Famine on the Wind", blame the late blight epidemic of potato for the defeat of the Germans by the British in World War I. During the growing season of 1916 and 1917 the weather in Germany was similar to that of Ireland in 1846. The holy water of plant pathology specific for potato blight was not available as the military leaders could not release the copper needed to make it. Because it was diverted to shell casings, electric wire and perhaps for brass buttons too. All the grain and potato went to the Army in 1916 and 1917. The soldiers were not hungry but their families at home were starving. Many of them were starved to death. The morale of the soldiers weakened, the military might weakened and it collapsed in 1918. *P. infestans* played a role. According to Carefoot aad Sprott (1967) it reminds us of an old saying that "For want of a nail a shoe is lost, for want of a shoe a horse is lost and for want of a horse the battle was lost".

Great Bengal Famine

Almost hundred years after the potato famine that killed almost 1.5 million Irishmen, another famine struck in Bengal halfway round the world and killed an estimated 2 million of its citizens (Padmanabhan, 1973). Dr. S. Y. Padmanabhan in his fine monograph on the disease (1973), stated that he was sent to Bengal to investigate the disease. He says "The author was appointed as a mycologist in Bengal when the famine was in its height. When he travelled on the 18th of October, 1943, he could see dead bodies and starving and dying persons all along the way. This horrendous situation continued through October, November and December". The dying continued until the new crop arrived in the summer of 1944. The war worsened the famine because Japanese army had occupied neighbouring Burma and thereby shut off the normal flow of rice from Burma (now called as Myanmar). The British were fighting with their backs to the wall and could not possibly provide enough rice from elsewhere. Thus you have a complex relationship between plant disease epidemics and war. A plant disease (ergot) helped the Turks to defeat the Russians in the eighteenth century. Another helped the Russians to defeat the British in the nineteenth century (the potato famine of Ireland) and the third helped the British to defeat the Germans in the Twentieth Century (again potato blight).

Coffee rust of Ceylon

The history of coffee and its rust has been treaten by Large (1940) and by Carefoot and Sprott (1967). The Arabs brought coffee from Ethiopia about 1000 AD. They found it stimulating. It helped them to outlast the endless rituals of Islam. The Islamic Turks had established Coffee hours by 1550 and the roasted coffee beans began to move into Europe to meet the appetites of the wealthy. Arabs did not allow live beans move out because they wanted to maintain a monopoly. Coffee provided foreign gold just as their oil does now. It is said that a Muslim from S. India on his holy pilgrimage to Mecca stole 7 live beans and brought them to South India and sowed them where these flourished. From India Coffee was taken to Ceylon. In 1835 the British were growing only 200 ha in coffee and by 18 0 they had about 200,000 ha in coffee and they exported 50 million kg of beans per year. In the meantime the Britishers learnt empirically to boil their drinking water to avoid intestinal fluxes. They found that the boiled water was better to drink if they boiled coffee with it. By 1870, in the last quarter of Victorias reign, they had become a nation of coffee drinkers. Then devastating rust disease of coffee struck Ceylon. It killed the coffee planted faster than these could be replaced. In 19 years after 1870 the yield dropped from 50 millions kg to essentially zero. Ceylons economy was completely broken. The thousands of South Indian labourers who had been imported to pick up the coffee beans were sent home without funds and the local Shingalese went hungry because they had no money to buy rice. Berkely gave the name *Hemileia vastatrix* to the pathogen. To endure the cold climate the Britishers needed a stimulant to replace coffee so what did they do ? They planted tea of course in place of coffee. By dint of herculean effort by 1880 the planters had covered about 140,000 ha with tea bushes. England put tea in its boiled water there upon became a nation of tea drinkers. They established the pleasan custom called elevenses tea at eleven O'clock. Americans call it the coffee break but their coffee comes from South America and no Ceylon. The disease has now jumped the Atlantic Ocean to Brazil and Columbia and rapidly spreading. What it will do only time will tell ? What effect it will have on the American coffee break ?

