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MICRO-ORGANISMS

- WHAT THEY ARE
- WHERE THEY GROW
- WHAT THEY DO

By C. R. Benjamin, W. C. Haynes, and C. W. Hesseltine

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WHAT THEY ARE, WHERE THEY GROW, WHAT THEY DO

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The world literally teems with micro-organisms of all kinds. They include viruses, bacteria, actinomycetes, algae, protozoa, fungi (also yeasts), and lichens. Some micro-organisms are ubiquitous, airborne forms that are much more difficult to avoid than to find. Others are so restricted in habitat that they may be found only on a particular part of the body of a single species of beetle.

It is estimated that there are 100,000 species of fungi alone. Fungi range from such ubiquitous forms as *Cladosporium*, which grows throughout the world on all kinds of plant material, to such specialized forms as *Chitonomyces*, which has had 16 species reported on a single aquatic beetle. Of these 16 species, 6 are restricted to male beetles and 9 to female. Not only are the species restricted to a single host and sex but they are also restricted to a single part of the host body.

Some kinds of bacteria have been found several miles above the earth surface or below in the mud of ocean floors. Both bacteria and blue-green algae grow in hot springs at temperatures of 75° C., and bacteria have been isolated from antarctic ice. Certain molds grow best on or near receding edges of snow on mountains. Some lichens normally grow on seemingly bare rock. Whatever the habitat, generally a variety of micro-organisms can be found.

In this publication these micro-organisms are described, references are made to their classification, some of their uses are indicated, and authoritative sources of information are cited.

VIRUSES AND RICKETTSIAE

Viruses and rickettsiae are members of the taxonomic class appropriately named Microtatobiotes, which means literally "smallest living things." Most viruses cannot be seen with the ordinary light microscope; they are ultramicroscopic and are only seen by means of an electron microscope. They pass through filters designed to retain bacteria, and in the past they were often spoken of as filterable viruses.

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Virus particles generally are less than 0.1 micron in diameter. On the other hand, rickettsiae are generally more than 0.1 micron in diameter, are retained by bacterial filters, and may be seen with the light microscope.

Both viruses and rickettsiae are parasites, depending on other living organisms for their growth and multiplication. As far as is known, none of them are free living. All hosts of rickettsiae are in the animal kingdom, but viruses find hosts among both plants and animals. Even bacteria and actinomycetes are attacked by viruses, which are designated bacteriophages and actinophages, respectively. Among the common virus diseases of man and animals are poliomyelitis, mumps, measles, foot-and-mouth disease, and rabies. Rickettsiae are causative agents of typhus, scrub typhus (tsutsugamushi disease), Q fever, and Rocky Mountain spotted fever, and are often transmitted by arthropod vectors.

Although phages may interfere with some industrial fermentations by destroying or weakening the fermentative agent, other viruses are industrially useful. Some are grown to make vaccines useful in the prevention and control of disease, such as the Salk polio vaccine and the rabies vaccine.

BACTERIA

Micro-organisms of the class Schizomycetes (fission fungi) are generally referred to as bacteria. To laymen they are better known as germs or microbes, terms that are less definitive because they may include viruses, molds, and yeasts.

Bacteria are larger than viruses and rickettsiae. Cells of the most important kinds in medicine, industry, and agriculture are from 1 to 3 microns in their largest dimension. Moreover, most bacteria are able to grow without living hosts or body fluids. They require essentially the same nutrients for growth as do higher forms of life; i.e., sources of carbon and nitrogen, essential mineral elements, and water. Many must also be provided with one or more vitamins, but many others make their own from ingredients at hand. Bacteria lack chlorophyll, the green matter of higher plants that enables them to utilize the sun's energy for the manufacture of food. A few varieties of bacteria have photosynthetic pigments chemically related to chlorophyll, which permit them to function like higher plants. Most bacteria can only utilize soluble nutrients, which enter cells through the semipermeable cell membrane.

Reproduction of bacterial cells is by simple cell division, or fission, rarely by budding. This characteristic differentiates bacteria from viruses, whose reproductive mechanism is so intimately associated with the living protoplasm of the host cells that the exact method is unknown. It also differentiates them from yeasts and molds, which reproduce by budding and full-fledged sexual processes.

Under the microscope bacterial cells are seen in three basic forms. Some are more or less spherical and are referred to as cocci. Others are cylindrical and are called rods. Curved rods are vibrios if comma shaped, and spirilla if helicoidal. The cells may occur in natural associations. Thus cocci may appear as strings of beads (streptococci),

grapelike clusters (staphylococci), pairs (diplococci), or singly. Rods, vibrios, and spirilla may also be arranged in chains and clusters of cells, but characteristic names are not commonly used for them.

Bacteria are ubiquitous. They are found in all types of soil and are most numerous in fertile soils. They inhabit all the waters of the earth, both fresh and salt, stagnant and free flowing. They are in hot springs and geysers, as well as in arctic and antarctic waters and soils. The surfaces of all plants and animals, as well as intestinal and urinary tracts of animals, are inhabited by bacteria. The air is laden with micro-organisms, including bacteria, and they have been recovered even from the air above the North Pole.

Bacteria are versatile and adaptable. By diligent searching it may be possible to find strains capable of attacking, and thereby modifying, almost any substrate and of growing under almost any set of conditions. Life as we know it is impossible without free or dissolved oxygen, except for certain bacteria that cannot grow in its presence. These are called anaerobic bacteria. Bacteria that fail to grow unless they have free access to the oxygen of the air are said to be strict aerobes. Still a third group of bacteria, said to be facultative, grow whether or not gaseous oxygen is available.

Most bacteria grow at a fairly narrow range of temperatures between 0° and 40° C. However, some can grow at about 55°, but fail to grow if the temperature falls to 30°. Such bacteria are said to be thermophilic. The osmotic pressure of body fluids, of lakes, streams, rivers, and oceans, and of vegetable juices is ideal for the growth of almost all bacteria, but the halophilic and osmophilic types grow in saturated brines and sirups.

Although bacteria generally cannot grow if the moisture content drops below 70 percent or if either temperature or osmotic pressure varies too greatly from their optima, some can survive unfavorable conditions because they form spores. Spores are specialized structures formed by relatively few bacteria. In spore form some bacteria can even withstand boiling water. For this reason, a temperature of 121° C. (250° F.) is used whenever it is desirable to destroy all life, as in preparing surgical instruments, gowns, gloves, and solutions for parenteral administration to patients in hospital and medical practice; as in sterilizing culture media and equipment in microbiology laboratories; and as in processing foods and beverages in the canning industry. Even then the time of exposure, which is governed by the size and nature of the products to be sterilized, must be at least 10 minutes, usually longer.

The systematic position of bacteria has long been a controversial matter. Some authorities believe they belong in the plant kingdom, others in the animal kingdom, and still others in an independent kingdom. Meanwhile, they are generally treated as if they belonged in the plant kingdom.

Several systems of bacterial classification are in current use, but the one followed by most bacteriologists, especially in the United States, is the system given in Bergey's Manual of Determinative Bacteriology (*4*).[2] According to it, bacteria are divided into 10 orders, 4 of which

[2] Italic numbers in parentheses refer to Literature Cited, p. 34.

include all the well-known bacteria of medical and industrial usefulness. The other six orders are largely unknown except to a few specialists.

No one knows how many species of bacteria there are. In Bergey's manual (*4*) about 1,500 species are listed in 208 genera.

Fortunately most bacteria are harmless or beneficial. It is unfortunate that most people's knowledge of bacteria is limited to those that cause disease. Almost everyone has been awed by stories of the Black Death, which decimated the populations of entire towns in Europe a few centuries ago, by the white plague, and by epidemics of diphtheria, typhoid fever, scarlet fever, and cholera. These diseases still rage in underdeveloped areas, and their still-present dangers must not be forgotten.

Even in ancient times bacteria as well as other micro-organisms were used to benefit mankind. Before food was preserved by refrigeration and before canning had been developed, men learned to ferment shredded cabbage to sauerkraut; milk to yoghurt, kumiss, and leben; milk curd to various cheeses; cucumbers and other vegetables to pickles; and wine and other alcoholic solutions to vinegar.

