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– Invited review –

Biology and Health Aspects of Molds in Foods and the Environment

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Abstract

Molds are eucaryotic, multicellular, multinucleate, filamentous organisms that reproduce by forming asexual and sexual spores. The spores are readily spread through the air and because they are very light-weight and tend to behave like dust particles, they are easily disseminated on air currents. Molds therefore are ubiquitous organisms that are found everywhere, throughout the environment. The natural habitat of most molds is the soil where they grow on and break down decaying vegetable matter. Thus, where there is decaying organic matter in an area, there are often high numbers of mold spores in the atmosphere of the environment. Molds are common contaminants of plant materials, including grains and seeds, and therefore readily contaminate human foods and animal feeds. Molds can tolerate relatively harsh environments and adapt to more severe stresses than most microorganisms. They require less available moisture for growth than bacteria and yeasts and can grow on substrates containing concentrations of sugar or salt that bacteria can not tolerate. Most molds are highly aerobic, requiring oxygen for growth. Molds grow over a wide temperature range, but few can grow at extremely high temperatures. Molds have simple nutritional requirements, requiring primarily a source of carbon and simple organic nitrogen. Because of this, molds can grow on many foods and feed materials and cause spoilage and deterioration. Some molds can produce toxic substances known as mycotoxins, which are toxic to humans and animals. Mold growth in foods can be controlled by manipulating factors such as atmosphere, moisture content, water activity, relative humidity and temperature. The presence of other microorganisms tends to restrict mold growth, especially if conditions are favorable for growth of bacteria or yeasts. Certain chemicals in the substrate may also inhibit mold growth. These may be naturally occurring or added for the purpose of preservation. Only a relatively few of the approximately 100,000 different species of fungi are involved in the deterioration of food and agricultural commodities and production of mycotoxins. Deteriorative and toxic mold species are found primarily in the genera *Aspergillus, Penicillium, Fusarium, Alternaria, Trichothecium, Trichoderma, Rhizopus, Mucor* and *Cladosporium*. While many molds can be observed as surface growth on foods, they also often occur as internal contaminants of nuts, seeds and grains. Mold deterioration of foods and agricultural commodities is a serious problem world-wide. However, molds also pose hazards to human and animal health in the form of mycotoxins, as infectious agents and as respiratory irritants and allergens. Thus, molds are involved in a number of human and animal diseases with serious implication for health.

Key words : molds, fungi, mycotoxins, food spoilage, food safety

INTRODUCTION

The kingdom Fungi includes eucaryotic organisms which range from microscopic, unicellular yeasts, to multicellular molds to the macroscopic mushrooms. Fungi are heterotrophic organisms which obtain food by absorption and require organic compounds for energy and carbon. In terms of food safety and food spoilage by fungi, we are concerned primarily with molds and yeasts. This paper discusses the properties of molds and examines the implications to health of molds in foods and the environment.

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MOLDS

Molds are eucaryotic, multicellular, multinucleate, filamentous organisms that grow as compact masses of intertwining, branching, hairlike filaments[1,2]. The total mold organism can frequently grow to macroscopic size and be visible to the unaided eye. Thus, the molds are not true microorganisms, but parts of mold structures are microscopic in size. Molds are sometimes referred to as microfungi because they are much smaller than the so called macrofungi, such as mushrooms. The individual mold filaments are called hyphae, (sing., hypha) and a mass of these branching filaments, forming a mold colony or portion of mold growth is referred to as a mycelium or the plural, mycelia. The hyphae may be submerged, or growing in a food substrate. The submerged hyphae, also called vegetative hyphae, serve to anchor the mold to the substrate and take up nutrients and water by absorption. In this respect they are not unlike plant roots. Hyphae that grow above the substrate, the visible part of the mold, are called aerial or fertile hyphae, because they give rise to reproductive structures known as conidiophores or sporangiophores which produce millions of spores ; conidiospores or conidia, and sporangiospores or sporangia.