Antibiotics

Few scientific discoveries have appealed to the public imagination to the same extent as that of penicillin. According to Late Prof. Brian (1946), penicillin has saved tens of thousands of lives in the few years that have elapsed since its discovery. According to Dr. Brian the isolation of penicillin depended on previous biological observations every bit as much as the manufacture of atom bomb depended on a centurys of physical research. In 1929, Fleming was working in St. Marys Hospital in London, on *Staphylococcus aureus* the bacterium responsible for boils and certain other diseases. In the course of this work, he was growing *Staphylococcus* on a nutrient jelly in a glassdish. Under such conditions the organism appears as numerous yellowish specks (colonies). Fleming had occasion to lift the lid of one of these dishes to examine the colonies of *Staphylococcus* more closely and a mould spore fell into the dish. This kind of accident happens frequently in bacteriological and mycological laboratories as the air around us almost always contains great numbers of bacteria, mould spores and other microorganisms. The mould spore grew into a green colony with a white edge. Fleming made the observation that the small bacterial colonies close to the mould colony were being lysed. He grew the mould in a liquid nutrient solution and examined the properties of the culture filtrate in some detail and found that it inhibits the growth of certain bacteria including many pathogenic ones at very low dilutions. Fleming also showed that the culture filtrate was not toxic to warm blooded animals. He gave the name penicillin to it but he was not able to isolate it in pure form. The fungus was *P. notatum*. The nutrient medium on which Fleming grew the mould was a complex broth made up of meat and peptone. Other workers subsequently (1932) showed that the penicillin was also produced on a synthetic medium consisting of sugar (glucose or cane sugar) with a few simple mineral salts, sodium nitrate, potassium phosphate, potassium chloride, magnesium sulphate and a little of iron sulphate. They also showed that penicillin is very unstable chemically and were not able to isolate it in pure form.

The first great contribution came from Oxford group of workers (Chain, Florey and others) who discovered a method whereby penicillin could be obtained in pure from. The Oxford workers were greatly helped by a fairly simple biological assay devised by Heatley. Essentially this was carried out as follows : A small quantity of the penicillin containing liquid is put in the glass tube. The antibiotic diffuses through the agar and produces a clear halo in the medium indicating the effectiveness of the antibiotic. It is not proposed to go in detail the methods of extraction of penicillin, purification and the refinements subsequently made but some references are worth mentioning about the difficulties encountered in the isolation. Earlier workers had found that penicillin could be extracted from the culture filtrate into certain organic solvents such as ether or amyl acetate if the culture filtrate was first made acidic. It was hoped that when the ether extract was evaporated penicillin would be left, but the free acid penicillin is too unstable to stand this treatment. The Oxford workers made the important discovery that the penicillin could be taken back to water by the shaking small quantity of alkali with it. By alternate procedure of taking it to organic solvent in acid conditions and by bringing back to water in alkaline conditions using progressively smallar volumes of liquid penicillin could be obtained in purer form. All the operations being done in the cold. The concentrated acquous penicillin was frozen in solid carbon dioxide (dry ice) and the water removed by applying high vacuum. Another improvement was made by Moyer who found that addition of 5-10 percent of corn steep liquor, the production of penicillin could be increased by five fold. Corn steep liquor is a by-product of starch manufacture ; it is a complex mixture of carbohydrates, proteins, degradation products of proteins and many other substances. A second improvement was in the improvement of the strain of *P. notatum*. Biotechnology has helped in the improvement of the strains and here is enough work to be done for the improvement of strains by concerted breading or other means to produce better strains. Several other fungal antibiotics have been discovered. The story of the discovery of penicillin is beautifully given by Brian (1946). Screening, selection and strain improvements have been discussed by Elander (1987).