These processes have been developed into modern industries. We have learned to domesticate a great variety of micro-organisms and to induce them to make products of value other than food. Among the products of bacterial fermentation, apart from foods, are acetic acid, lactic acid, acetone, butyl alcohol, 2-ketogluconic acid, alpha-ketoglutaric acid, dextran, vitamin B_{12}, various enzymes, and antibiotics. In addition, vaccines, antisera, and antitoxins are produced in quantities sufficient to protect the populations of the world against the dread diseases of past generations.

ACTINOMYCETES

Actinomycetes are considered to be bacteria by some microbiologists and to be fungi by others. Still others consider them to be a distinct, isolated group, but related to both bacteria and fungi.

Actinomycetes include forms found in soil and water, although a few are animal and plant pathogens. By far the largest group produces branched mycelium and spores, which function similarly to asexual spores in fungi. The spores are not heat resistant as in the bacterial genera *Bacillus* and *Clostridium*, but they function as units of reproduction as well as cells to carry the species through periods of adverse growth conditions, such as drying. Like bacteria, the spores and the mycelium are never more than 2 or 3 microns in diameter. Like bacteria, various genera are attacked by numerous phages. In some forms sporangia develop, and the spores may be motile by means of flagella. Whether or not sexual reproduction occurs is not yet conclusively proved.

According to Bergey's manual (*4*), there are four families in the order Actinomycetales—Mycobacteriaceae, Actinomycetaceae, Streptomycetaceae, and Actinoplanaceae. These families embody nine genera and a considerable number of species, with the largest number in *Nocardia* and *Streptomyces*. The first family may be considered as more removed from the order than the last three, which are closely

related to one another. Classification of both families and genera is based exclusively on morphology, whereas that of species may depend partly on morphology but also on physiological characteristics.

In the large and important genus *Streptomyces*, classification of groups of species seems to be based best on (1) morphology of the sporophores, (2) color of the aerial part of the colony, or (3) combinations of these two characteristics. In this genus, spores germinate to form germ tubes, which then develop a colony of substrate mycelium exactly like fungi. The colonies are slower growing than most fungi, are powdery on the surface, and have a strong soil odor. From the substrate mycelium, aerial stalks are formed that branch in a characteristic fashion. Ultimate hyphal branches, at least, form spores simultaneously. A study of the spores and their development, using the electron microscope, has shown many interesting species differences in *Streptomyces*. More and more evidence indicates that these forms should be studied as fungi.

The actinomycetes produce a great variety of insoluble and water-soluble pigments. *Nocardia* attacks paraffin, phenol, and other petroleum-derived materials. *Streptomyces* forms a wide variety of antibiotic substances, some of which are of great economic importance like streptomycin, chlorotetracycline, oxytetracycline, tetracycline, chloramphenicol, and erythromycin. Some species are known to transform steroids and to produce vitamin B_{12}. As far as we know, all species can be preserved successfully in a lyophilized state provided mature spores are used.

ALGAE

Algae are the simplest of the chlorophyllous plants. Probably they were the first photosynthetic organisms in the history of life on earth and are considered the first link in the food chain of life. They are nearly omnipresent and are numerous almost any place that has sufficient moisture, such as on tree trunks, soil, rocks, and walls, and of course throughout fresh-water and marine habitats. In addition, certain algae grow in such unusual places as snow or hot springs. Frequently they grow in association with other plants or on mollusks and fish.

Algae are cellular organisms. The multicellular ones can have a fairly complex structure, but because they have no jacket of sterile cells surrounding the sex organs, they are separated from the mosses and liverworts. A few of the simpler algae are difficult to distinguish from protozoa, fungi, or bacteria, but unlike the bacterial groups, the vast majority of algae contain chlorophyll and can thus utilize the energy of sunlight to synthesize organic matter and their own protoplasm from carbon dioxide, water, and inorganic substances. The numerous exceptions generally are related to other algae by their structure, life history, and storage products; but some organisms are arbitrarily assigned to the algae or protozoa.

Generally each algal cell—or alga, where unicellular—is either surrounded by a gelatinous sheath or embedded in a gelatinous matrix. The cell wall is composed of cellulose and pectins and occasionally has in it chitin, silica, and tannins. The protoplast contains pigment bodies, nucleus, cytoplasm, and vacuoles. Each cell usually holds

some form of reserve food, like starch grains, sugar crystals, or oil droplets.

Pigmentation and the nature of the photosynthate, or kind of reserve food, are usually correlated in the various groups of algae. Frequently pigmentation is used as a basis for dividing algae into the larger categories of classification. On this basis there are blue-green, grass-green, yellow-green, brown, and red algae, and all contain chlorophyll, carotene, and xanthophyll. All these groups, except the grass-green algae, have additional pigments that mask the green of the chlorophyll. Other groups of algae contain the euglenoids, cryptomonads, and dinoflagellates.

Generally seven or eight algal groups are recognized by taxonomists, and these groups currently are considered to be of "division" rank. In all, there are probably about 18,000 described species. A good taxonomic review is that by Papenfuss (*24*), and detailed references to taxonomy and morphology may be found in Smith (*27*) and Fritsch (*10*).

Algae are of considerable practical importance. The free-floating forms, or phytoplankton, occur in great numbers and are the main source of food, directly and indirectly, for fish and other aquatic animals. Since oxygen is a byproduct of photosynthesis, algae supply oxygen to their aquatic environs during the day. However, if they are present in great numbers, they can be undesirable. Pond scum and red tide are examples, as well as slime accumulations at drinking fountains, in reservoirs, and at swimming pools.

The diatoms, members of the division or phylum Chrysophyta, are of interest because of their siliceous cell walls, or shells. They occur in large numbers in both fresh and salt water, and when they die their shells fall to the bottom of the lake or ocean. Great beds of these shells have been built up and are called diatomaceous earth. Diatomaceous earth has several uses, such as a filtering agent, insulating material, polishing powder, and a carrier for nitroglycerin in dynamite.

The most important groups of algae economically are the seaweeds, consisting of the brown algae with their giant kelp and of the red algae with their agarophytes, or agar producers. Although seaweeds have been used as sources of soda ash, potash, iodine, and food, their chief importance today arises from the phycocolloids that can be extracted from them. According to Idson (*15*), U.S. annual output of seaweed colloids increased from $2 million in 1945 to about $10.3 million in 1955. Gross sales for the industry are expected to be $20 million per year by 1975.

The three commercial seaweed colloids are agar, carrageenin, and algin. Several others, including fucoidan, laminoran, hypnean, eucheuman, furcellaran, agaroid, porphyran, and iridophycan, have little or no known commercial importance as yet, although some are said to be easily obtainable. Agar and carrageenin come from red algae, principally *Gelidium* and other genera for the former and *Chondrus* for the latter. Algin is obtained from brown algae, or kelp, and a major source is members of the genus *Macrocystis*.

An extensive treatment of the phycocolloids and their uses can be found in the book edited by Whistler and BeMiller (*28*), in which several chapters are devoted to products from red and brown algae.

Some algae are readily used in culture studies. Notable are certain of the unicellular green algae, such as members of the genus *Chlorella*, which have been utilized widely for physiological studies. Certain green algae produce sterols and vitamins in addition to their chlorophyll, carotenes, carbohydrates, proteins, and fats. Favorable results have been obtained in their use as food and feed supplements, as reported by Morimura and Tamiya (*23*). Under proper conditions of illumination and agitation, some algae also grow well in deep-tank fermentors, as shown by Pruess et al. (*25*). A pertinent reference to algal culture is the monograph edited by Burlew (*5*).

PROTOZOA

Among animals, only protozoa are unicellular and for this reason are placed in a separate phylum. Within a single protozoan cell all life functions are carried out that are found in the Metazoa or higher animals. Only a few protozoa are large enough to be observed with the naked eye. Approximately 15,000 to 20,000 species of protozoa are known (*18*). Unlike bacteria and many fungi, most studies on protozoa have been made on material not freed of other protozoa or bacteria.

Free-living protozoa are chiefly found in water, both fresh and salt, at temperatures ranging from freezing to 70° C., and to a less extent in soil and decaying organic material. During unfavorable growth conditions, cysts are formed that allow the organism to survive until growth conditions are again favorable.