Conidiospores are produced free by special cells on the ends of conidiophores and are not enclosed in any type of structure (Fig. 1). Conidia are microscopic in size, very light-weight and are very dry. The conidia are not easily wetted and are hydrostatic, tending to associate with, and behave like dust particles.

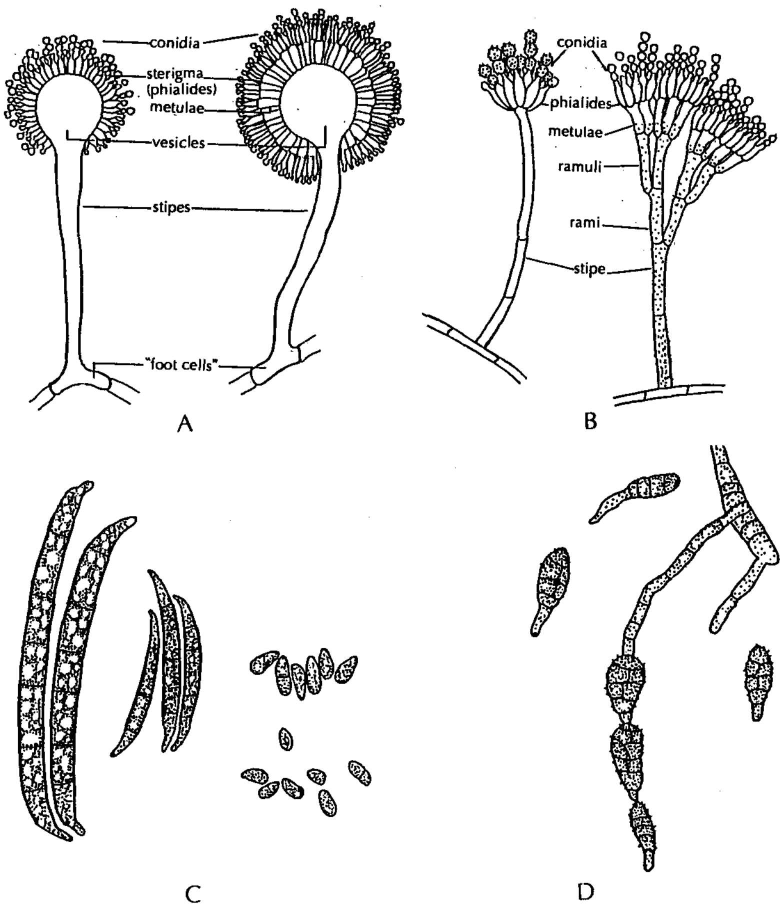

Fig. 1. Examples of conidiophores and conidia or conidiospores of several different mold genera, *A. Aspergillus, B. Penicillium, C. Fusarium* macroconidia and microconidia and D. *Alternaria*.

Fig. 2. Sporangiophores and sporangiospores common to *R-hizopus* and *Mucor.*

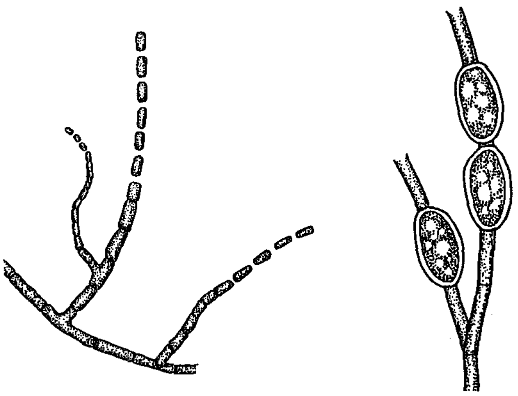

Fig. 3. Arthrospores(A) and chlamydospores(B).