Moulds in Brewery Industry

Fungi are very important in fermentation,. in production of alcohol, beverages, organic acids and various fermented products. Yeasts are the most important in this capacity to ferment sugars to produce alcohol. The manifestations of the activities of yeasts have been known for thousand of years, ever since the first toast was proposed over a shell full of wine, and the first loaf of leavened bread was baked. Man has been interested in alcohol from the remote past. The yeast ferment glucose to yield alcohol and carbon dioxide :

$$C_6H_{12}O_6 \xrightarrow[\text{enzymes}]{\text{Yeast}} 2C_2H_5OH + CO_2 + \text{Energy}$$

Most common substrates throughout the world are corn, molasses, sugarbeets, potatoes, and grapes. The strains of yeasts carry out a vigorous fermentation in which the yeast cells are carried to the top of the liquor and form a surface pellicle. These are commonly used in the manufacture of typical English beers. The bottom yeasts ferment more slowly and stay at the bottom of the liquor. These are used in the manufacture of so called "lager" beer such as fermentation brought about by *Saccharomyces carlsbergensis*. Alcoholic beverages are prepared either by fermentation or distillation. Beer is brewed from malt substitute and is a product of fermentation. The ethyl alcohol in this beverage is derived from malt sugars through the fermenting action of *S. cerevisae* and *S. carlsbergensis*. The little flavour is imparted by the addition of hops. Beer is 90% water and ethyl alcohol ranges from 3-8%. The beverage also contains proteins, minerals and CO_2. Wines are produced from grapes, logan berries or other fruits and is also the fermentation product brought about by *S. illipsoides*. The alcoholic content in wines is generally 14% or less. In certain wines the alcoholic content may be raised to 25% or so by the addition of alcohol. The alcoholic beverages grouped under the general scheme of spiritios liquors includes whisky, brandy, rum, gin, vodka and absinthe. In it the alcoholic content may be upto 50% by volume.

It is wrong to speak of alcohol as a stimulant, that so called stimulation due to effects represents the first stage in the depression that it brings about. Alcohol causes a warm feeling but lowers blood temperature. Alcohol is high energy food which does not have to be digested. It stimulates the flow of saliva and gastric juices and increases the excretion of urine. It dialates the blood vessels and brings about an increase in the rate of heat and blood pressure, when the percentage of alcohol is 0.05% (of the total blood) the area controlling inhibitions is lost and there is loss of self discipline. When it reaches 0.1% the area of brain controlling motion is affected. When it reaches 0.2% the mid brain controlling emotional behaviour loses control. Higher concentration senses are affected which may lead to coma and death follows when the concentration is around 0.6 to 0.7%. (heart beat and breathing is affected). Alcohol gets converted into acetaldehyde in the liver and this which is most toxic. Acetaldehyde brings about the sorosis of the liver. Alcohol also directly affects the tissues such as mouth, wind pipe, throat and stomach. The tissues become inflamed and may undergo serious damage.

Fermented Food Stuffs

Hesseltines (1965) stated that millenium of fungi are used as food and in fermentation and provides detailed information on tempeh, sufu, miso and other fermented food stuffs and varied ones (ragieto) are already sold in the markets. Large quantities of fermented products like jalebies, murch, Punjab warries, papdams, and toddy are consumed daily in Asian countries. The fungi responsible for these fermentations are *Torulopsis*, *Candida* and *Trichosporon pullulans* for idli, *Saccharomyces bayanus* for jalebies, *Hansenula anomala* var. *anomala* for kanji, *H. anomala* var. *schaeggii*, *N. rouxinianus* and *R. arrhizus* for murcha, *Candida* sp. and *S cerevisae* for toddy (Batra and Milner, 1974). There are several oriental and other food products. The organisms involved and the substrates used and the food products produced are given in Table 1.

Table 1. List of fermented food products produced by useing fungi

Name of the food product	Substrate	Organism Involved in fermetation
Idli	Rice	*Torulopsis, Candida, Trichosporon pullulans*
Jalebies	Mung Pulse	*Saccharomyces bayanus*
Kanji	Urd Pulses	*Hansenulla anamola* var. *anamola*
Murcha		*H. anomala* var. *schneggi* and *Mucor rouxianus*
Punjabi waries & papadams	Urd/Mung	*Candida* sp. and *S. cerevisae*
Toddy	Sugars in the Toddy palms trees	*S. cerevisae*
Tempeh	Dehulled soyabean grits	*Rhizopus oligosporus*
Shou or soya souce	Soyabean	*Aspergillus oryzae, Saccharomyces rouxii* and *Lactobacillus dilbrueckii*
Lao chao	Rice	*Rhizopus chinensis* and *Endomycopsis* sp.
Hamanatoo	Soyabeans	*Aspergillus oryzae*
Ontzom	Peanuts	*Neuorspora sitophila*