Many other protozoa live in or on other organisms. A few protozoa have been isolated and grown in pure culture, but extremely complex nutritional conditions are required for growth. Defined culture media are very complex. The ciliate *Tetrahymena* has been extensively studied for its growth requirements and nutrition and has been used for the assay of some amino acids. Culture media for the growth of protozoa need not necessarily be liquid. We have seen amebae, flagellates, and ciliates grown on an agar medium. In some instances a semisolid medium for small ciliates is preferred to a solid medium.

Like the more primitive micro-organisms in other groups, some protozoa cannot be readily distinguished from algae. The genus *Euglena*, which is motile and holophytic (able to use carbon dioxide and light to manufacture carbohydrates), is studied by both protozoologists and algologists. Also, the Myxomycetes is treated in textbooks on mycology and on protozoology.

The protozoa have three kinds of locomotion. In some, for example amebae, temporary projections of part of the cytoplasm, called the pseudopodia, are formed. Others swim by means of a flagellum, which is a filamentous extension of the cytoplasm that is highly flexible and so fine that it is often difficult to see even under a microscope. The third kind of locomotion is by cilia, which are rather short projections of the ectoplasm.

Most protozoa reproduce asexually by binary fission, which takes place longitudinally, although many ciliates divide transversely during reproduction. Binary fission is the division of the single protozoan cell or body into two nearly equal daughter cells. In some, multiple

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division occurs so that several daughter cells result. Sexual reproduction also occurs. In some, gametes fuse to form a single cell or zygote. In others, two individuals conjugate for the purpose of exchanging nuclear material.

Protozoa are extremely sensitive to slight changes of temperature, light, acidity, chemical composition of the water, and kinds and amounts of food. Some protozoa apparently cannot live unless their food consists of bacteria or other minute protozoa. If a small amount of hay is placed in a beaker of water and kept in the laboratory, a whole progression of protozoa species will be observed. Those seen active during the first few days will disappear completely in a week or so.

When one collects protozoa, practically every source of water, soil, and organic material will vary in the kinds and number of species, and these will change radically even in a single collecting location depending on the season of the year. Some flagellates have an absolute symbiotic relationship to other animals. Thus the termite when freed of its intestinal flagellates dies even when its gut is filled with wood chips. When the flagellates are removed from the termite intestine, they likewise die.

Protozoa offer one advantage over algae in pure culture work—light is not required for growth. Cell for cell, protozoa have more complex cellular structures than any other group of micro-organisms, as seen with the light microscope. Protozoa cells may contain definite points in which food is ingested—contractile vacuoles, food vacuoles, several nuclei, and eye spots.

Protozoa are often divided into several classes based on the mode of locomotion. The first of these is the Mastigophora, or protozoa that possess one or more flagella. Some of the forms in this class have plantlike characteristics, like chlorophyll, and some can carry out photosynthesis in the presence of light, but when light is withdrawn, they may carry on a saprophytic nutrition as colorless protozoa do.

The second class is Sarcodina, or amebae that lack a definitive pellicle or cell covering and can form pseudopodia. Their principal means of classification is based on the types of pseudopodia they form.

The third class is Sporozoa, in which the protozoa without exception are parasitic and form spores in their development. Their hosts are scattered throughout the animal kingdom.

The remaining protozoa are forms that possess cilia sometime during their life. In the class Ciliata, cilia are present throughout the entire life cycle. Their classification is based on the nature of the nuclei and on the arrangement of cilia. The second class of protozoa with cilia is the Suctoria. At maturity they do not have cilia.

Apart from their use for biological studies, protozoa are important in reducing bacteria in soil and sewage. Many protozoa are eaten by microscopic animals and small fish, which in turn become part of the food chain for larger animals. Still other protozoa because of their skeletons are sources of diatomaceous earth. Some of the parasitic forms cause serious diseases in man and animals, such as amebic dysentery and trypanosomiasis.

FUNGI

Among the largest of the categories of micro-organisms are the fungi, which perhaps contain more complex morphological forms and more species than any of the other groups except possibly the algae. According to Ainsworth (*1*), there are more than 4,000 genera of fungi and probably 100,000 species. Fungi are in air, soil, water, plants and animals, and decaying organic matter. Mostly they are aerobic.

Yeasts, molds, and mushrooms are common names used for some types of fungi. Yeast usually refers to fungi that reproduce asexually by budding, but may include forms from any of the four major classes of fungi. Mold implies any fungus with a mycelium. Mushroom refers to fungi that produce large macroscopic fruiting bodies. In culture, mushrooms produce mycelia and can be called molds. In scientific writing it is better not to use these three terms. Two general modern textbooks on mycology are Bessey (*3*) and Alexopoulos (*2*).

Fungi are usually divided into the following classes: Phycomycetes, Ascomycetes, Basidiomycetes, Fungi Imperfecti, and Myxomycetes. The system most generally followed is that of Martin (*22*).

Phycomycetes

The class Phycomycetes contains the most primitive fungi and represents about 245 genera and more than 1,300 species. The mycelium is coenocytic; i.e., there are many nuclei within the cell walls, and septa in the mycelium are relatively few or absent. The lower forms of Phycomycetes are chiefly aquatic and usually require special techniques for isolation involving baits. Reproduction is by means of motile sporangiospores, or zoospores, except in the four orders where nonmotile conidia or sporangiospores are formed. Sexual reproduction is unknown in some genera, but when present it is represented by oospores or zygospores. In any studies with Phycomycetes it must be remembered that there are few septa, and consequently care must be taken not to break the mycelium into too small fragments or much of it will be killed.

Not typically encountered in water are three of the higher orders—Peronosporales, which are mostly obligate parasites on higher plants; Mucorales, which are mostly saprophytic forms, although a few are parasitic on other fungi; and Entomophthorales, which are often parasitic on insects, although they can be grown in pure culture. Unlike other classes of fungi, Phycomycetes are named without regard to whether the sexual stage is known or unknown.

Much work on morphogenesis and physiology has been done on members of the order Blastocladiales, especially the genera *Allomyces* and *Blastocladiella*. Among many of the genera producing motile cells, much work remains to be done on the conditions for growth in pure culture and on the problems of producing sufficient material for physiological and biochemical studies.

The groups most widely studied in pure culture for their products and physiology are found in the order Mucorales. Genera such as *Rhizopus* produce large amounts of fumaric and lactic acids. Mu-

corales have been used in steroid transformations, for the assay of vitamins and antifungals, and for microbiological study because of the many forms readily available. They are especially useful, since large amounts of mycelium are produced in a few hours on chemically defined media. Asexual spores develop readily and rapidly for inoculum.

Phycomyces has been studied extensively because of its extremely long sporangiophores, which respond to light. They are often 20 cm. long and yet exist as single cells. This genus has been used in many studies on the biosynthesis of carotenes. Under certain conditions some species, especially in *Mucor*, may take on a yeastlike phase.

As far as is known, all genera of the Mucorales produce sexual spores known as zygospores. Some species are homothallic (zygospores are produced from a colony derived from a single sporangiospore) or heterothallic (zygospores are formed only when two opposite mating types are brought together). One disadvantage to genetic studies of this order of fungi is the fact that spores are multinucleate. Also, the zygospores are very difficult to germinate.

The Mucorales grow almost everywhere and are easily isolated by removing some of the hyphal tips or by isolating a few spores from a fruiting stalk. Mucorales often occur among the first organisms during incubation of various substrates. Because they grow rapidly and utilize simple sugars, they are sometimes referred to as the sugar fungi.

The Entomophthorales have a coarse mycelium made up of hyphae of reduced length. Typically, conidia are formed in abundance and these are forcibly discharged. In spite of the fact that a number of species are parasitic on insects, growth of these forms is relatively easy both on solid and in liquid media.

The following orders in the Phycomycetes seem to offer the best opportunities for biochemical and physiological studies: Mucorales, Entomophthorales, Blastocladiales, and Saprolegniales. In addition, the family Pythiaceae of the order Peronosporales should be considered. The first two orders have nonmotile spores and are typically terrestrial. The other groups have soil or aquatic forms and motile spores. The family Pythiaceae has several important parasites of higher plants, with infection beginning usually in the root system of the host. A great many Phycomycetes offer practically a virgin field for the study of nutritional and growth-factor requirements.