Thus, these spores are readily spread through the air and are disseminated on air currents to new surfaces and habitats. If the spores settle where conditions are favorable for growth, they rapidly germinate and begin to form a new mold colony. Sporangiospores are spores that are produced in an enclosed sac-like structure, called a sporangium, at the end of the sporangiophores (Fig. 2). These spores are released into the air when the sporangium ruptures. Conidia and sporangiospores are common in the molds found in

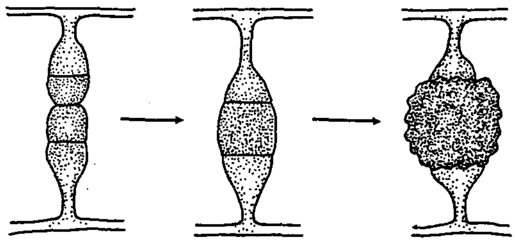

Fig. 4. Formation of a zygospore.

foods. In addition, individual molds may form other types of spores, such as arthrospores, resulting from fragmentation of septate mycelia, and chlamydospores which result from a thick wall that develops around individual mycelial cells(Fig. 3). These spores are all referred to as asexual spores, that is they are formed by non-sexual means and without exchange of any genetic material by two different molds. Many of the molds that are important in foods reproduce in this way, without sexual stages in their life cycles, and are placed in a group known as the *Fungi Imperfecti*. These are molds whose sex cycles are not known[3,4].

Some of the molds important in foods also reproduce by sexual means in addition to asexual means. They form other types of spores, by sexual processes, including ascospores and zygospores. These are referred to as "Perfect" molds, or the perfect or teleomorphic state. Ascospores are usually formed in some type of an enclosed saclike structure located down in the mycelial mass. These structures are known as asci (sing., ascus). The asci are enclosed in a fruiting body called an ascocarp. An ascocarp that is spherical or flask shaped and with no opening is known as a cleistothecium. A spherical or flask shaped ascocarp with an opening (ostiole) is called a perithecium. A saucer or cup shaped ascocarp that is open is known as an apothecium. A zygospore is formed when the tips of two hyphae come together and their contents fuse. The zygospore develops as a thick-walled structure between the two hyphal tips (Fig. 4). All sexual spores result from the fusion of two haploid nuclei[5].

OCCURENCE

Molds are ubiquitous organisms, which are found everywhere[2~4]. The natural habitat of most molds is the soil where they grow on and break down decaying vegetable matter. Molds rot wood, leaves and other organic debris to form humus in the soil. During growth on organic debris, molds form their spores which are readily picked up by air currents and widely disseminated. When there is decaying organic matter in an area, there are often high numbers of mold spores in the atmosphere of the environment. Thus most plant materials including grains and seeds will have a microflora that includes mold spores. In addition some grains and seeds will be colonized by mold while yet in the field, and these too will become a part of the microflora of these products. Many of these molds are capable of growth within the seed under proper conditions. Molds therefore are common contaminants of food and animal feed materials, and occur throughout the environment[3].

GROWTH REQUIREMENTS

Molds can tolerate relatively harsh environments and adapt to more severe stresses than most microorganisms[6,7]. Molds require less available moisture for growth than bacteria or yeasts and can grow on substrates containing concentrations of sugar or salt that bacteria can not tolerate. Molds can also grow on drier substrates than bacteria and can survive in dehydrated environments. Molds can tolerate and grow in high concentrations of acid and over a wide pH range (2.0~9.0). Since molds are slower growing than bacteria and yeasts, the dry or acidic conditions that inhibit the growth of these organisms, especially bacteria, favor the growth of molds. If competitive growth of bacteria is eliminated by either low moisture or low pH, mold growth will be enhanced. Thus mold growth in grains and animal feeds with lowered moisture content may sometimes occur if the moisture content is not too low. Some molds can obtain moisture from the atmosphere, as well as the respiration of other organisms, such as insects, and begin to grow at even low moisture levels. Once mold growth begins it likely will continue, being fed by moisture released through its own respiration.