Proteins from Fungi

Keeping in veiw the ever increasing human population and lack of corresponding increase in food production, there is a growing need of more and more protein for human nutrition. The importance of single cell proteins was much realised during World War II. The earlier work on fungal protein was done by Pringsheim and Lichtensten in 1920. They fermented straw with *Aspergillus fumigatus*. In addition to use of bacteria (*Hydrogenomonas, Methanomonas, Methylomonas*) green algae(*Chlorella* and *Scenedesmus*), blue green alga (*Spirulina*), the use of yeasts particularly *Rhodotorula* and *Saccharomyces* has long been established. Continuous culture of *Candida* on hydrocarbons from petroleum which was started by British Petroleum at Grangemouth, Scotland in 1971. It was producing 4000 tons of protein concentrate per year for use as animal feed by the mid 1970. According to Roth (1982) the extent to which microbial proteins can be competitive with conventional proteins sources cannot be determined at this stage. There is an enormous scope for the biotechnologists in this area of both in the strains (better ones) and the technology involved in its harvesting and conversion into eatable form of proteins. Microbial biomass as a protein source has been discussed by Olsen and Allermann (1987).

Organic Acid Fermentation

The commercial production of organic acids by mycelial fungi, in most instances, limited to members of the genus *Aspergillus*. The organic acids produced by fungi are gluconic acid (*A. niger*), citric acid (*A. niger*), itaconic acid (*Rhizopus oryzae*), gallic acid (*A. niger*), L-malic acid (*Scizophyllum commune*), and trans-apoxy succinic acid (*A. fumigatus*, and *Paecilomyces varioti*). These organic acids are used industrially in various ways, for example, gluconic acid in the manufacture of tooth paste, itaconic acid in the mauufacture of plastics. Gluconic acid is used in the preparation of tablets of calcium gluconate when calcium becomes more soluble in this form to lactating mothers. Citric acid is used in the manufacture of citrates, flavouring ingredients, effervescent salts, in ink, in dyeing, in silvering mirrors as well as medically. Organic and amino acid fermentation has been critically discussed by Meers and Milson (1987).

Fungi as Agents of Diseases in Humans and Animals

The first fungus disease was described in around 1880. It is now more than 100 years that the medical mycology has made rapid advancements. Some of the important diseases are superficial and cutaneous mycosis, subcutaneous mycosis, systemic mycosis. Fungi also cause diseases in birds. The most important is the *Aspegillus flavus* infections in the lungs of birds. There are diseases of the fishes also. The most important is the *Saprolegnia parastica* and *S. ferax*.

Entomogenous Fungi

Fungi growing on insects are known as entomogenus fungi. One good example of an entomogenous fungus is the fungus *Coelomomyces* sp. which is parastic on mosquito larvae. Entomogenous fungi have great potential for biological control of insects. Some entomogenous fungi with insect hosts are given in Table 2.

Fungi Parasitic on Other Fungi

Then there are fungi which are parastic on other fungi. The mycoparasites are separated into two major groups based on the mode of parasitism i.e. necrotrophic and biotrophic. The necrotrophic ones contact their hosts, excrete a toxic substance, which kills the host cells, and utilises the nutrients that are released. On the other hand a biotrophic one is able to obtain nutrient from living cells.

