Microorganisms Important in Food Microbiology

Food Microbiology (MIC4003a)

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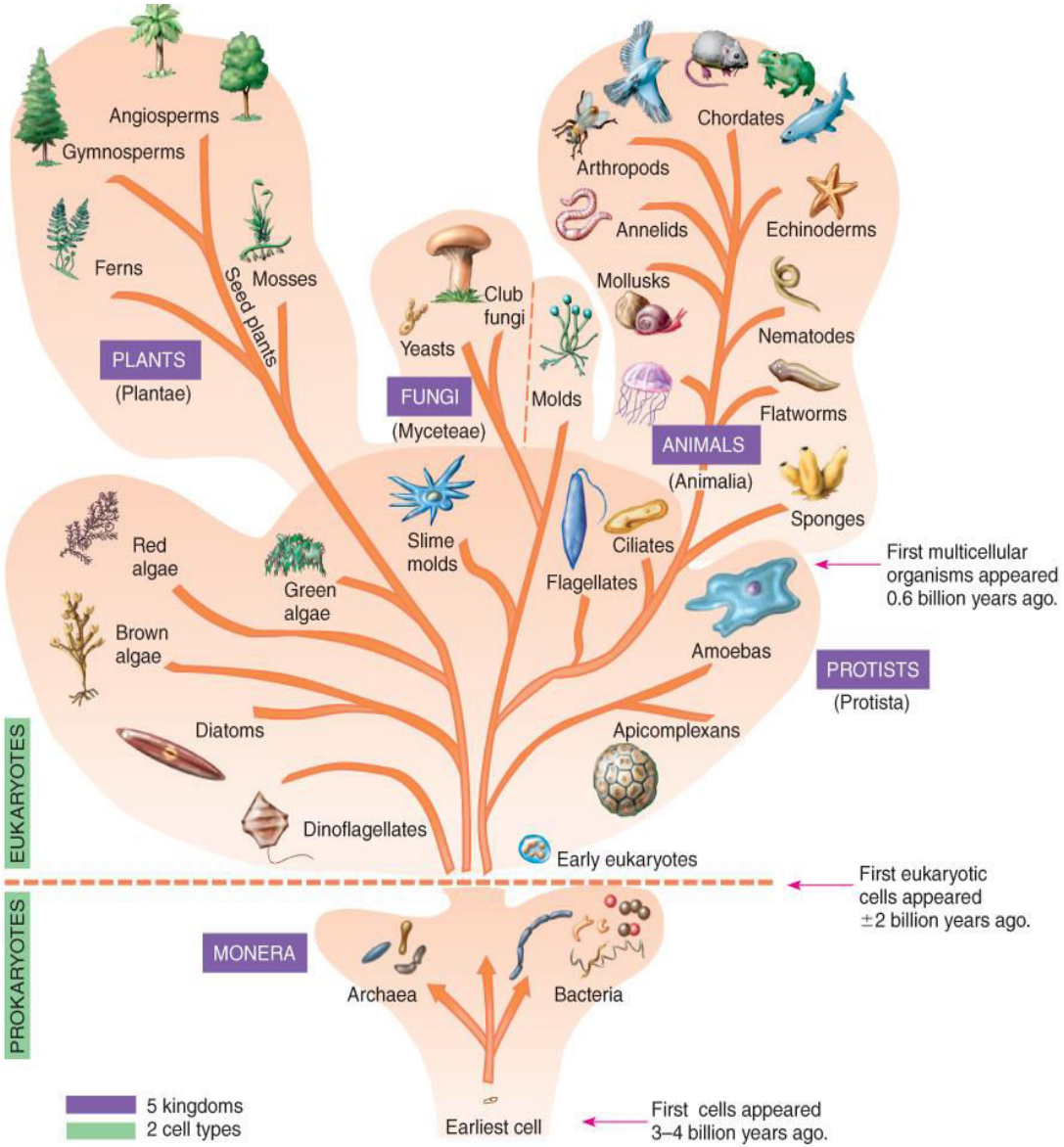
Introduction

Food microbiologists must become acquainted with the microorganisms important in foods.

- **Molds**
- **Yeast**
- **Bacteria**

Whittaker five kingdom classification



Characteristics of Fungi

Different Body form

- **Unicellular**
- **Filamentous** (tube-like strands called hypha (singular) or hyphae (plural)
- **Mycelium** - aggregate of hyphae
- **Sclerotium** - hardened mass of mycelium that generally serves as an overwintering stage.
- **Multicellular** such as mycelial cords, rhizomorphs, and fruit bodies (mushrooms)

- **Saprophytes or saprobes**- feed on dead tissues or organic waste (decomposers).
- **Symbionts**-mutually beneficial relationship between a fungus and another organism.
- **Parasites**- feeding on living tissue of a host.

MOLDS

- Mold growth on foods with its **fuzzy or cottony appearance**, sometimes colored. Usually food with a moldy or “mildewed” food is considered unfit to eat.

- While it is true that molds are involved in the spoilage of many kinds of foods, special molds are **useful in the manufacture of certain foods** or ingredients of foods.

- Molds have been **grown as food** or feed and are employed to produce products used in foods, such as amylase for bread making or citric acid used in soft drinks. Some mold do produce various **toxic metabolites** (mycotoxins).

GENERAL CHARACTERISTICS OF MOLDS

The term “mold” is a common one applied to certain **multicellular, filamentous fungi** whose growth on foods usually is readily recognized by its fuzzy or cottony appearance. The main part of the growth commonly appears white but may be colored or dark or smoky. Colored spores are typical of mature mold of some kinds and give color to part or all of the growth. The **thallus**, or vegetative body, is characteristic of **thallophytes**, which lack true roots, stems, and leaves.

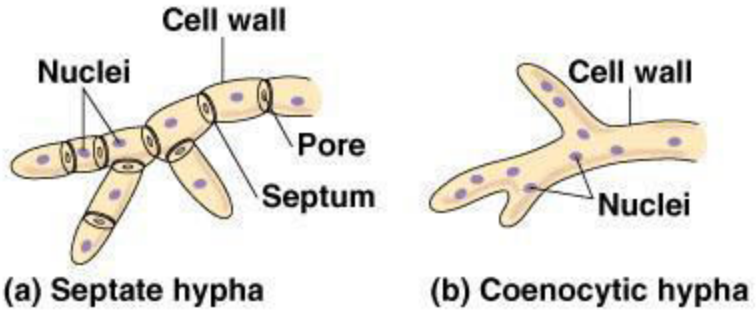
Morphological Characteristics

- **Hyphae and Mycelium:** The mold thallus consists of a mass of branching, intertwined filaments called hyphae (singular hypha) , and the whole mass of these hyphae is known as the mycelium.
- The hyphae may be submerged or growing within the food or aerial or growing into the air above the food.
- Hyphae also may be classed as **vegetative** or growing, and hence involved chiefly in the nutrition of the mold or **fertile**, involved in the production of reproductive parts.
- In **most molds** the **fertile hyphae** are **aerial**, but in **some molds** they may be **submerged**.
- A few kinds of molds produce **sclerotia (singular sclerotium), which are tightly packed masses of modified hyphae, often thick-walled, within the mycelium.**
- These sclerotia are considerably more resistant to heat and other adverse conditions.

Different types of molds

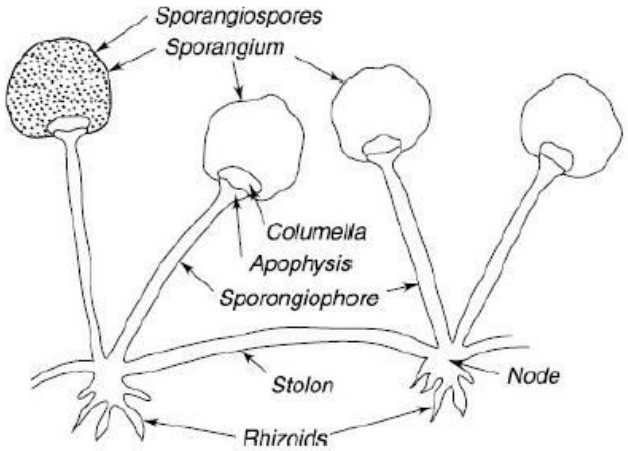
Septate- with cross walls dividing the hypha into cells. Septate hyphae increase in length by means of division of the tip cell (**apical growth**) or of cells within the hypha (**intercalary growth**), the type of growth being characteristic of the kind of mold.