Most molds are highly aerobic, that is they require oxygen for growth, and an abundant supply of oxygen enhances growth. Carbon dioxide on the other hand inhibits mold growth, and if the CO_2 concentration gets high enough it can totally prevent mold growth[7]. Some molds are better adapted to growth in elevated concentrations of CO_2 than are others.

Molds also grow over a wide temperature range, but most have their optimum growth temperatures between 25 and 35° C (75~94° F). Mold growth is especially rapid under conditions of high temperatures and high humidity. Some molds can grow at temperatures as low as 0 to 5° C (32 to 41° F) and a few species can even carry on growth processes just below freezing. A few molds can grow at extremely high temperatures such as above 60° C (140° F).

Molds have simple nutritional requirements. They can utilize a range of organic substrates, from simple to complex, requiring primarily a source of carbon and simple inorganic nitrogen[2,3]. Molds are capable of synthesizing their own vitamins and growth factors. Molds can readily utilize simple carbon sources such as glucose and other sugars, as well as complex carbohydrates such as starch and cellulose. Molds can also utilize inorganic nitrogen in the form of nitrate and ammonium salts, and organic nitrogen such as found in proteins, amino acids and nucleic acids. Because molds possess a vast array of hydrolytic enzymes they are capable of dissimilating and utilizing a very wide array of substrates. Thus most organic materials are subject to deterioration by molds if the conditions of moisture and temperature are not limiting.

MOLDS AND FOOD SPOILAGE

Molds are very efficient in converting nutrients into cell material and mycelial biomass. If a substrate is composed of nutrients in moderate amounts, most of the substrate will be converted to cell biomass and products of primary metabolism for essential life processes. If nutrients are available in large or excessive amounts, a variety of breakdown products may be excreted into the medium and storage reserves of carbohydrates and lipids may accumulate in the mycelia. At some point in the life cycle of the mold, when growth slows and conditions are appropriate, the mold may convert these carbohydrates and lipids to alcohols, organic acids and complex heterocyclic biochemical compounds. This process is known as secondary metabolism, since the metabolites or compounds produced have no apparent purpose in essential life processes. These "secondary metabolites" may thus accumulate in the substrate causing off-flavors and other problems. The filamentous hyphae of the molds are well adapted to growth over surfaces and through or into porous and solid substrates. The hyphae provide a large surface area relative to the mold biomass to excrete enzymes that break the substrate down into available nutrients, which in turn are absorbed back into the hyphae. The nutrients may be transported to the actively growing hyphal tips where they are utilized for energy and to produce primary metabolites, and form new active cytoplasm. The nutrients may also be used for maintenance of cellular activities or be converted to cellular storage reserves and secondary metabolites.

As a result of the metabolic activity of molds in a substrate a number of consequences can occur which may be desirable or undesirable. If the substrate is a food or an animal feed, the biochemical activities of the mold may result in deterioration and spoilage as the substrate is broken down, and undesirable by products accumulate causing off-flavors, loss of dry matter and nutrients, as well as other problems. Particularily in drier substrates, such as grains and animal feeds, such deterioration will result in spoilage. Some of the secondary metabolites of certain molds are compounds that are toxic to humans and animals. These toxic substances are collectively known as "mycotoxins"[8-12].

Control of mold through processing and storage

Mold growth is affected by a number of factors including atmosphere, moisture content, relative humidity, temperature, microbial competition and chemicals in the substrate[13]. Since molds are very tolerant of acidic conditions, and have few nutritional requirements, the pH and nutrient content of a substrate can not be used to greatly affect the ability of molds to grow.