Table 2. Successful examples of entomogenous fungi utilized for the biological control

Entomogenous Fungi	Insect Hosts		Country
	Common Name	Zoological Name	
Aschersonia aleyrodis	Citrus whitefly	Dialeurodes citri	Japan
A. placenta	Cirtus whitefly	Dialeurodes citri	Soviet union
Aschersonia sp.	Greenhouse whitefly	Trialeurodes vaporariolum	Soviet union
Beauveria bassiana	Colorado potato beetle	Lepinotarsa decemlineata	Soviet union
B. bassiana	Codling moth	Carpocapsa pomonella	Soviet union
B. tenella (B.bronghiart i)	Soyabean beetle	Anomala rufocuprea	Japan
B. tenella	Larger strated chafer	Anomala costata	Japan
Hirsutella thompsoni	Citrus rust mite	Phyllocoptruta oleivora	USA
Metarhizium anisopliae	Rhinoceros beetle	Oryctes rhinoceros	Tonga
M. anisopliae	Black rice bug	Scotinophara lurida	Japan
Nomuraea rileyi	Tobacco budworm	Heliothis virescens	USA
Paecilomyces farinosus	Citrus ground mealy bug	Rhizoecus kondonts	Japan
P. fumosoroseus	Peach fruit moth	Carposina niponensis	Japan
P. lilacinus	Potato tuberworm	Phithorimae opeculella	Japan
Verticillium lecanii	Brown soft scale	Coccus hesperidum	Czechoslovakia

Fungus Gaderns of Insects

Any one with at least a passive interest in biology is aware that fungi being plants lack chlorophyll and cannot photosynthesise, live on other organisms or on decaying matter. It is less well known that many insects are similarly dependent on fungi. Indeed, there are insects that tend to elaborate gardens of a fungus, controlling the growth of the plant according to their own specialiged needs. The association of fungi with insects is beautifully given by Batra and Batra (1967).

Mycorrhizal Fungi

These were discovered by the German Botanist Frank in the year 1885. Most woody plants depend upon mycorrhizal fungi for absorption of nutrients from soil. Formerly it was through that the mycorrhizal fungi are important in the forest ecosystem but it is being realized now that they are equally important in the agricultural systems in crop plants. The mycorrhizal fungi can be broadly classified into ectomycorrhizae, and endomycorrhizae. The ectomycorrhiza are present in temperate to boreal forests. Species of *Boletus*, *Cortinarius*, *Russula*, *Tricholoma*. *Amanita* etc. are the partners. Endomycorrhiza can be divided into two groups : (1) those with septate fungi and (2) those with unseptate fungi. Many endotrophic mycorrhizal fungi develop arbuscles and vesicles (vesicular and arbuscular fungi nick named as VAM). There is an enormous amount of interest in these fungi these days throughout the world and it may not be possible to discuss this aspect in details here. Then there are the lichens. The fungi in association with algae. They have an important role to play in the economy of nature.

Fungi as Allergens

Some hypersensitive people develop asthma as a result of fungal allergy. The genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, *Fusarium*, *Caphalosporium*, *Trichoderma* *Chaetomium*, *Phoma*, *Botrytis*, *Mucor* *Rhizopus*, the yeasts, rusts and smut spores cause various types of allergies in humans.

Nematode Trapping Fungi

There are several fungi that attack and kill nematodes. There are various types of mechanisms by which these fungi trap their nematode hosts. For much of the knowledge of these fungi we owe to Charles Drechslter, C. I. Duddington and G. L. Barron. Work has also been done on the ultrastructure of these fungi (Dowsett and Reid, 1977 ; Dowsett *et. al.* 1977 ; Heintz and Pramer, 1972). Various ingenious methods have been developed by these fungi to entrap and kill their nematode hosts.

Fungi Producing Mycotoxins

The mycotoxin problem first came to the knowledge of public attention in groundnut in 1960, when poultry farms in U. K. suffered severe losses of young turkeys and ducks, more than 100,000 young turkeys died in a few months from an apparently new disease that was named "turkey × diasae" (Blount, 1961 : Asplin Carnaghan, 1961). *Aspergillus flavus* was indentified as being the mould responsible for producing a group of highly toxic substances, when it grows in ground nuts, known collectively as aflatoxin. Several mycotoxins have been discovered and some important ones and the organisms producing them and the diseases that they also produce toxic substances in it. This area of mycology is receiving lot of attention from medical mycologists in our country and abroad.

Fungi as Sources of Vitamins

Saccharomyces cerevisae and some other yeast are the good sources of vitamin B-complex and riboflavin. Because of their high vitamin and satisfactory amount of protein the yeasts are considered as a valuable food throughout the world. Ergosterol, which contains vitamin-D is synthesized by quite a good number of fungi.