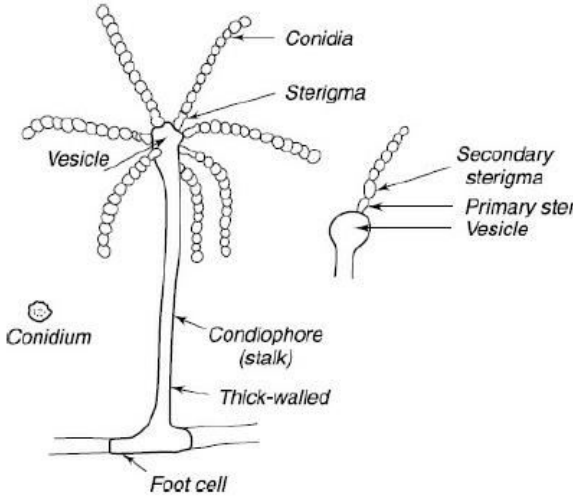
Non-septate or coenocytic- with the hyphae apparently consisting of cylinders without cross walls. The nonseptate hyphae have nuclei scattered throughout their length.

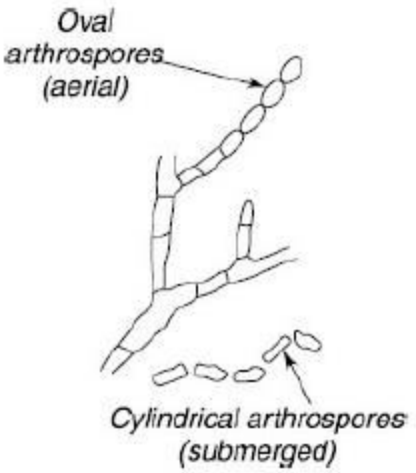


Special mycelial structures

- **Rhizoids** or **holdfasts** of *Rhizopus and Absidia*
- **Foot cell** in *Aspergillus, and the* dichotomous
- **Y-shaped branching** in *Geotrichum*







Reproductive Parts

Molds can grow from a transplanted piece of mycelium

Reproduction of molds is chiefly by means of asexual spores.
Some molds also form sexual spores. Such molds are termed “perfect” and are classified as either *Oomycetes or Zygomycetes* if nonseptate, or *Ascomycetes or Basidiomycetes* if septate, in contrast to “imperfect” molds, the Fungi Imperfecti (typically septate), which have only asexual spores.

- **Asexual reproduction**
- **Sexual reproduction**

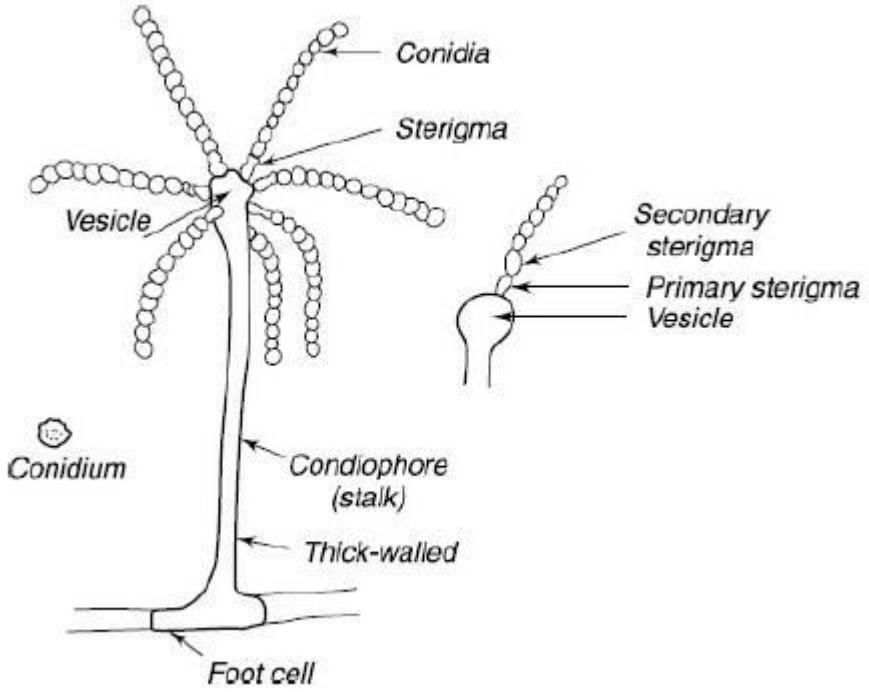
Asexual reproduction

Asexual Spores: The asexual spores of molds are produced in large numbers and are small, light, and resistant to drying. They are readily spread through the air to alight and start new mold thallus where conditions are favorable. The three principal types of asexual spores are

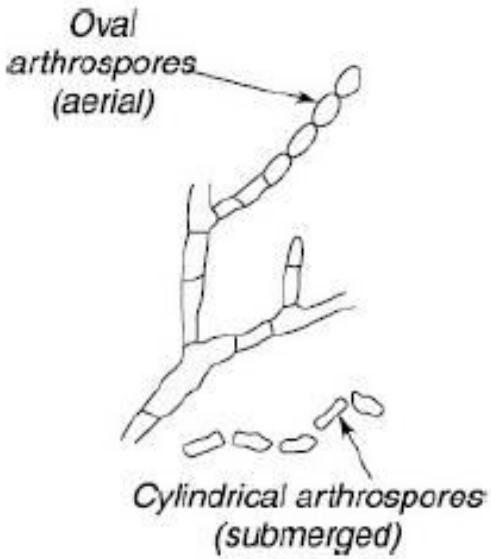
(1) Conidia (singular conidium)
(2) Arthrospores, or oidia (singular oidium)
(3) Sporangiospores

Conidia are bud from special fertile hyphae called conidiophores and usually are in the open, and not enclosed in any container.

Arthrospores are formed by fragmentation of a hypha, so that the cells of the hypha become arthrospores.



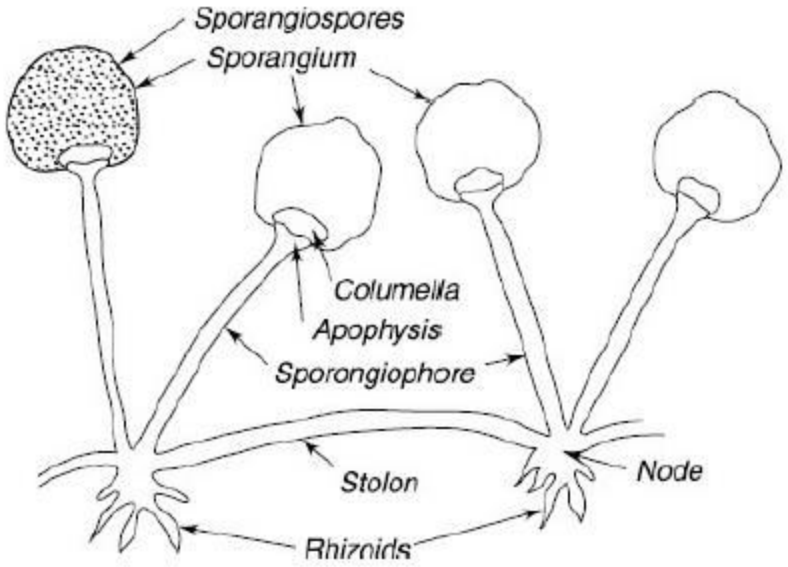
Aspergillus



Geotrichum

Sporangiospores- are formed in a sporangium or sac at the tip of a fertile hypha, the sporangiophore.

Chlamydospore is formed by many species of molds when a cell here and there in the mycelium stores up reserve food swells and forms a thicker wall than of surrounding cells. This Chlamydospore or resting cell can withstand unfavorable conditions better than ordinary mold mycelium and under favorable conditions can grow into a new mold.



Sexual reproduction

The molds which can produce sexual spores are classified on the basis of the manner of formation of these spores and the types produced.

- The **nonseptate** molds (**Phycomycetes**) that produce **oospores** are termed **Oomycetes**. These molds are mostly aquatic however, included in this group are several important plant pathogens, the "**downy mildews**" which cause late blight of potatoes and buckeye rot of tomatoes. The oospores are formed by the union of a small male gamete and a large female gamete.

- The **Zygomycetes** from zygospores by the union of the tips of two hyphae which often appear similar and which may come from the same mycelium or from different mycelia.

- Both oospores and zygospores are covered by a tough wall and can survive drying for long periods.