Moisture and temperature however, are probably the two most critical factors affecting mold growth that can be used as control factors, and it is difficult to discuss one without the other. The moisture content of a substrate is less meaningful in understanding the effect of water on mold growth than is water activity (a_w). Water activity has taken the place of moisture content as the most useful expression of the availability of water for microbial growth. The lower the a_w, the less water is available to molds for growth. Water activity, and not absolute water content is what microorganisms encounter and what determines their ability to grow. Moreover the a_w of the substrate is affected by the relative humidity (RH) of the environment in which the substrate is found. Relative humidity refers to the moisture in the atmosphere surrounding the substrate, and a_w is a property of the substrate and its moisture content. In a closed system, the water activity of a substrate and the relative humidity of the atmosphere surrounding it will be in equilibrium.

The concept of water activity is important in understanding how molds may grow in a seemingly dry substrate. Conditions such as high humidity of a microenvironment affected by insect respiration can cause the a_w of a small portion of a substrate to rise to a level that may permit mold growth. Once growth has been initiated, respiration of the mold will also contribute to increasing a_w of the surrounding substrate. In this way the mold growth become a spreading, self-perpretuating process, with the pocket of growth becoming ever larger. This is what causes "hot spots" in a mass of stored grain or animal feed.

The minimum water activities at which micrc organism associated with foods and feeds will grow have been studied extensively. The minimum water activities for the general groups of microorganisms are given in Table 1. Halophilic bacteria, xerophilic molds and osmophilic yeasts are adapted to growth at very low water activities. Most spoilage and toxigenic molds grow over an a_w range of 0.72 to 0.94. Mold growth is completely inhibited at a_w values

Table 1. Minimum water activity requirements of microorganisms

Microorganism	Minimum a_w
Most Spoilage Bacteria	0.90
Most Spoilage Yeasts	0.88
Most Spoilage Molds	0.80
Halophilic Bacteria	0.75
Xerophilic Molds	0.65
Osmophilic Yeasts	0.60

below 0.65. This would be equivalent to moisture contents well below 20%.

Temperature and water activity interact to affect microbial growth. If temperature is near the optimum growth temperature of a mold, the range of a_w over which that mold can grow will be greatest. And at any temperature the ability of a mold to grow will be reduced as the a_w is reduced. Conversely if the a_w of a substrate is high, molds will be able to grow over a wider range of temperatures and will be able to grow at lower temperatures.

Atmospheric gases, other than moisture, also affect mold growth[7]. Molds require oxygen(O_2) to grow, and are inhibited by increasing concentrations of carbon dioxide (CO_2) or decreasing concentrations of oxygen from those found in air. A concentration of 40% CO_2 in air depresses fungal growth, but the CO_2 level must be increased to more than 90% to completely inhibit growth. Likewise reducing the O_2 content of air to less than 2.0% depresses mold growth, but to prevent growth the O_2 level must be reduced to 0.2%. Complete replacement of air with a nitrogen atmosphere will also inhibit mold growth. Controlled atmosphere (CA) storage of commodities has been studied as a means of preventing mold deterioration. Both the CO_2 and O_2 concentration are usually controlled. Controlled atmosphere with 10% CO_2 and 2.0% O_2 greatly increases the time for mold growth to begin and reduces the amount of growth.

The presence of other microogranisms tends to restrict fungal growth if conditions are favorable for the growth of the other microorganisms[2,3]. Bacteria and yeasts are capable of more rapid growth than molds and tend to overgrow molds. The rapid growth of bacteria on fresh meat, for example, is the probable reason molds are rarely seen growing on these substrates. Lactic acid bacteria have been shown to be competitive with molds, and to suppress aflatoxin production. Molds are likewise competitive with each other, and under certain conditions one mold may prevent the growth of another mold or may alter its growth patterns and metabolism. Competition by bacteria and yeasts, and between mold species is affected by the microenvironment of the substrate. The a_w, RH and temperature all impact on competition and growth and will determine which organism or group of organisms will predominate.