Ascomycetes

The Ascomycetes comprise perhaps the largest class of true fungi, or Eumycetes. Ainsworth (*1*) conservatively estimates the number of valid species to be 15,000, or one-third of the 45,000 so far described. According to him, these 15,000 species are contained in 1,700 genera, of which 850 are monotypic; i.e., containing only 1 species.

Although the Ascomycetes are a large class and have many diverse organisms, they all have one structure in common, the ascus. It is a membranous, saclike structure produced by sexual processes (i.e., processes involving nuclear fusion and reduction), in which ascospores are formed. The ascus may lie single and naked, as in such simple

forms as the Hemiascomycetes, or it may be grouped with others in fruit bodies, or ascocarps, of varying complexity. The young ascus typically starts out as a binucleate cell, in which the nuclei fuse, divide meiotically, and redivide, forming in most species eight haploid ascospores by the process called free-cell formation.

The number of ascospores is usually constant within a species, but the number per ascus varies within the class from one, as in *Tuber candidum* Harkn., to more than a thousand (rare), as in *Thelebolus stercoreus* Tode ex Fr. (*3*). The asci containing fewer spores than eight, as in the Saccharomycetaceae, are sometimes considered reduced forms; those containing more than eight, as in the Dipodascaceae, often are considered somewhat primitive forms.

Asexual reproduction in the Ascomycetes is common and usually comprises one or more of the conidial types found in the class Fungi Imperfecti. *Emericellopsis*, for example, has a conidial state classifiable in the form genus *Cephalosporium; Microascus* has the *Scopulariopsis* type; and *Byssochlamys* has the *Paecilomyces* type. *Ceratocystis galeiformis* Bakshi has three conidial types—*Leptographium*, *Graphium*, and *Cephalosporium*. Some ascomycete genera have conidial types that would be classifiable in a single genus of the Fungi Imperfecti. *Gibberella*, *Nectria*, *Hypomyces*, and *Calonectria* have a *Fusarium* type of conidial apparatus; *Eurotium*, *Sartorya*, and *Emericella* all have an *Aspergillus* type; and both *Physalospora*, a pyrenomycete, and *Tryblidiella*, a discomycete, have a *Diplodia* type.

Conidial states often are inconspicuous where associated with the ascocarpic or perfect state on natural substrate, but may predominate or even be the only state present in culture. *Eurotium* is one of the cleistothecial genera with the *Aspergillus* type of conidial apparatus. It was reported as *Mucor herbariorum* on natural substrata as early as 1780 by Wiggers, but no association was made with a conidial state until 74 years later when DeBary (*8*), interested in life cycles, proved that its conidial state was an *Aspergillus*.

Some strains in culture are sensitive to substrate and temperature changes, and under proper combinations of conditions they vary from being almost entirely cleistothecial to being entirely conidial. This classification does not mean, however, that the name *Aspergillus* can be abolished or removed from its proper placement in the Fungi Imperfecti, because numerous species apparently do not have perfect states and may never have. Neither can the ascomycetous genus or species in which such strains would belong, if they formed perfect states, be predicted with any degree of certainty.

The Ascomycetes are classified primarily on the basis of the morphology and development of their sexual states. The early classificational systems emphasized gross morphology, whereas later treatments stressed the nature of the asci and other microscopic structures. Traditionally the class is divided into two main groups or subclasses, the Hemiascomycetes, containing yeasts and other families that do not produce their asci in ascocarps, and the Euascomycetes, containing the balance of the class that does form ascocarps.

Due partly to the size of the class, there are no recent comprehensive taxonomic treatments of the species. However, Martin (*22*) presents a key to families, and Bessey (*3*) gives keys to families and

many of the common genera. Gäumann (*11*, *12*) includes keys to families with characters that perhaps are more nearly indicative of the evolutionary development of the families, but they are rather difficult to use.

Hemiascomycetes (Yeasts)

The subclass Hemiascomycetes contains the orders Taphrinales and Endomycetales and seven families (*22*). The Taphrinales, with two families and six genera, are plant parasites with no known industrial application. The genus *Taphrina* contains most of the 125 species and will grow in culture.

The order Endomycetales contains the other five families: Pericystaceae, Ascoideaceae, Saccharomycetaceae, Endomycetaceae, and Spermophthoraceae. Except for the Spermophthoraceae, which contains the riboflavin-producers *Ashbya* and *Eremothecium*, the only two families containing organisms of known technological importance are the Saccharomycetaceae and the Endomycetaceae, which contain the ascosporogenous or so-called true yeasts. The nonascosporogenous or false yeasts are in the class Fungi Imperfecti, since they apparently form no sexual states and are contained in the two families Cryptococcaceae and Sporobolomycetaceae. Inasmuch as these two families are generally discussed along with ascosporogenous yeasts, they are so treated here.

Yeast is a rather general, indefinite term. It refers to the unicellular-growth form of true fungi, which develop moist, rather mucoid colonies. Yeasts have no true mycelium, but on certain media some form pseudomycelia made up of chains of vegetative cells.

The two most comprehensive treatments of yeasts are the monograph by Lodder and Kreger-van Rij (*19*) and The Chemistry and Biology of Yeasts edited by Cook (*7*). The latter volume contains a classification by Lodder et al. (*20*) of both the ascosporogenous and the anascosporogenous yeasts. All the ascospore-forming yeasts are treated by Lodder et al. (*20*) in the single family Saccharomycetaceae, which includes the Endomycetaceae of Gäumann (*11*) and Martin (*22*). Lodder et al. differentiate among 17 genera, of which *Eremascus* and *Endomyces* are not considered yeasts because they form true mycelia. The most important genus of the ascosporogenous yeasts is *Saccharomyces*, which contains the so-called brewing and bakers' yeast strains so important to the food and fermentation industries.

The two families of anascosporogenous yeasts contain 13 genera, 2 of which are not yeasts in the strict sense. These families have some strains that are undoubtedly imperfect states or heterothallic forms of the true yeasts, some strains that are probably imperfect states of Basidiomycetes, and several human pathogens. The most important genus for technological purposes is *Torulopsis*, which is a convenient source of protein.

Euascomycetes

The subclass Euascomycetes is characterized by its asci being formed in or on a specialized fruiting body called an ascocarp. Ascocarps range from a few strands of sterile hyphae incompletely covering the

asci, as in the family Gymnoascaceae, through such complicated structures as those in the morels, in which there is differentiation into a stipe and cap, much as in mushrooms. The four main types of ascocarps are as follows: The cleistothecia are sterile hyphae or pseudoparenchymatous cells enveloping the asci completely and leaving no opening for escape or discharge of the ascospores. The perithecia are similar to the cleistothecia, but they have pores or slits for spore discharge. The locules superficially resemble the perithecia immersed in a rather extensive sterile hyphal mass, or stroma, but they are really just cavities hollowed out of the stroma. The apothecia are disk- or cup-shaped structures largely exposing the asci upon maturity.

The Euascomycetes contains 14 orders, 60 families (*22*), and many species that have never been studied from an industrial standpoint or even grown in culture. However, many important plant pathogens, such as the powdery mildew, apple scab, Dutch elm disease, and ergot organisms, belong here, as do the highly edible truffles and morels. Like the downy mildews of the Phycomycetes, the powdery mildews are obligate parasites and have never been successfully cultured. The morels and truffles have a mycorrhizal relationship with higher plants, but the morels, and possibly the truffles, can be grown in culture in a mycelial state.

Several members of this subclass are of industrial interest, but they are mostly airborne species or ones readily isolated from soil, and they often have prominent conidial states. The genus *Chaetomium* has long been important as an agent of deterioration. *Neurospora* is extremely useful for genetic studies. *Emericellopsis* produces an antibiotic of the penicillin type useful against typhoid fever. *Gibberella* produces a plant-growth regulator. Several organisms belonging to the genera *Ophiobolus*, *Sporormia*, *Eurotium*, *Peziza*, *Calonectria*, and *Gibberella* are utilized in the microbiological conversion of steroids. As fermentation research expands, the number and variety of Ascomycetes useful to industry undoubtedly will increase.

Basidiomycetes

The class Basidiomycetes is estimated to encompass about 550 genera and 15,000 species. All Basidiomycetes have in common a basidium, which typically produces four basidiospores. In the basidium nuclear fusion is followed by reduction division, so that the basidiospores are haploid. Hence, the basidium represents the perfect or sexual state of all fungi in this class.