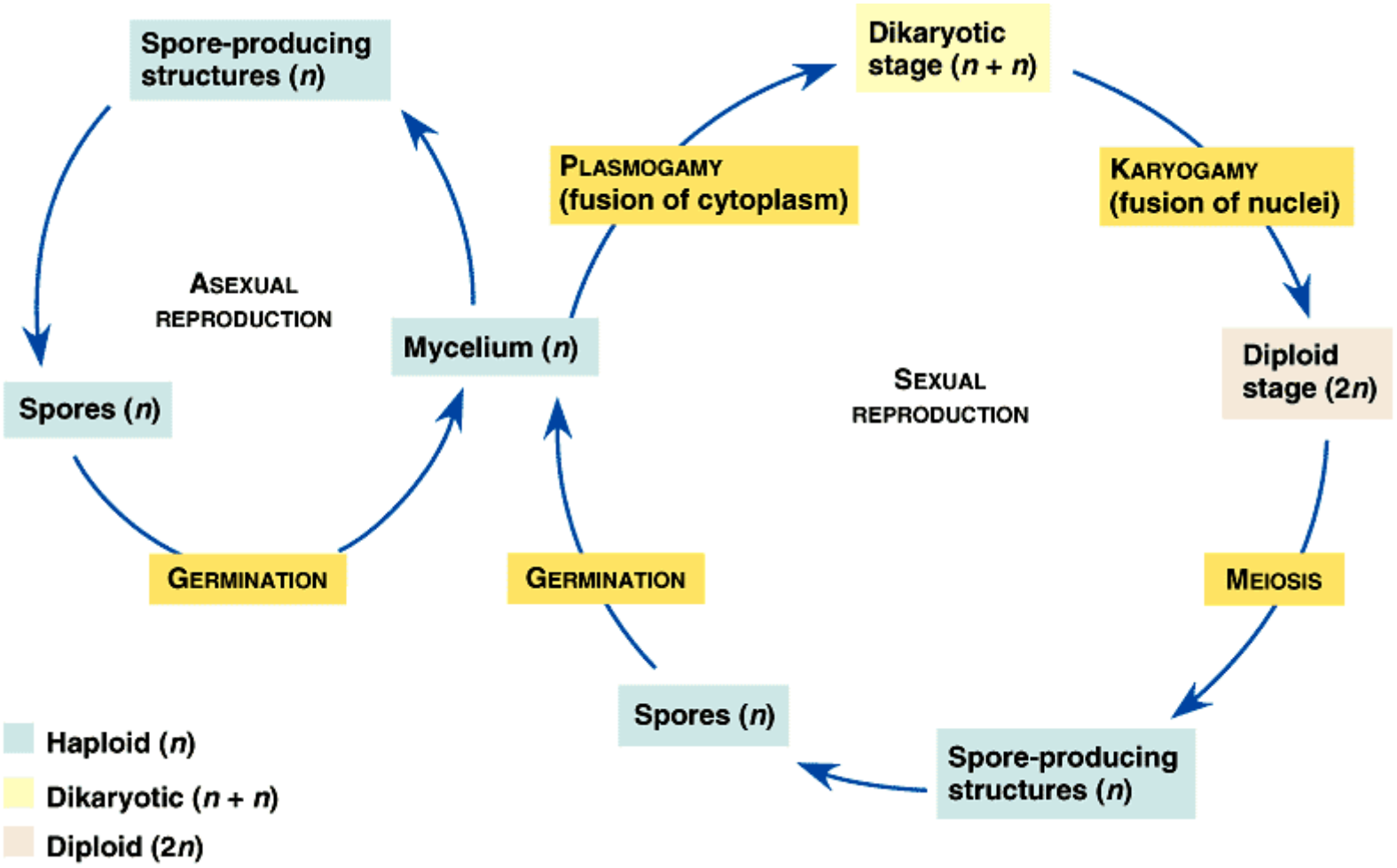
- The **Ascomycetes** (septate) from sexual spores known as **ascospores, which are formed after the union of two cells from the same mycelium or** from two separate mycelia.

- The ascospores resulting from cell division after conjugation are in an **ascus or sac with usually eight spores per ascus.**

- **The asci may be single or may be grouped** within a covering called an **ascocarp formed by branching and intertwining adjacent hyphae.**

- The **Basidiomycetes** which include most mushrooms, plant rusts, smuts, etc., form a fourth type of sexual spore, the **basidiospore**.

Life cycle of a Mold



Physiological Characteristics

- **Moisture Requirements- In general most molds require less available moisture than do most** yeasts and bacteria. It has been claimed that below 14 to 15 percent total moisture in flour or some dried fruits will prevent or greatly delay mold growth.
- **Temperature Requirements-** Most molds be considered **mesophilic** i.e., able to grow well at ordinary temperatures. The optimal temperature for most molds is around 25° to 30° C, but some grow well at 35° to 37°C or above, e.g., *Aspergillus* spp., and some at still higher temperatures.
- **Psychrotrophic- they grow fairly well at temperatures of refrigeration,** and some can grow slowly at temperatures below freezing. Growth has been reported at as low as −5° to −10°C.
- A few are **thermophilic, they have a high optimal temperature.**

Oxygen and pH Requirements: Molds are aerobic, they require oxygen for growth, this is true at least for the molds growing on foods. Most molds can grow over a wide range of hydrogen-ion concentration (pH 2 to 11). But the majority are favored by an acid pH.

Food Requirements: Molds in general can utilize many kind of foods, ranging from simple to complex. Most of the common molds possess a variety of hydrolytic enzymes, and some are grown for their amylases, pectinases, proteinases, and lipases.

Inhibitors compounds inhibitory to other organisms are produced by some molds, such as **penicillin** from *Penicillium chrysogenum* and **clavacin** from *Aspergillus clavatus*. Certain chemical compounds are mycostatic, inhibiting the growth of molds (sorbic acid, propionates, and acetates are examples), or are specifically fungicidal, killing molds.

CLASSIFICATION AND IDENTIFICATION OF MOLDS

Molds are plants of the kingdom Myceteae. They have no roots, stem, or leaves and are devoid of chlorophyll.

The following criteria are used chiefly for differentiation and identification of molds:

1. Hyphae septate or nonseptate

2. Mycelium clear or dark (smoky)

3. Mycelium colored of colorless

4. Whether sexual spores are produced and the type: oospores, zygospores, or ascospores

5. Types of asexual spores: sporangiospores, conidia, or arthrospores (oidia)

6. Characteristics of the spore head
a. Sporangia: size, color, shape, and location
b. Spore heads bearing conidia: single conidia, chains, budding conidia, or masses, shape and arrangement of sterigmata or phialides, gumming together of conidia

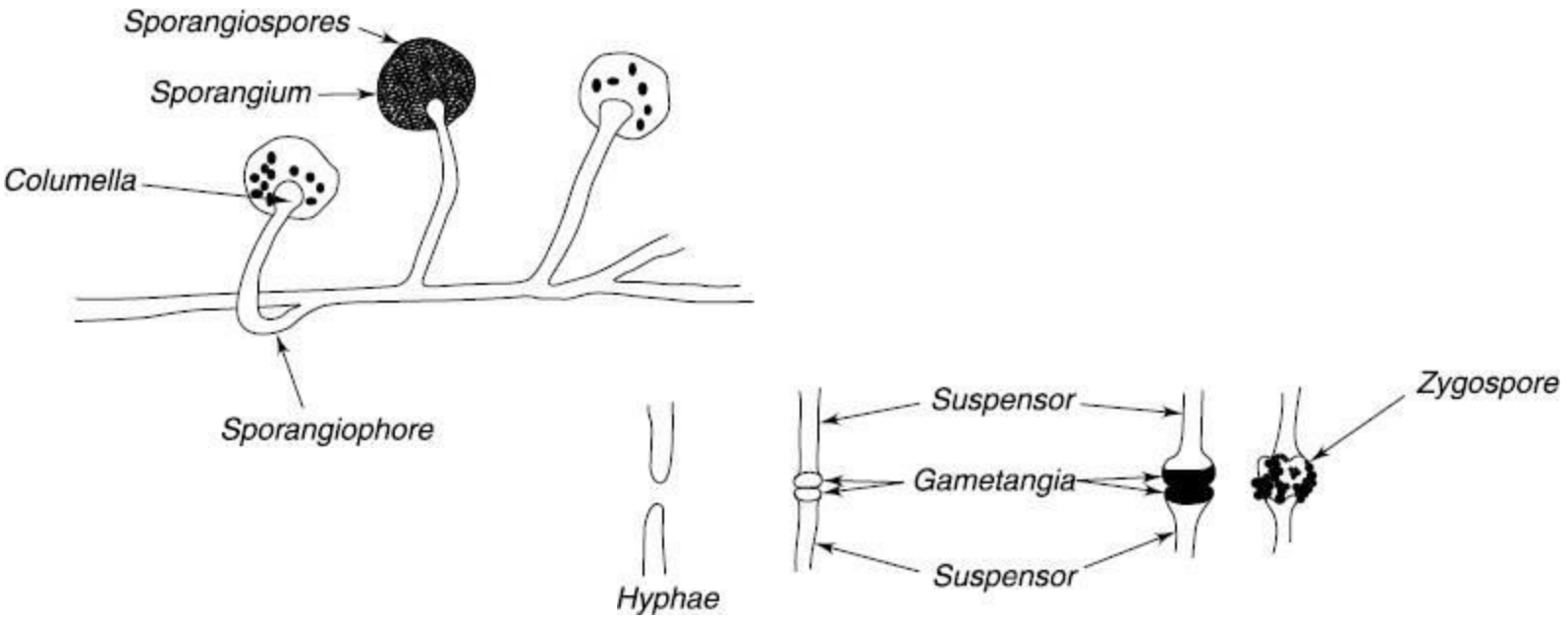
7. Appearance of sporangiophores or conidiophores: simple or branched, and if branched the types of branching, size and shape of columella at tip of sporangiophore whether conidiophores are single or in bundles