Ambrosia Fungi

Fungi, e. g. yeasts, *Ambrosiozyma, Ascoidea and Dipodascus* spp., Hyphomycetes etc., that grow in tunnels of ambrosia beetles (woodboring Scolytidae) and serve as food for larvae and adults. Many are specific for the particular insects (Batra, 1963). Ambrosia beetles inhabit recently dead trees, freshly cut logs, newly sawn lumber, and occasionally the stems and branches of living trees and shrubs. The female ambrosia beetle usually has the job of preparing the home, which is simply a tunnel or gallery excavated in the wood. The gallery may be communal, the eggs beings laid in groups in large numbers in a single-vaulted chamber, where the young grow up into females, or it may be in the shape of a tunnel in which each egg is laid in a separate niche cut in the tunnel wall. Regardless of the type of dwelling they construct, none of ambrosia beetles eat anything but the spores of a fungus they cultivate. They were given the name of ambrosia beetles because when they were first studied, about a hundred years ago, an acute student recognized that they fed upon material that seemed to form of itself on the walls of their tunnels. The investigator, for all his acumen, was innocent of any knowlede of fungi, a not surprising state of affairs about 1840. The material on which these beetles fed seemed to have no earthy source, was called as ambrosia, and the beetles which lived on it as ambrosia beetles (Christensen, 1965).

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Another aspects of fungi that cannot fail to challenge today's students is the matter of waste, both the prevention of food and fibre waste from moulding and the purposeful disposal of solid waste by moulding and composting. The discoveries of Huge Miehe (1907, 1930), the German botanist who set out to explain and control the heating and spontaneous combustion of damp hay around the turn of the present century. He,more than any other investigator, is responsible for revealing the role of thermophilic fungi (Cooney and Emerson, 1964) as well as other microorganisms in the microbial thermogenesis of large masses of organic matter.

Fungi as Food for Ants Belonging to the Genus Atta

Species of ants belonging to the genus have peculiar habit of carrying the large proportions of leaves and then gather them at a particular place. Ants do not eat these leaves directly but allow the mold to grow on them and then eat the molds. Nutrient rich fungal cells are eaten by the ants. The fungi mainly belong to the genus *Agaricus* and other mushrooms. Fungi as source of hormones : Kurosawa a Japanese plant pathologist, working in Formosa in the early 1920s undertook an investigation of the fungus *F. moniliforme* (*Gibberella fujikuroi*) which had earlier been implicated as causative agent of a disease in rice known as foolish seedling disease "Bakanaeobyo" (in japanese). Kurasawa denomstrated that the sterile culture filtrates from the fungus grown on liquid media, when applied to healthy rice seedlings, accelerated the growth of the seedlings similar to that characteristic of the disease. Thus a new group of chemical substances was discovered and we now know that there are several such compounds having basic gibbane structure. Their role in plants is well know but they have an equally important role to play in certain host parasite combinations such as the bakanae disease of rice.

There are several aspects of these molds which I might not have discussed for want of time and space but it would be worthwhile to discuss the impact of biotechnology in relation to molds : John Naisbitt has divided the industrialized civilization through technology based eras, from chemical change (plastics) to an atomic age (nuclear enery) and microelectronics (computers) and presently we are passing through an era of biotechnology.

What is biotechnology ? Biotechnology in its simplest meaning means the utilization of plant, animal or microbial cells to produce wide variety of products. But there are others who think that biotechnology means the package of power ful techniques used for alternation and manipulation of life forms and the application of engineering principles to such life forms for processing of the materials to provide the desired end products.

Biotechnology is the slogan these days. Fungal biotechnology is the application of fungi for the manufacturing processes such as production of pharmaceuticals, feed stuffs and fermentation. There are two important contributions in the area of fungal biotechnology. One is by Smith *et. al.* (1980) and the other is by Benett and Lasure (1985). The latter work deals with the gene manipulations in fungi.