When basidiospores germinate, the mycelium is formed, which is known as the primary or haploid mycelium. Later, diploidization occurs and the mycelium becomes dikaryotic; i.e., nuclei of opposite mating reaction are associated in the same cell but continue to divide independently and multiply without nuclear fusion. This dikaryotic mycelium is often characterized by clamp connections or swollen regions at the septa, in which the earlier cell formed is swollen and appears to bend or clamp over the next cell beyond it. The mycelium in nature may become very extensive and may live for years. During periods of proper temperature and moisture, it produces fruiting

bodies, which may range from microscopic to structures weighing 20 or 30 pounds.

Some species are strictly saprophytic, others are parasitic, but can be grown free of their host, and still others are obligate parasites. Many Basidiomycetes cause the decay of wood, whereas many others are symbiotically associated with the roots of plants.

The rusts (order Uredinales) are all obligate parasites on higher plants. They have not been grown in pure culture. There are about 5,000 known species. Some rusts have as complex a life history as any fungi known. For example, wheat rust (*Puccinia graminis* Pers.) produces uredospores on wheat. These spores may germinate to infect additional wheat plants and again produce more uredospores. Eventually the mycelium-producing uredospores will produce a second type of spore, teliospore, which germinates to produce a basidium and basidiospores. The basidiospores then infect the barberry, forming spermatia, and after fertilization the dikaryotic phase is established, which then forms aeciospores on the barberry. Aeciospores are carried by the wind to wheat plants, and the cycle is repeated. Both hosts, which are unrelated, are required to complete the life cycle of the wheat rust. Studies of plant tissue culture and the rust growing on it offer a promising field for research.

The smuts (order Ustilaginales) are parasitic fungi and are practically always found on flowering plants. This group consists of about 40 genera and 700 species. Often smuts produce large tumorlike growths in the host, as in corn smut. Unlike the rusts, smuts can be grown in pure culture on both solid and liquid media without much difficulty. Often the mycelium fragments or even appears yeastlike in culture. Pure cultures can be maintained by lyophilization. For purposes of identification, it is important that the smut fungus be studied from the host material. Work on smuts in culture has shown that they produce a large number of interesting compounds, ranging from lysine to ustilagic acid.

Fungi in the order Tremellales are especially abundant in the Tropics. Most of them are saprophytic on wood, and their fruiting bodies are generally gelatinous when wet, hence the name "trembling fungi." When dry, they become inconspicuous and hornlike. Although little studied in pure culture as yet, their growth in pure culture is not difficult. Carotenes have been identified already in some forms.

The Polyporales, or bracket fungi, are easily grown in culture on such media as malt agar. Almost all genera grow in dead or living trees and are the most important group of fungi responsible for timber decay. As far as we know, all genera and species can be grown in pure culture, but it is difficult to retain viable cultures because they lack spores. Identification depends on the nature of the fruiting bodies, which are producing basidiospores. In some, spores are simply borne on the smooth surface of the fruiting structure, others on a wrinkled or roughened surface, and still others inside pores or tubes, which have openings to the air on the lower side of the sporocarp. In one group, spores are borne on the surface of toothlike outgrowths.

All fungi that produce gills and are macroscopic are placed in the Agaricales. This group is commonly called mushrooms or gill fungi. In the more restricted sense there are about 4,000 species in approxi-

mately 100 genera. The mycelium in nature may be extensive in development and may live for years. Many forms are saprophytic, although a few are parasitic. Like the bracket fungi, many species may be grown in pure culture; some even will produce normal sporocarps under these conditions. Many forms are eaten, although a few species are poisonous, especially some members of the genus *Amanita*. Like the other Basidiomycetes, identification is based on study of the mature fruiting body. The commercial mushroom belongs to this group of fungi. Of considerable importance is the fact that many species develop a vegetative mycelium in association with living plants, apparently benefiting both the fungus and the plant.

The last major group of Basidiomycetes, composed of several orders with fewer genera than in the previous groups, is the Gasteromycetes or puff balls. In these fungi large fruiting bodies may occur. The group has, as a common characteristic, basidia enclosed during their development of basidiospores and often permanently so. In puff balls both basidia and basidiospores are formed internally. When the spores are mature and the fruiting body is dry, spores are released by weathering and action of the wind. Approximately 110 genera are known and about 700 species. Most Gasteromycetes appear on the ground, although a few are found on dung or decaying wood. Few Gasteromycetes have been grown in pure culture or studied for either their products or biochemical activity.

Fungi Imperfecti

The last major group of true fungi is the "form class" Fungi Imperfecti, or Deuteromycetes, comprising about 1,350 genera and 11,000 species of imperfect fungi; i.e., without a known "perfect" or "sexual" state. Most of these fungi cannot be assigned with certainty to any of the other classes. Included are many important plant and animal pathogens, as well as a great variety of saprobic, or saprophytic, forms generally found decaying vegetation, deteriorating fabric and equipment of all kinds, "molding" fruits and vegetables, and inhabiting soil and water.

The terms "form class" and "form genus" are used when referring to members of this class, because such groupings do not necessarily contain closely related organisms. True relationships are best determined by study and comparison of perfect states and entire life cycles, and in the absence of such information groupings are generally highly artificial. In the case of Phycomycetes and Uredinales (rusts), however, imperfect states are so characteristic that species can be and are assigned to positions in those groups. The Fungi Imperfecti consists of imperfect forms of Ascomycetes and certain Basidiomycetes. Most of them are undoubtedly conidial states of Ascomycetes.

Ainsworth (*1*) stated that of the 11,000 species, possibly a third of them have named perfect states, a third have perfect states not yet named, and the rest have no such states. In any event when the perfect state is known and named and when the connection between the two states is proved, the conidial form is removed from the Fungi Imperfecti and the imperfect-state name dropped. Where the relationship is not definitely proved, or where there is such a difference in the

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time of development or substrate conditions that often only the conidial state is present, it is desirable from a practical standpoint to retain the conidial-state name among the Fungi Imperfecti, because in identifying the organism it would be sought there. Thus we sometimes find two names referring to the same organism. The gibberellin producer, for example, although preferably called by its perfect-state name, *Gibberella fujikuroi* (Saw.) Wr., is sometimes called *Fusarium moniliforme* Sheldon when only the conidial state is present.

The morphology of the imperfect fungi varies a good deal and is generally used as the basis for classification. Mycelial hyphae are septate and similar to those of the Ascomycetes and Basidiomycetes. Except for a few that form no spores, the various species develop one or more types of conidia, which are commonly borne on special mycelial branches of varying complexity. These special branches, or conidiophores, may arise singly or in clusters, variously termed "coremia," "synnemata," and "fascicles." Some species form their conidia in or on special fruiting structures termed "pycnidia," "acervuli," and "sporodochia." These special structures may or may not be produced in culture, and identification of species sometimes depends on their presence.

Conidia are many. They may be almost any color, shape, or size. Some are produced singly; others are arranged in chains, heads, or mucoid droplets. Some are pinched off the ends of conidiophores; others are formed in or at the tips of special flasklike hyphal tips called phialids. They may be warted, spiny, or even have various kinds of appendages. Some are single-celled; others can be two-celled or even many-celled, with the cells arranged in various characteristic patterns, which aid in identifying genera and species.

The imperfect fungi are usually classified in the following orders: Sphaeropsidales, which contains the four families forming pycnidia; Melanconiales, which has the single family forming acervuli; Moniliales, which includes the remaining six families forming conidia; and Mycelia Sterilia, which has the few genera not producing conidia. Most of the so-called common molds are located in the Moniliales, which contains the two families of imperfect yeasts, in addition to such familiar genera as *Aspergillus*, *Penicillium*, *Alternaria*, *Cladosporium*, and *Fusarium*.

Almost all the common imperfect fungi can be cultivated readily on agar media. Some that produce great numbers of spores are often even laboratory pests, contaminating sterile agar or cultures of other organisms. Members of the Sphaeropsidales and Melanconiales generally sporulate less readily than many of the Moniliales in artificial culture, occur less commonly as soil forms, and therefore are present in fewer numbers in culture collections. Consequently, their industrial potential is less understood than that of the Moniliales. Poor sporulation in culture often is due to the use of media too rich in nutrients. Where this is true, as with many pycnidial strains, such weak but natural media as hay extract agar, soil extract agar, or cornmeal agar markedly increase sporulation.