8. Microscopic appearance of the asexual spores, especially of conidia: shape, size, color, smooth or rough; one-, two-, or many-celled

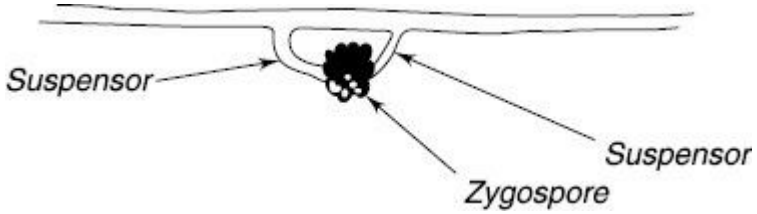
9. Presence of special structures (or spores): stolons, rhizoids, foot cells, apophysis, chlamydospores, sclerotia, etc.

Molds of Industrial Importance

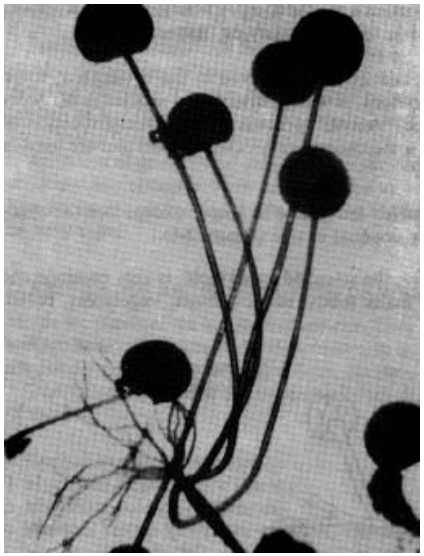
Mucor are involved in the spoilage of some foods and the manufacture of others. A widely distributed species is *M. racemosus. M. rouxii is used in the* **Amylo process** for the **saccharification of starch**, and mucor help ripen some chesses (e.g., Gammelost) and are used in making certain Oriental foods.



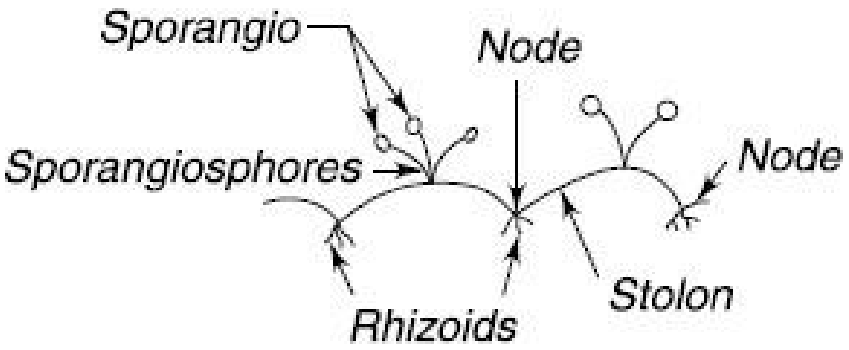
Zygorrhynchus: These soil molds are similar to Mucor except that the **zygospore suspensors** are markedly **unequal in size**.



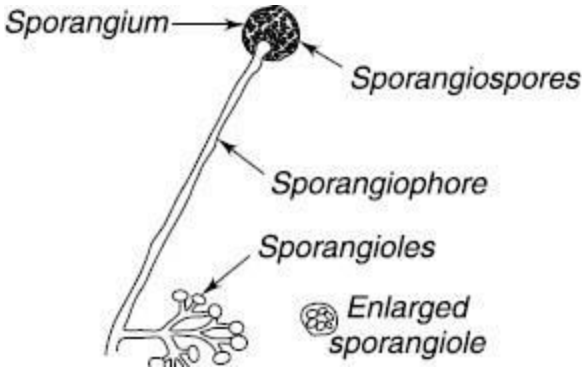
Rhizopus stolonifer **the so-called "bread mold" is very common and is involved in the** spoilage of many foods: berries, fruits, vegetables, bread, etc.



Absidia similar to *Rhizopus* except that sporangia are small and pear-shaped.

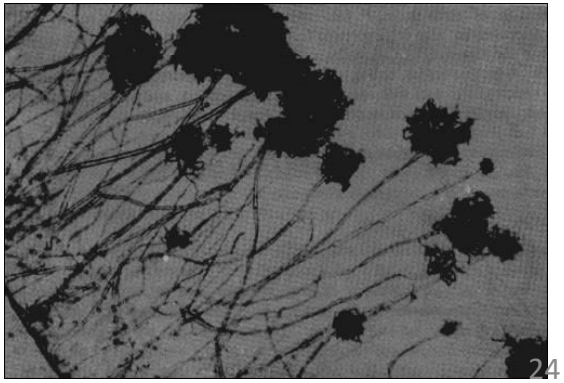


Thamnidium elegans is found on meat in chilling storage, causing **"whiskers"** on the meat.



Aspergillus- Raper and Fennell (1965) list eighteen groups of aspergilli and recognize 132 species.

- **The *A. glaucus group*, with *A. repens*** as an important species, is often involved in food spoilage. **Conidia of this group are some shade of green, and ascospores are in asci within yellow to reddish perithecia.**
- The ***A. niger*** is widespread and may be important in foods. **The spore-bearing heads are large, tightly packed, and globular and may be black, brownish-black, or purple-brown**. **Many strains have sclerotia**, colored from buff to gray to blackish. **Selected strains are used for the commercial production of citric and gluconic acids**.
- The ***A. flavus-oryzae*** group includes molds important in the marking of some Oriental foods and the production of enzymes, but molds in this group often are involved in the spoilage of foods. **Conidia give various yellow to green shades to the spore heads, and dark sclerotia may be formed.**



Penicillium- This is another genus that is widespread in occurrence and important in foods.

- The genus is divided into large groups on the basis of the **branching of the spore-bearing heads** or **penicilli**.
- These heads or **verticillata** are a whorl or cluster of three or more elements: sterigmata, metulae and branches.
- ***P. expansum*** *the* **blue-green spore mold**, causes soft rots of fruits.

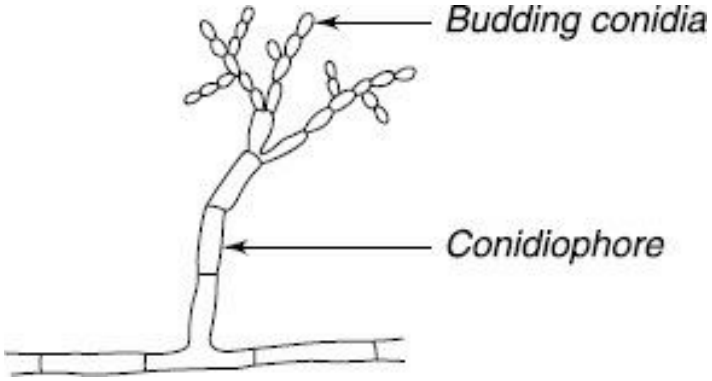
Other important species are ***P. digitatum*** *with* **olive or yellowish green conidia** causing a soft rot of citrus fruits, ***P. italicum*** called the **"blue contact mold"** with blue-green conidia also rotting citrus fruit, ***P. camemberti*** with **grayish conidia** useful in the ripening of Camembert cheese.