Applications of Fungal Biotechnology

John F. Paberdy in his presidential address to the members of British Mycological Society (1989) states that "The rate of expansion of knowledge base in the biological sciences in the past two decades has been massive. Technical developments have been made in a wide range of techniques used by the experimentalists ranging from pressure liquid chromatography to microscopy, and from electrophoretic methods which provide opportunities for separating chromosomes to enzyme manipulation to alter DNA molecules. At the organizational level major advances have been made in culture systems for both plants and animal cells and for microbial cells. One aspect of these technical developments has been the isolation of and manipulation of naked cells or protoplasts. This technology is applicable to all biological systems, not the least the fungi. Using the modern techniques of biochemistry, molecular biology and microscopy many secrets of life of fungi could be revealed. The last ten years have seen a major stride in this direction, the next ten years will be period of anticipation." The other important technique on biotechnology is the production of recombinant DNA. Recombinant DNA technology has given a boost in this area.

Protoplast fusion is a technique whereby the genomes of different strains, either isogenic or non-isogenic or of different species can be brought together as the first step towards recombination. Transformation of protoplasts provides opportunities for the manipulation of individual genes of specific DNA sequences derived from a given gene opening up the whole field of molecular genetics of fungi.

The first indication of the utilization of protoplast fusion in fungus biotechnology is the improvement of Cephalosporin C titre in recombinants of the two high titre strains of *C. acremonium* that has been crossed using the fusion techniques (Hamlyn and Ball, 1979). But the most important of these investigations has been recounted by Lein (1937) for *Penicillium chrysogenum*. Confronted with two raised titre strains, each of which had certain defects, the Panlabs group set about crossing them using selective markers other than morphological differences. In the absence of selection it was necessary to screen the large numbers of progeny for the rare recombinants. This was done to good effect with the recovery of two recombinants in which desirable characters had been brought together. Transformation DNA mediated transformation was first described in

S. cerevisae in 1978 (Beggs, 1978; Hinen, Hicks and Fin, 1978). Recombinant mammalian proteins have been produced in *Aspergillus nidulans.*

Genetic manipulation of fungi has led to the insertion of the genes for making cellulolytic enzymes which are of major potential use in hydrolysis of renewable cellulosic biomass. True lignases have been demonstrated and cloning system for the white rot fungi *Schizophyllum commune* (Seligy *et. al.* 1983) and *Phanerochaete chrysosporium* (Peterson *et. al.*, 1984 ; Rao and Reddy, 1984) have recently been developed. Commercial fungal carbohydrases, the organism producing them and their applications are given in Table 3.

Table 3. Some fungal sources of industrial enzymes

Fungus	Type of enzyme
Aspergillus oryzae	Amylase, pectinase, protease, adenylic acid, deaminase.
A. niger	Amylase, amyloglucosidase, pectic enzymes, protease, glucose oxidase, maringinase.
Rhizopus sp.	Amyloglucosidase, Lipase, rennet protease, pectic enzymes.
Penicillium chrysogenum	Glucose oxidase.
P. citrinum	5 prime, phosphodiesterase
Mucor sp.	Rennet protease
Trichoderma viride	Cellulase

The single cell, cell proteins is the area which has already been discussed and here fungal biotechnology has much to offer. Another area where fungal biotechnology has been utilised is in the area of preventing senescence in organisms and strains which are useful industrially. Esser and Keller (1976) have shown that the occurrence of senescence in *Podospora* may be prevented by the synergistic action of two genes (linked genes). The ageing may be impeded when the mycelial area grown on media containing inhibitors of mito-chondrial functions like ethedium bromide on the antibiotics streptomycin or liamulin. Podospora work opens up possibilities of preventing senescence in useful strains of fungi and is full of great practical importance.

I have discussed the activities of these moulds but it is not possible to include every thing which these moulds are capable of doing. I will like to end my lecture with the quotation of Prof. B. O. Dodge, "As species of plants and animals start disappearing from the surface of the globe, fungi will always be there to dispose of the last remains". Moulds have been there on the scene much before the man appeared on the scene and doing well long before man appeared many of them are pretty certain to be around in the remote future, long after man has played his part and groped his way into dark wings or fallen through the trap door on the stage (Christensen, 1965).

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