A few of the imperfect fungi have long been used commercially for the manufacture of cheese and the production of citric acid, but it was the antibiotics industry that stimulated fermentation research to ex-

plore some of the possibilities of more widespread utilization of the fungi and their products. *Aspergillus* and *Penicillium* seem to be especially versatile genera, yielding important organic acids, antibiotics, and enzymes. However, other imperfect genera are also gaining recognition, especially in the microbiological conversion of steroids. Members of the genera *Fusarium*, *Cladosporium*, *Trichoderma*, *Hendersonia*, *Trichothecium*, *Curvularia*, *Botrytis*, and *Nigrospora* have been found to accomplish desirable transformations of the steroid molecule by hydroxylation, epoxidation, or dehydrogenation. This tailoring of molecules by micro-organisms seems to be not only a trend that will continue but one that will expand considerably in the future.

Myxomycetes

The class Myxomycetes comprises a small closely related group of primitive organisms, which have been placed in both the animal and plant kingdoms, but which apparently are more closely related to the true fungi. They are of little economic importance, if any, and according to Ainsworth (*1*) number only about 400 species. They have been called Myxothallophyta, Mycetozoa, Myxogastres, Phytosarcodina, or slime molds by various authors referring to them either singly or in association with other groups of uncertain affinities.

The Myxomycetes are widely distributed, free-living forms, which are found in damp or wet places, such as on old logs or other decomposing plant material. They resemble the true fungi in their lack of photosynthetic pigments and in their food reserves. They differ from them in their growth or assimilative phase, which consists of a single large protoplast called a plasmodium. It is multinucleate, motile, and naked.

The life cycle is rather unusual. A germinating spore produces one to four ameba-like, flagellated, swarm cells, which may function immediately as gametes by uniting in pairs or which may, by loss of their flagella, become "myxamebae" and undergo divisions before copulation. The resultant zygote is ameboid and grows directly into a plasmodium living in or moving slowly on a substratum. The plasmodium ingests micro-organisms, spores, or bits of dead plant and animal material and increases in size, generally to several centimeters in diameter. Nuclear fusion may occur at zygote formation or later. After several nuclear divisions and sufficient growth, the plasmodium humps up and becomes converted into one or more sessile or stalked sporangia or spore cases. Meiosis occurs before spore formation, which then completes the life cycle.

The chief characters for the classification of the Myxomycetes are the method of spore development; the type of fruit body; the structure of the spore case and the threadlike material, or capillitium, inside; the calcium carbonate, where present; and the size, color, and ornamentation of the spores (*1*). Although the Acrasiales, which includes the genus *Dictyostelium*, and the Labyrinthulales, with *Labyrinthula*, have sometimes been classified along with the Myxomycetes, their affinities are highly uncertain, and the class Myxomycetes is generally restricted to the slime molds that have a true plasmodium. A good

classification of the Myxomycetes is the monograph of the North American species by Martin (*21*).

LICHENS

Lichens are an unusual group of micro-organisms, because each one is always composed of two separate living components; namely, a fungus and an alga living in association with each other to give the gross appearance of a single plant. Even though two micro-organisms are involved, they are named as a single plant.

In many lichens special outgrowths are formed, which are composed of a fungal mycelium and a few algal cells that together act as a reproductive body. The fungus component is usually an ascomycete, most often a form belonging to the Discomycetes. The fungus typically reproduces by forming ascospores. A few lichens have their fungus components as basidiomycetes. The algae usually belong to the blue-green or to the green algae. The component organisms have been grown independently of each other.

Typically, lichens grow on soil, trunks and branches of trees, and rocks. Rarely are they found in water. Many problems of their nutrition and growth are unsolved.

According to one authority, there are about 400 genera and 16,000 species of lichens in the world. Two references on the classification of these organisms are by Zahlbruckner (*29*) and Fink (*9*).

Lichens produce a variety of lichen acids and pigments like litmus. In the Far North some lichens are utilized for food by grazing animals.

RELATIONSHIP OF TAXONOMY TO TECHNOLOGY

In the mid-1940's microbiological studies in pure culture on growth, physiology, and products, except for pathogens, were limited to a small number of bacterial genera and less than 12 genera of fungi. Only a few soil microbiologists had ever investigated saprophytic actinomycetes. To the fermentologist then, genera such as *Ustilago*, *Curvularia*, and *Wojnowicia* were unknown. Twenty years later the diversity of micro-organisms used in microbiological investigations appears to parallel roughly the tremendous research activity and industrial development in the field of microbiology. Developments in the antibiotic, vitamin, and steroid fields have made it necessary to look at all kinds of micro-organisms not hitherto investigated.

It is necessary in any microbiological research group to have a collection of a great variety of micro-organisms and to have at least one person supervising it who is versed in their identification and ecology. This combination is necessary for two reasons. First, to keep a collection of micro-organisms vigorous and pure, someone needs to be familiar with their appearance and know the salient species characteristics. Second, to obtain more diverse and new types of organisms without repetition, a taxonomist is needed who is intimately acquainted with the habitats of micro-organisms in nature. Undoubtedly new developments will depend largely on the discovery of new strains of micro-

organisms, which will produce in their metabolism new reactions and new products. Taxonomy and nomenclature are, therefore, basic to both fundamental and applied microbiology.

In 1928 when A. Fleming, a British bacteriology professor, first isolated the *Penicillium* strain that produced the antibiotic penicillin, the name *P. rubrum* Biourge was applied to it. Later, after study of this strain, C. Thom, a U.S. Department of Agriculture mycologist, found that it was actually *P. notatum* Westl. When this correction was made, it became apparent that one should look for better penicillin-producing strains among the isolates of *P. notatum.* With this knowledge of the correct classification, it was possible to find a second strain that produced penicillin in submerged culture and thus opened up the practical industrial-scale production of penicillin in time for use during the last years of World War II.

Certainly if penicillin were formed in this species and if the classification of the genus *Penicillium* were anywhere near a natural one, the next place to look would be in the near relatives of *P. notatum.* This assumption proved correct, for the same antibiotic was found to occur in the species *P. chrysogenum* Thom, which belongs in the same series of species as *P. notatum.* From an isolate of *P. chrysogenum* came the strain that is the ancestor of the highly developed strains used today to manufacture penicillin.

A similar case, but one in which identification did not turn out so well, was the report in 1918 that *Aspergillus fumaricus* Wehmer was able to convert sugar to fumaric acid with yields of 70-percent conversion of the sugar. C. Wehmer, who reported this discovery and named the species, did not distribute it. His strain was not fully described, and no one knows what kind of *Aspergillus* he worked with. To date no one has obtained yields of fumaric acid as high as he did from an *Aspergillus.* Had a more careful description been prepared and had the culture been carefully preserved, it might be available today.

Another example of how elucidation of the taxonomy of a new organism has aided microbiological studies is the work of J. H. Grosklags and M. E. Swift on the fungus that produces the antibiotic synnematin. This fungus was originally isolated and classified as a species of *Cephalosporium.* Later these same investigators examined the particular strain on a variety of media and discovered that it formed ascocarps. Since the genus *Cephalosporium* is based on the imperfect state, it was necessary, according to the International Code of Botanical Nomenclature (*16*), to transfer this species to the perfect-state genus, namely *Emericellopsis.* They then examined living cultures of the six other taxa in the genus *Emericellopsis* and found that all can produce an antibiotic inhibiting the growth of *Micrococcus* and *Salmonella.* Consequently, a considerable number of strains became available for study.

The correct identification of a micro-organism may have far-reaching consequences and greatly affect subsequent work. A striking example resulted from the first antibiotic discoveries in the genus *Streptomyces*, which had received little attention before 1940, except by a few soil microbiologists and pathologists. Yet, after it became apparent that this genus of actinomycetes was a source of new antibiotics, probably no other group of micro-organisms has been so

intensively isolated and screened for new products. The rewards in new commerical antibiotics have been tremendous, as seen in the production of streptomycin, chloramphenicol, chlorotetracycline, oxytetracycline, erythromycin, tetracycline, and in the subsequent discoveries that some antibiotics stimulate animal growth, that they may also be used in the preservation of food, and also that actinomycetes are a source of vitamin B_{12}.