- A few species form asci with ascospores in cleistothecia and a few exhibit sclerotia and therefore have caused trouble in canned acid foods.

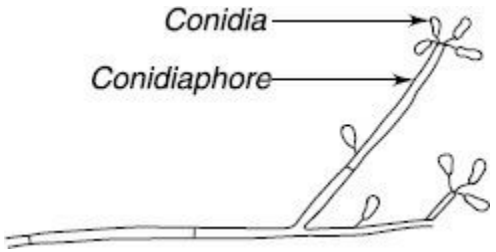
Trichothecium roseum is a "**pink mold"** which grows on wood, paper, fruits such as apples and peaches and vegetables such as cucumbers. This mold is easily recognized by the **clusters of two-celled conidia** at the ends of short, erect conidiophores. Conidia have a **nipplelike projection** at the point of attachment, and the smaller of the two cells of each conidium is at this end.

Geotrichum (Oospora or Oidium): Species may be white, yellowish, orange or red with the growth appearing first as a firm, felt like mass that later becomes soft and Creamy. *Geotrichum candidum (Oospora lactis)* often called the **"dairy mold"** gives white to cream-colored growth. The hyphae are septate and in common species are dichotomously branched. The **asexual spores are arthrospores** (oidia) which may appear rectangular if from submerged hyphae and oval if from aerial hyphae.

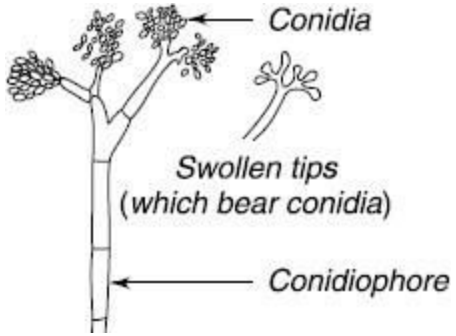
Neurospora: **This genus has been described under various names** because of the confusion concerning its classification but most mycologists believe that it should be classed among the perfect molds (producing sexual spores) and call the genus *Neurospora. Neurospora (Monilia)* the most important species in foods, sometimes is termed the **"red bread mold"** because its pink, loose-textured growth often occurs on bread. It also grows on sugarcane bagasse and on various foods. **The perfect or ascosporogenous stage is very rarely seen.**



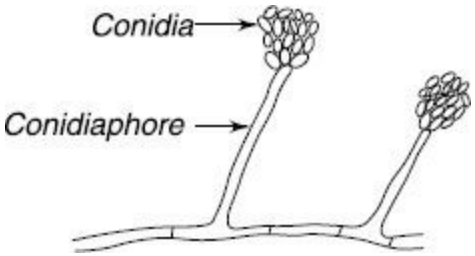
Sporotrichum **is saprophytic species and found growing on** chilled meats. It causes **"white spot."**



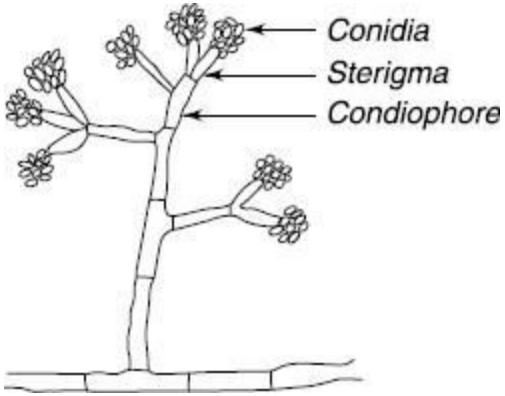
Botrytis **- *It causes a disease of grapes*** but may grow saprophytically on many foods.



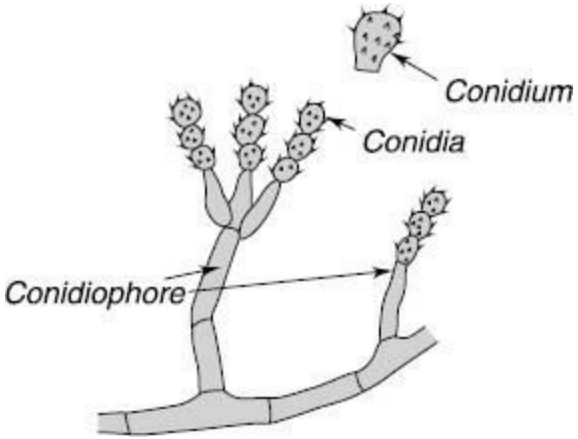
Cephalosporin acremonium



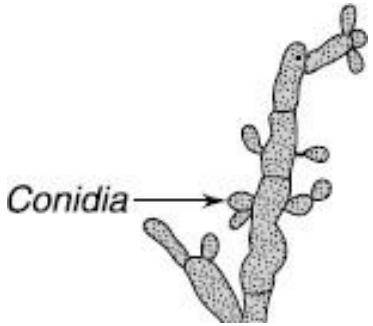
Trichoderma viride: **The mature mold plant is bright green** because the balls of green conidia are glued together and tufts of with hyphae (sterile) stick up well above the conidiophores.



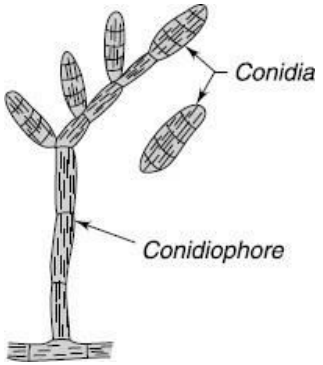
Scopulariopsis brevicaulis **is a common species. This genus may be confused** with *Penicillium* for both have brushlike penicilli and chains of spores cut off from the sterigmata **but the conidia of *Scopulariopsis are never green***. Conidiophores may be branched or unbranched in *Scopulariopsis* and the branching usually is irregular. *The* spore-bearing heads may vary from complex branching systems with penicilli to single sterigmata arising from short branches of aerial hyphae. The spores are distinctive in microscopic appearance and are **not green but commonly yellowish-brown,** they are lemon-shaped, thick walled, **spiny**, and pointed at one end, with a thick ring at the opposite end. Colonies are brownish and cottony.



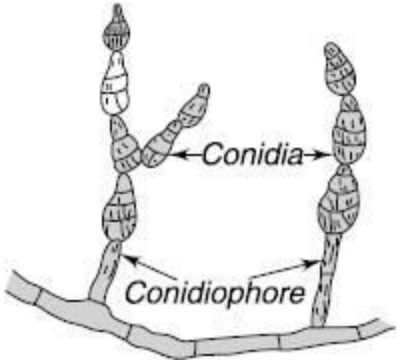
Cladosporium herbarum: These **dark molds** cause ***"black* spot"** on a number of foods on cellar walls etc. Colonies of *C. herbarum* are restricted in growth and are thick, velvety and olive to gray-green.



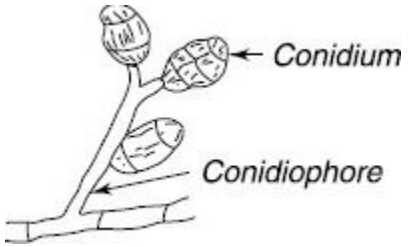
Helminthosporium: **Species of this genus are for the most part plant pathogens but** may grow saprophytically on vegetable materials.



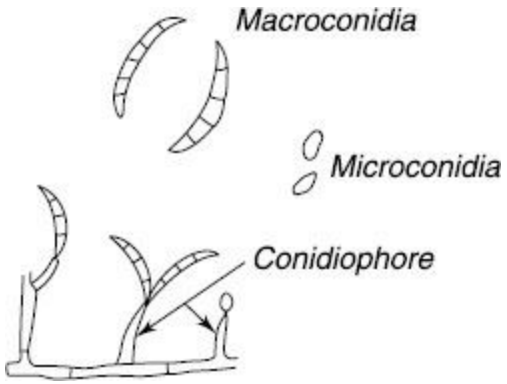
Alternaria: ***Molds of this genus are common causes of the spoilage of foods. A. citri*** (rotting citrus fruits), *A. tenuis* and *A. brassicae* are common species. The mass of mycelium usually is dirty gray-green but hyphae often look nearly colorless under the microscope. The brown, many-celled conidia are in a chain on the conidiophore.