Mold growth is also affected by chemicals in the substrate which have antimicrobial or antifungal properties[6,14]. These chemicals may be naturally occurring compounds in the substrate, or they may be added for the purpose of preservation. Naturally occurring substances such as benzoic acid in cranberries, and components of essential oils in herbs and spices may restrict or prevent fungal growth. Mold growth may also be prevented in foods and feeds by the addition of antifungal or antimycotic chemicals. These substances may be certain organic acids, or salts of these acids, such as sorbic, propionic and benzoic among others, antibiotics such as natamycin, chemical dyes such as gentian violet in the case of poultry feeds, antioxidants or combinations of these and various other chemicals. In most cases the levels of the chemicals used are such that they are fungistatic, that is they prevent or delay mold growth, but do not kill or completely inhibit growth for an indefinite period of time.

Specific genera of molds

There are about 100,000 different species of fungi, though relatively few of these are directly involved in the deterioration of food and agricultural products, and/or mycotoxin production in these products. Genera that are of particular interest because of their involvement in deterioration of foods and agricultural commodities, and potential mycotoxin production are as follow : *Aspergillus, Penicillium, Fusarium, Alternaria, Trichothecium* and *Trichoderma*. Other genera, important primarily as spoilage organisms, include *Rhizopus, Mucor,* and *Cladosporium*. The most significant mycotoxin producing molds are considered to be numerous species in the genera *Aspergillus, Penicillium* and *Fusarium*. *Alternaria, Trichothecium* and *Trichoderma* contain a few individually toxic species that are important.

For detailed description of these and other genera of molds, it is necessary to refer to specific taxonomic keys for each genus. Taxonomic keys for identification of different mold genera will be found in Beuchat[3], Klich and Pitt [15], Nelson et al.[16], Pitt[17], Pitt and Hocking[18], and Samson and von Reenen-Hoekstra[5].

Recognizing molds

Molds are recognized primarily as visible fuzzy, cottony or colored growth on the surfaces of substrates such as foods or other organic materials. In grains, seeds, and animal feeds however, such growth may not always be visible in this manner. Internal mold contamination and growth in nuts, seeds and grains may not always be visible as filamentous growth. However, mold growth in nuts whole grains or seeds often results in discoloration and altered appearance. In corn (including popcorn) for instance, invasion and growth of mold in the germ area may produce darkened, greenish or bluish colored germ areas in affected kernels. When this growth is due to certain blue colored *Aspergillus* or *Penicillium* species, and a bluish line appears in the germ area, the condition is known as "blue eye". In other grains such as wheat, white to pinkish discolored and shriveled kernels may indicate mold invasion. Likewise with nuts, shriveled or misshapen kernels may be due to mold, though that is not the only cause of such problems.

Health implications

Mold deterioration of foods and agricultural commodities is a serious problem world-wide. Besides deterioration of food and feed products by causing damage that reduces quality, grades and prices, resulting in economic loss, the presence of molds in foods and commodities pose hazards to human and animal health[8-12].

A serious hazard associated with mold growth in foods and feeds is the possible production of mycotoxins, substances which are toxic and potentially carcinogenic. Additional hazards arise from the presence of the molds themselves. A wide spectrum of human and animal diseases are attributable to molds, ranging from mycotic abortion to aflatoxin poisoning in animals, and from allergic reactions to life-threatening systemic infections in humans. In recent years, invasive mycoses have become a more frequent and important cause of morbidity and mortality in humans, particularly in individuals who are immunosuppressed as a result of organ transplantation, underlying disease (AIDS), chemotherapy, radiation therapy or age. The environment, including foods, is the source of these molds. Certain occupational niches, such as grain handling and storage, pose yet additional hazards to agricultural workers through exposure to high levels of mold spores and endotoxins in grain dusts. These dusts may also be a significant source of exposure to mycotoxins. Various lung diseases in farmers and their food producing animals are associated with molds and grain dust. Thus molds can affect human and animal health by the production of mycotoxins, by causing infections and by causing allergenic reactions. Given the widespread distribution of molds in the environment and the broad range of effects on health, the implications for human and animal health are serious and increasing in importance.

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