Micro-organisms, as they occur in their wild state, are restricted to certain ecological habitats and some, at least, are restricted to geographical areas. An understanding of their growth requirements, the substrates they occur on or in, and their activities in the wild state gives clues as to which micro-organisms may best be suited to solve a particular problem or to give a particular product. Fungi that grow first on a substrate rich in sugars are the best organic acid producers. *Aspergillus* and *Rhizopus* are usually the first and the most rapid-growing forms on decaying plant material, and their members are the best producers of citric, gluconic, and fumaric acids. Bacteria that ferment unpasteurized milk produce the most lactic acid. On the other hand, such organisms as algae tend to grow in water where nutrients are low. Consequently, most media devised for algae have small amounts of salts and a very low organic content.

At the Northern Regional Research Laboratory much work was done to develop dextran. It was known that the best organisms to produce dextran gums were members of the bacterial genus *Leuconostoc*. There were many references that material high in sugar, when contaminated with members of this genus, became thick and viscid. Knowledge of this characteristic led to a survey of strains of *Leuconostoc* and to the development of a process to make dextran by using a culture of *Leuconostoc mesenteroides* (Cienk.) v. Tiegh. Solvents, such as alcohol, acetone, and butanol, are produced by organisms, some of which live under anaerobic conditions.

In any search for a new product or process a mere random screening program should be tempered by a review of which micro-organism might best offer a possible solution. When no clues are available, then a random screening must be attempted. For example, a search for an organism to destroy lignin would not include any of the sugar fungi, but rather would concentrate on those forms that grow on and in wood. Likewise, a search for an organism suitable for the assay of an unknown growth factor would concentrate on organisms difficult to grow on any medium and incapable of growing on a defined medium in which all the known growth factors are added.

Understanding the life cycle of micro-organisms should be considered in selecting an organism for fundamental studies. For example, B. O. Dodge's pioneering work on *Neurospora* showed that this organism was ideally adapted to genetical studies, and further work was carried out by G. W. Beadle and E. L. Tatum. On the other hand, a genus such as *Mucor* would offer extremely poor material for genetical studies, since all the reproductive structures are multinucleate and the diploid state, zygospore (the cell resulting from nuclear fusion), often resists germination. The same also applies to mutation work. Organisms with a single nucleus and a single set of chromosomes are probably much easier to mutate than cells of an organism with many nuclei.

Micro-organisms can mutate under natural conditions. *Staphylococcus aureus* Rosenbach has mutated until it resists all the common commercially produced antibiotics. Wheat rust develops new races that attack wheat varieties developed by plant breeders to resist rust. The same type of natural mutation and selection undoubtedly occurs in cultures of micro-organisms grown under different conditions from those encountered in nature and must be constantly guarded against. Too, there is always the possibility that cultures may be contaminated with other micro-organisms.

SOURCES OF MICRO-ORGANISMS

Despite the variety of natural sources and the ubiquity of micro-organisms, they are abundant only in habitats favorable to their growth. Such habitats or substrata would be the ones to investigate in a search for new types. Viruses and rickettsiae, for example, are strictly parasitic and can be grown only in tissue culture. Since rickettsiae are parasitic only on animals, they would be sought for isolation from some animal showing symptoms of a rickettsial disease. Viruses, depending on the type desired, would be sought and isolated from living plants or animals. If a bacteriophage were desired, it would be sought in old lysogenic bacterial cultures or substrata such as sewage, feces, or polluted streams.

Pathogenic bacteria, fungi, and protozoa would, like the viruses and rickettsiae, be sought in their respective infected hosts for the most part, or in cases of intestinal parasitism, commensalism, or symbiosis, by isolation from excreta. The isolation of many parasitic strains is made easier by their lack of host specificity and by their generally ready growth in artificial culture. Among the fungi, notable exceptions would be the powdery mildews (Ascomycetes) and the rusts (Basidiomycetes), which have never been successfully grown in artificial culture. Organisms that may not grow in pure culture or are difficult to obtain often can be maintained in two-member culture with bacteria; for example, certain protozoa, algae, Myxomycetes, and Acrasiales.

Most algae and protozoa are free living and can be found where there is sufficient moisture. Stagnant pond water is a good source of fresh-water protozoa and algae, but slow-moving or fast-moving streams, hot springs, ocean water, soil, or dung may be better sources for particular types. Many algae are submerged aquatics, but also a large number contribute, with protozoa, to make up fresh-water and marine plankton.

Because of the tremendous competition for survival, many micro-organisms survive in nature only because of certain advantageous enzyme systems. Thus, *Morchella* occurs exclusively in the Temperate regions of the world and is associated with only particular kinds of trees. Furthermore, its fruiting is restricted to but a week or so in the spring of each year. Since reliably identified isolates can only be obtained from the fruiting structures, their availability is likewise restricted.

Perhaps the best natural sources of saprobic bacteria, actinomycetes, and fungi are soil, dung and other excrement, sewage, decomposing

vegetation, humus, and polluted water. For yeasts, frass and exudates of various sorts may also be good. Moist, tropical soil is probably the best single source for isolating the greatest number and diversity of species, although bacteria would be present in higher numbers in dung. It has been estimated that the number of bacteria in fertile soil is often as high as 100 million per gram, representing 6 to 12 genera and perhaps twice that number of species. The numbers of actinomycetes and fungi might total more than a million, including mycelial fragments and spores, and the number of species represented might easily reach 75 to 100. The greatest number of micro-organisms are in the upper 2 inches of soil. A variety of fungi can be isolated directly from bits of dead wood, leaves, or debris; even more can be found after incubating such materials in moist chambers.

CULTURE COLLECTIONS

Although many micro-organisms can be isolated rather readily from nature by a competent microbiologist, often a particular strain is desired because of its unique properties. It may be a strain used for a particular assay, for the production of a particular product in high yields, for a particular enzyme system, or for checking the report of others on the behavior of a particular organism; or it may be the type strain one wishes to study for comparative purposes. Some organisms are geographically isolated, such as the fungus *Blakeslea trispora* Thaxt., which can be found only in the Tropics. Where specific strains are desired, it is best to acquire them from a culture collection.

Culture collections are of two kinds. The first is the large collection with hundreds of cultures of many different species and genera. The second is the specialized collection with a few to many strains usually limited to a single group or genus and maintained by an investigator who has spent a lifetime in isolating different forms and assembling all the type cultures available from collections around the world.

There are several large general collections of micro-organisms in the United States. Foremost is the American Type Culture Collection in Washington, D.C., which uses the letters ATCC preceding its culture numbers. According to the sixth edition of its catalog, the American Type Culture Collection has over 4,350 strains available for sale. This collection was established in 1924. It consists of bacteria, fungi, yeasts, bacteriophages, viruses, and a few algae and protozoa. It includes cultures of both animal and plant pathogens.

Two government collections that are more specialized are the ARS (Agricultural Research Service) Culture Collection, U.S. Department of Agriculture, located at Peoria, Ill., and the Quartermaster Culture Collection at Natick, Mass.

The ARS Culture Collection has micro-organisms that are useful or related to useful strains employed in industrial microbiology. Some are strains that produce such products as penicillin; others are used for the assay of vitamins and antibiotics, or serve as type strains for comparative taxonomic purposes. No catalog is issued for this collec-

tion, which has more than 9,000 strains. In addition, this collection has in lyophil about 14,000 other strains on a nonpermanent basis. The ARS collection consists of bacteria, yeasts, actinomycetes, and molds. The strain numbers are preceded by the letters NRRL (Northern Regional Research Laboratory). Among the bacteria, *Pseudomonas* and *Bacillus* cultures are especially numerous. Yeasts and actinomycetes are very complete. In the fungi the genera *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, and other Mucorales are especially complete. The ARS collection was started when C. Thom studied the manufacture of Roquefort and Camembert cheese in the U.S. Department of Agriculture shortly after 1900. It was not formally established until about 1940.