Stemphylium- The conidia are dark and multicellular but have fewer cross walls than those *of Alternaria* and are rounded at both ends.



Fusarium- Molds of this genus often grow on foods. The species are very difficult to identify and the appearance of growth is variable.



YEAST AND YEAST LIKE FUNGI

- Like mold the term “yeast” is commonly used but hard to define. As used here it refers to those fungi which are **generally not filamentous but unicellular and ovoid or spheroid and which reproduce by budding or fission**. Yeasts may be useful or harmful in foods.

- Yeast fermentations are **involved in the manufacture of foods such as bread, beer, wines, vinegar**, and surface-ripened cheese, and yeasts are grown for enzymes and for food.

- **Yeasts are undesirable when they cause spoilage of fruit juices, sirups, molasses, honey, jellies, meats, wine, beer and other foods.**

General Characteristics of Yeasts

Morphological Characteristics

Yeasts may be spherical to ovoid, lemon-shaped, pear-shaped, cylindrical, triangular or even elongated into a false or true mycelium. They also differ in size. Visible parts of the structure are the cell wall, which may be metachromatic, albuminous or starchy. Special staining is necessary to demonstrate the nucleus.

Reproduction

Asexual Reproduction

- Most yeasts reproduce asexually a process in which some of the **protoplasm bulges out the cell wall**, the bulge grows in size and finally walls off as a new yeast cell (eg.-*S. cerevisiae*).

- In some yeasts notably some of the **film yeasts** the bud appears to grow from a **tube like projection from the mother cell**. Replicated nuclear material is divided between the mother and daughter cells.

- A few species of yeasts **reproduce by fission** and one reproduces by a combination of fission and budding (eg- *Schizosaccharomyces pombe*).

Sexual reproduction

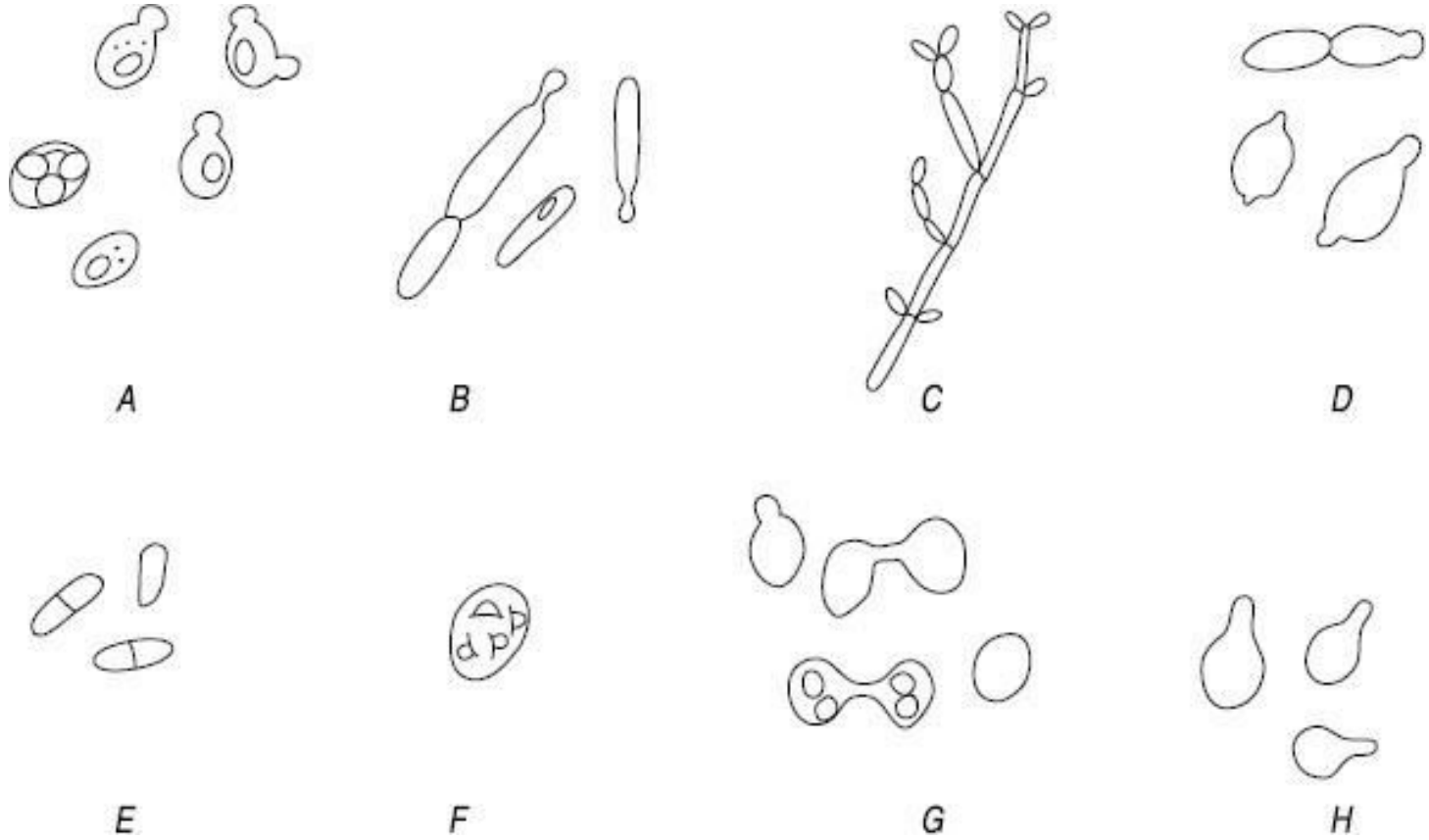
- Sexual reproduction of "true" yeasts *(Ascomycotina)* results in the **production of ascospores** the yeast cell serving as the ascus.

- The formation of ascospores follows **conjugation of two cells in most species of true yeasts** but some may produce ascospores without conjugation followed by conjugation of ascospores or small daughter cells.

- **The usual number of spores per ascus and the appearance of the ascospores are characteristic of the kind of yeast.**

- The ascospores **may differ in color, smoothness or roughness of their walls and in their shape** (round, oval, hemispherical, angular, fusiform, or needle-shaped).

Yeasts of different shapes



(A) Saccharomyces cerevisiae, with budding cells and one **ascus with four ascospores, *(B) Candida yeast***, with elongated cells*, (C) Candida, showing pseudomycelium, (D) apiculate (lemon-shaped) yeast,* ***(E)*** **Schizosaccharomyces**, *multiplying by fission, (F) Hansenula, with ascospores shaped like derby hats,* ***(G)*** **Zygosaccharomyces** showing **conjugation** with ascus and four ascospores*, (H) flask-shaped yeasts.*

Cultural Characteristics

- Growth as a film on the surface of liquid media suggests an **oxidative or film yeast** and production of a carotenoid pigment indicates the genus ***Rhodotorula***.

- However, the appearance of the **growth is important when it causes colored spots on foods**. It is difficult to tell yeast colonies from bacterial ones on agar plates the only certain way is by means of microscopic examination of the organisms.

- Most young yeast colonies are moist and somewhat slimy, most colonies are whitish but some are cream-colored or pink.

- **Fermentative yeasts** usually grow throughout the liquid and produce cardon dioxide.

Physiological Characteristics

- Most common yeasts grow best with a plentiful supply of available moisture. But since many **yeasts grow in the presence of greater concentrations of solutes** (such as sugar or salt) than most bacteria, it can be concluded that these yeasts require less moisture than the majority of bacteria.

- These ***aw* values will vary with the nutritive properties** of the substrate, pH, temperature, availability of oxygen, and presence or absence of inhibitory substances.

Classification and Identification of Yeasts

The true yeasts are in the subdivision *Ascomycotina* and the false or asporogenous yeasts are in the subdivision Fungi Imperfecti or *Deuteromycotina.*

1. Whether ascospores are formed.

2. If they are spore-forming:

(a) The method of production of ascospores:

(1) Produced **without conjugation** of yeast cells (parthenogenetically). Spore formation may be followed by (a) conjugation of ascospores or (b) conjugation of small daughter cells.

(2) Produced after **isogamic conjugation** (conjugating cells appear similar).

(3) Produced by **heterogamic conjugation** (conjugating cells differ in appearance).

(b) Appearance of ascospores, shape, size, and color. Most spores are spheroidal or ovoid, but some have odd shapes, e.g., most species of *Hansenula, which look like derby hats.*

(c) The usual number of ascospores per ascus: one, two, four, or eight.

3. Appearance of vegetative cells: shape, size, color, inclusions.

4. Method of asexual reproduction:
(a) Budding.
(b) Fission.
(c) Combined budding and fission.
(d) Arthrospores (oidia).
5. Production of a mycelium, pseudomycelium, or no mycelium.
6. Growth as a film over surface of a liquid (film yeasts) or growth throughout medium.
7. Color of macroscopic growth.
8. Physiological characteristics (used primarily to differentiate species or strains within a species):
(a) Nitrogen and carbon sources.
(b) Vitamin requirements.
(c) Oxidative or fermentative: film yeasts are oxidative, other yeasts may be fermentative or fermentative and oxidative.
(d) Lipolysis, urease activity, acid production, or formation of starch like compounds.

Yeasts of Industrial Importance

Genus Schizosaccharomyces: These yeasts, which **reproduce asexually by fission** and form **four or eight ascospores per ascus after isogamic conjugation** have been found in tropical fruits, molasses, soil, honey, and elsewhere. A common species is *S. pombe.*

Genus Saccharomyces

- Cells of these yeasts may be round, ovate, or elongated and **may form a pseudomycelium**.
- Reproduction is by **multipolar budding** or by **ascospore formation**, which may follow conjugation or may develop from diploid cells when these represent the vegetative stage.
- The ascospores **one to four per ascus** are usually round or ovate.
- The leading species *S. cerevisiae* is employed in many food industries, with special strains used for the leavening of bread, as top yeasts for wines, and for the production of alcohol, glycerol etc.
- **Top yeasts are very active fermenters and grow rapidly at 20°C**. **The clumping of the cells and the rapid evolution of CO2 sweep the cells to the surface**, hence the term top yeast. **Bottom yeast do not clump, grow more slowly, and are best fermenters at lower temperatures (10° to 15° C)**.

- ***S. cerevisiae var. ellipsoideus*** is a high-alcohol-yielding variety used to produce industrial alcohol, wines, and distilled liquors.
- *S. uvarum* a bottom yeast is used in making beer.
- *S. fragilis* and *S. lactis* because of their ability to ferment lactose may be important in milk or milk products.
- *S. rouxii and S. mellis* are osmophilic.

Genus Kluyveromyces: These yeasts reproduces by **multilateral budding** and ascopores are liberated upon maturity.

Genus Zygosaccharomyces: Some workers consider this a subgenus of *Saccharomyces*. These yeasts are notable for their ability to grow in high concentrations of sugar (**osmophilic) and are involved in the spoilage of honey, sirups, and** molasses and in the fermentation of soy sauce and some wines. *Z. nussbaumeri grows in honey.*

Genus Pichia: These oval to cylindrical yeasts may form **pseudomycelia. Ascospores are round or hat-shaped** and these are **one to four per ascus**. A **pellicle is formed on liquids**, e.g., *P. membranaefaciens* grows a pellicle on beers or wines.

Genus Hansenula: **These yeasts resemble *Pichia* in appearance but are usually more** fermentative, although some species form pellicles. Ascospores are hat- or Saturn-shaped.

Genus Debaryomyces: **These round or oval yeasts from pellicles on meat brines. Ascospores** have a warty surface. *D. kloeckeri* grows on cheese and sausage.

Genus Hanseniaspora: These lemon-shaped (apiculate) yeast grow in fruit juices. ***Nadsonia*** yeasts are large and lemon-shaped.

False Yeasts *(Fungi Imperfecti)*

Genus Torulopsis: **These round to oval fermentative yeasts with multilateral budding cause** trouble in breweries and spoil various foods. *T. sphaerica* ferments lactose and may spoil milk products. Other species can spoil sweetened condensed milk, fruit-juice concentrates, and acid foods.

Genus Candida: **These yeasts from pseudohyphae or true hyphae, with abundant budding cells or** blastospores, and may form chlamydospores. Many form films and can spoil foods high in acid and salt. *C. utilis* is grown for food and feed. *C. krusei* has been grown with dairy starter cultures to maintain the activity and increase the longevity of the lactic acid bacteria. Lipolytic *C. lipolytica* can spoil butter and oleomargarine.

Genus Brettanomyces: These ogive or arch-shaped yeasts **produce high amounts of acid** and are involved in the late fermentation of Belgian lambic beer and English beers. **They also are found in French wines**. *B. bruxellansis and b. lambicus* are typical species.

Genus Kloeckera: These are imperfect apiculate or lemon-shaped yeasts. *K. apiculata* is **common on fruits and flower** and in the soil.

Genus Trichosporon: These yeasts bud and **form arthrospores**. They grow best at low temperatures and are found in breweries and on chilled beef *T. pullulans is a common species.*

Genus Rhodotorula: These red, pink, or yellow yeasts may causes discolorations on foods, e.g., colored spots on meats or pink areas in sauerkraut.

Morphological Characteristics of Bacteria in Food

One of the first steps in the identification of bacteria in a food is microscopic examination to ascertain the **shape, size, aggregation, structure, and staining reactions** of the bacteria present. The following characteristics may be of special significance.

Encapsulation

- The presence of capsules or slime may account for **sliminess or ropiness of a food**.

- Capsules serve to increase the resistance of bacteria to adverse conditions, such as heat or chemicals.

- To the organism they may serve as a source of reserved nutrients. Most capsules are polysaccharides of dextrin, dextran, or levan.

Formation of Endospores

- Bacteria of the genera *Bacillus, Clostridium, Desulfotomaculum, Sporolacto-bacillus (rods), and Sporosarcina (cocci)* share the ability to form endospores.

- The primary interest to the food microbiologist are the spore forming species of the genera ***Bacillus*** (aerobic and some facultative anaerobic) and ***Clostridium*** (anaerobic).

- Endospores are formed at an **intracellular site**, are very refractile and are resistant to heat, ultraviolet light and desiccation.

- Lysis of the vegetative cell releases the free endospore, which may remain dormant with no detectable metabolism for years.

➢ Sporulation usually appears in the late logarithmic phase of growth, possibly because of nutrient depletion or product accumulation.

➢ During this transition of vegetative cell to spore, the spore becomes refractile, there is a **massive uptake of Ca2+ ions** and **synthesis of dipicolinic acid (DPA)** occurs, a compound absent from vegetative cells.

➢ The acquisition of heat resistance by the forming spore is closely correlated to the formation of DPA and the Ca 2+ uptake.

Formation of Cell Aggregates

It is characteristic of some bacteria to form long chains and of others to clump under certain conditions. It is more difficult to kill all bacteria in intertwined chains or sizable clumps than to destroy separate cells.

Cultural Characteristics Important in Food Bacteriology

Bacterial growth in and on foods often is extensive enough to make the food unattractive in appearance or otherwise objectionable. Pigmented bacteria cause discolorations on the surfaces of foods films may cover the surfaces of liquids, growth may make surfaces slimy or growth throughout the liquids may result in undesirable cloudiness or sediment.

Physiological Characteristics Important in Food Bacteria

- The food bacteriologist is concerned with the growth and activity of bacteria in foods and with the accompanying chemical changes.
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- These changes include hydrolysis of complex carbohydrates to simple ones, hydrolysis of proteins to polypeptides, amino acids, and ammonia or amines, and hydrolysis of fats to glycerol and fatty acids.

- Oxidation reduction reactions which are utilized by the bacteria to obtain energy from foods yield products such as organic acids, alcohols, aldehydes, ketones and gases.

- A knowledge of the factors that favor or inhibit the growth and activity of bacteria is essential to an understanding of the principles of food preservation and spoilage.

Genera of Bacteria Important in Food Bacteriology

Genus Acetobacter: **These bacteria oxidize ethyl alcohol to acetic acid. They are rod-shaped and** motile and are found on fruits, vegetables, souring fruits, and alcoholic beverages. They are a definite spoilage problem in alcoholic beverages.

Genus Aeromonas: **These are gram-negative rods with an optimum temperature for growth of 22** to 28 C. They are facultative anaerobes and can be psychrophilic. They are frequently isolated from aquatic environments. A *hydrophila* can be a human pathogen, it is also pathogenic to fish, frogs, and other mammals.

Genus Alcaligenes: **As the name suggests, an alkaline reaction usually is produced in the medium** of growth. A. *viscolactis* causes ropiness in milk, and *A. metalcaligenes* gives a slimy growth on cottage cheese. These organisms come from manure, feeds, soil, water, and dust. This genus also contains organisms which were formerly classified in the genus *Achromobacter.*

Genus Alteromonas: **Several former species of *Pseudomonas* are now classified as *Alteromonas.*** They are marine organisms that are potentially important in seafoods.

Genus Arthrobacter: **A predominant soil organism, it is inert in most foods. However, some** species can grow at 5 C and would be considered psychrotrophs.

Genus Bacillus

- The endospores of species of this aerobic to facultative genus usually do not swell the rods in which they are formed.

- Different species may be mesophilic or thermophilic, actively proteolytic, moderately proteolytic, or nonproteolytic, gas-forming or not, and lipolytic or not.

- In general the spores of the mesophiles e.g., *B. subtilis,* are less heat-resistant than spores of the thermophiles. Spores of the obligate thermophiles e.g., *B. stearothermophilus* are more resistant than those of facultative thermophiles e.g., *B. coagulans.*

- The actively **proteolytic species** usually may also sweet-curdle milk; *B. cereus* is such a species. The two chief acid and gas forming species, *B. polymyxa* and *B. macerans* sometimes are termed *"aerobacilli."*

- Several strains, as recognized by their American Type Culture Collection (ATCC) number, are important as test organisms in sterility testing.

- *Bacillus pumilus (ATCC 27142) is* recommended for determining the suitability of gamma radiation sterilization.

- *B. stearothermophilus (ATCC 7953) is recommended for testing procedures involving steam* sterilization *B. subtilis (ATCC 6633) is also used for steam sterilization procedures and as the* test organism for penicillin detected in milk.

- *B. subtilis var. niger (ATCC 9372) is recommended* for ethylene oxide sterilization testing.

Genus Brevibacterium:

***B. linens* is related to *Arthrobacter globiformis* and may be synonymous.** *B. linens* may be important in the **surface smear of certain cheeses**, *e.g., brick or Limburger,* where the culture produces an orange red pigmentation and helps ripening.

Genus Brochotrix

These are gram-positive rods which can form long filamentous like chains that may fold into knotted masses. The optimum temperature for growth is 20° to 25° C, but growth can occur over a temperature range of 0° to 45° C depending on the strain. Growth can occur between pH 5.0 and 9.0 and in the presence of 6.5 to 10.0 % NaCl. The organisms will not survive heating at 63° C for 5 min. They **can spoil a wide variety of meats** and meat products when they are stored aerobically or vacuum packed and held refrigerated. *B. thermosphacta is the only species listed.*

Genus Campylobacter

These bacteria were originally classified in the genus Vibrio. They are oxidase-positive, catalase-positive, gram-negative, curved, and S-shaped or spiral shaped. They prefer reduced oxygen tension. Several strains of *C. fetus subsp. jejuni have been associated with* gastroenteritis in humans (Chapter 24).

Genus Clostridium: The endospores of species of this genus of **anaerobic to microaerophilic bacteria** usually swell the end or middle of the rods in which they are formed. All species are **catalase-negative**. Many species actively **ferment carbohydrates** with the production of acids (usually including butyric) and gases (usually carbon dioxide and hydrogen).

- ***C. thermosaccharolyticum*** is an example of a saccharolytic obligate thermophile; this organism causes gaseous spoilage of canned vegetables.
- **Putrefaction of foods** often is caused by mesophilic, proteolytic species, such as *C. lentoputrescens* and *C. putrefaciens.*
- The violent disruption of the curd in milk by *C. perfringens* or similar species results in a "stormy fermentation" of milk, and the lactate fermenting.
- *C. butyricum* is a cause of late gas in cured cheese. The soil is the primary source of *Clostridium spp.,* although they also may come from bad silage, feeds, and manure.

Genus Lactobacillus

- **The lactobacilli are rods, usually long and slender, that form chains in most** species. They are microaerophilic, (some strict anaerobes are known), are catalase negative and gram-positive, and ferment sugars to yield lactic acid as the main product.
- They ferment sugar chiefly to lactic acid if they are **homo fermentative**, with small amounts of acetic acid, carbon dioxide, and trace products; if they are **hetero fermentative**, they produce appreciable amounts of volatile products, including alcohol, in addition to lactic acid.
- The homo fermentative lactobacilli with optimal temperatures of 37 C or above include *L. bulgaricus*, L. helveticus, L. lactis*, L. acidophilus, L. thermophilus*, and L. delbrueckii. L. fermentum is the chief example* of a heterofermentative lactobacillus growing well at higher temperatures.

Genus Pseudomonas

A number of species of Pseudomonas can cause food spoilage. These bacteria are **gram-negative**, usually **motile**, **rods and aerobic**. Characteristics of some of the *pseudomonas* species that make them important in foods are

(1) Their ability to utilize a large variety of noncarbohydrate carbon compounds for energy and their inability to use most carbohydrates

(2) Their ability to produce a variety of products that affect flavor deleteriously

(3) Their ability to synthesize their own growth factors or vitamins

(4) The proteolytic and lipolytic activity of some species

(5) Their ability to grow well at low (refrigeration) temperatures,

(6) Pigment production by some species, e.g., the greenish fluorescence by pyoverdin of *Pseudomonas fluorescens and white, creamcolored,* reddish, brown, or even black *(P. nigrifaciens*)* colors of other species,

(7) their resistance to many disinfectants and sanitizers used in the food industry.

Genus Photobacterium: The genus includes coccobacilli and occasional rods which can be luminescent. They are not widespread however, *P. phosphoreum has been known to cause* phosphorescence of meats and fish.

Genus Flavobacterium: The yellow- to orange-pigmented species of this genus may cause discolorations on the surface of meats and be involved in the spoilage of shellfish, poultry, eggs, butter, and milk.

Genus Erwinia: The species of this genus are plant pathogens that cause necrosis, galls, wilts, or soft rots in plants and therefore damage the plants and vegetable and fruit products from them. *E. carotovora* is associated with the market disease called "bacterial soft rot". in potatoes, sugar beets etc.

Genus Propionibacterium: Members of this genus may be found in foods. These bacteria are small, nonmotile, gram-positive and anaerobic to aerotolerant rods that often are coccoid and sometimes in chains. In Swiss cheese certain species (e.g., *Propionibacterium freudenreichii)* ferment the lactates to produce the gas that helps form the holes, or eyes, and also contribute to the flavor. Pigmented propionibacteria can cause color defects in cheese.

Groups of Bacteria Important in Food Bacteriology

Lactic Acid-Forming Bacteria, or Lactics

The most important characteristic of the lactic acid bacteria is their ability to ferment sugars to lactic acid. This may be desirable in making products such as sauerkraut and cheese but undesirable in terms of spoilage of wines. Because they form acid rapidly and commonly in considerable amounts, they usually eliminate for the time being much of the competition from other microorganisms. The major genera include *Leuconostoc, Lactobacillus, Streptococcus, and Pediococcus.*

Acetic Acid-Forming Bacteria

Most of acetic acid bacteria now belong to one of two genera, ***Acetobacter*** *and* ***Gluconobacter****.* Both oxidize ethyl alcohol to acetic acid, but *Acetobacter is capable of oxidizing acetic acid* further to carbon dioxide. Characteristics that make the acetic acid bacteria important are

(1) Their ability to oxidize ethanol to acetic acid, making them useful in vinegar manufacture and harmful in alcoholic beverages

(2) Their strong oxidizing power, which may result in the oxidation of the desired product, acetic acid, by undesirable species or by desirable species under unfavorable conditions, this oxidizing power may be useful, as in the oxidation of D-sorbitol to L-sorbose in the preparation of ascorbic acid by synthetic methods

(3) Excessive sliminess of some species, e.g., *Acetobacter aceti subsp, suboxydans*, that clog vinegar generators.*

Proteolytic Bacteria

➢ This is a heterogeneous group of actively proteolytic bacteria which produce **extracellular proteinases**.

➢ All bacteria have proteinases inside the cell, but only a limited number of kinds have extracellular proteinases, eg.-*Clostridium, Bacillus, Pseudomonas* and *Proteus.*

➢ Some bacteria termed "**acid-proteolytic**" carry on an acid fermentation and proteolysis simultaneously. *Streptococcus faecalis var, liquefaciens and Micrococcus caseolyticus are acid-proteolytic.*

➢ Some bacteria are **putrefactive**; i. e., they decompose proteins anaerobically to **produce foul smelling** compounds such as hydrogen sulfide, mercaptans, amines, indole, and fatty acids. Most proteolytic species of ***Clostridium*** are putrefactive.