The Quartermaster Culture Collection was established during World War II to maintain micro-organisms involved in deterioration research of such diverse materials as electrical insulation, paints, wool, cotton, leather, and plastics. A catalog of its cultures, issued in 1950, indicates that it has primarily fungi, although it also has a considerable number of bacteria. Its culture numbers are preceded by the letters QM.

Of special interest is the collection of algae established in 1953 at Indiana University, Bloomington. This collection has representatives of all the various groups of algae. Its catalog was issued in 1956 as well as a helpful account of the maintenance of algal cultures and a list of many of the most suitable media for algal stock culture.

Several large general collections exist outside the United States. The Centraalbureau voor Schimmelcultures at Baarn, Holland, was founded in 1906, and is recognized for its completeness in numbers of genera and species of fungi. According to its most recent catalog, published in 1957, it contains 9,693 strains of micro-organisms representing 6,294 species. It maintains many strains of fungi in which the only interest has been taxonomic. Included in the fungi is a large and complete collection of yeasts. Some strains of actinomycetes are kept, but no bacteria.

In England the Commonwealth Mycological Institute at Kew, Surrey, maintains a large collection of fungi. Its last catalog, issued in 1957, in addition to listing its holdings, contains the addresses of special collections in England; namely, fungi and yeasts pathogenic to animals and man, wood-rotting fungi, yeasts, industrial bacteria, bacteria pathogenic to plants, bacteria pathogenic to man and animals, and dairy bacteria.

A general catalog of the cultures of micro-organisms maintained in Japanese collections was issued in 1953 and gives the sources of about 22,000 cultures in 144 organizations. Among the largest general collections in Japan is the Institute for Fermentation at Osaka, which was established in 1944. According to a 1956 catalog, it maintains 2,391 strains, divided among molds, yeasts, brewery yeasts, and bacteria. A second collection is the Japanese Type Culture Collection, Nagao Institute, Kitashinagawa, Tokyo, which has molds, yeasts, and actinomycetes.

In Canada the Prairie Regional Research Laboratory at Saskatoon, Saskatchewan, maintains a large collection of micro-organisms, but it does not issue a catalog of its cultures.

Among the special collections of micro-organisms is that of *Bacillus* strains assembled from all over the world by N. R. Smith for his studies leading to the monographing of the genus. Another famous collection is the actinomycete strains assembled by S. A. Waksman at the Institute of Microbiology, Rutgers University, New Brunswick, N.J. Often such specialized collections are more complete in the limited area they cover than can be found in any of the general collections. Usually cultures can be obtained from special collections upon request, although one should recognize the burden upon a curator in time and equipment required to provide transfers.

In addition, all major fermentation companies have large collections of micro-organisms, but their scope, size, and contents are not revealed. Usually no cultures are distributed except the few strains deposited with general collections for assay of their products or for patent applications.

In using strains of micro-organisms from culture collections, several procedures should be followed after the culture is received. Adequate stocks should be prepared to maintain the strain and from which to prepare inoculum for experimental work. The culture's identity should always be verified. The culture should be studied to see that it is free of all contaminants, contains no aberrant strains resulting from mutation, and is in a vigorous, growing condition. If research on a particular strain is published, the author should clearly state the source of his culture and the number under which he received it. Some published research has little value, because no reference is made to the source of the culture; consequently, results of other published research on the same strain cannot be correlated with new information.

MAINTENANCE OF CULTURES

The procedure by which both the life and the taxonomic, physiologic, and morphologic integrity of a culture are conserved is called maintenance. Culture maintenance is the concern of everyone who works with living cultures, especially the personnel of culture collections. A prerequisite to success in maintaining cultures is a knowledge of the micro-organisms to be conserved. Some bacterial physiologists concentrate on one or a few strains or species of related bacteria and need relatively little information to maintain their stock cultures. At the other extreme are curators of large culture collections who need a broad knowledge about many kinds of micro-organisms in order to conserve successfully their collections so that they may distribute authentic, viable cultures. These collections sometimes include bacteria, actinomycetes, yeasts, molds, and even algae, viruses, and protozoa.

Every viable culture has a life expectancy within which effective action must be taken if viability is to be retained. With certain strains of micro-organisms maintained in lyophil (freeze dried), the life expectancy may be a matter of years and no immediate action is required. The life expectancy of *Lactobacillus leichmannii* Bergey et al. in stab or deep broth culture may not exceed a week. With this species and with other fastidious types, not only must action be taken soon after receipt of the culture but it must be repeated at weekly in-

tervals until a method that prolongs the life expectancy of the culture is substituted.

The type of action that is taken, at least as a preliminary, always includes transfer of the culture to a fresh nutrient medium, followed by incubation under conditions suitable for its normal development.

Choice of a medium and a set of conditions for growing a new accession usually is not difficult. Some donors describe the medium upon which they have grown the culture and some even suggest an incubation temperature. More often, however, a name—more or less complete—is all the information that is with a culture when it is received. Selection of a medium and conditions for growing a culture is then up to the recipient.

An experienced microbiologist can usually make a fairly good guess as to whether the name on an incoming agar or broth culture is reliable and, if not, which genus is most likely. With this much information he can generally select a suitable medium. Most common bacteria will grow on nutrient agar slants of 0.3-percent beef extract, 0.5-percent peptone, 2-percent agar, and water, all adjusted to pH 7. Oxygen relationships will be discovered by visual inspection. Incubation at 25° to 30° C., although not necessarily optimal, probably will be satisfactory.

Of course, with fresh isolates the necessary information will have been acquired during isolation, purification, and identification. However, there are many fastidious bacteria that require different media, restricted access to air, higher or lower incubation temperatures, or incubation in the light. A similar situation applies to other microorganisms, where one or two media and sets of growth conditions suffice for the growth of most types, but where special media and conditions are needed for large numbers of less common types.

Once the culture has been successfully cultivated on laboratory media, there are several possible methods of prolonging viability. The most common is to store a mature plate, slant, or broth culture in the refrigerator as long as is safe from the standpoint of longevity. Refrigerated cultures of many bacteria and yeasts will remain viable for several months, but it is usually recommended that no more than 3 or 4 months be allowed to elapse before making fresh transfers for continued storage. Sporulated cultures of actinomycetes and many molds will also remain viable for several months if kept refrigerated.

With many bacteria the period of storage may be extended by depressing the cotton plug and using a rubber stopper in the tube to reduce evaporation. In tubes protected only by cotton plugs a culture may lose its viability because the medium dries up; whereas if evaporation is reduced, many cultures will continue to be viable for longer periods.

In the past such methods of maintaining stock cultures were the most common, and they still are widely used. However, if there are many cultures, these methods are tedious, time consuming, and therefore costly. The greatest drawback is that continued serial transfer often leads to degeneration of cultures. Sometimes the degeneration is quantitative, as with 2-ketogluconic acid-producing strains of *Pseudomonas reptilivora* Cald. & Ryers. During the 2 to 3 years when one of the cultures was being transferred at 3- to 4-month intervals, the yield of acid declined a third.

At times degeneration involves such diagnostic features of cultures as the production of aerial mycelia and of spores by actinomycetes; the production of dextran by leuconostocs, streptococci, and lactobacilli; and the formation of sexual spores by molds and yeasts. The pathogenicity and antigenicity of many plant and animal disease-producing micro-organisms are often either lost or modified when maintenance entails serial transfer.

With certain bacteria, yeasts, and molds, oiled cultures sometimes are practical for prolonging the life of stock cultures. An ordinary slant culture is converted to an oiled culture by covering it with sterile mineral oil. If the medium is carefully and completely immersed in oil, the culture may remain intact for several months or even years. Certain disadvantages and dangers of this method, however, have restricted its use in many laboratories. First, oil clings to the loop, hook, or needle used to remove a part of the growth and spatters when the wire is flamed. Also, there is considerable danger in transferring pathogenic micro-organisms, which may be disseminated by the spattering oil. Even if these disadvantages were to be overcome, it is obvious that this method is but a modification of the serial-transfer refrigerator-storage method and therefore has its disadvantages also.

The use of soil cultures is advantageous in maintaining certain molds, actinomycetes, and bacteria. For this purpose fertile garden soil is sterilized in test tubes by autoclaving at 121° C. for an hour or more. The sterile soil is then moistened with about one-third of its volume of a broth culture or with a suspension of spores of actinomycetes or molds. The broth culture should contain a high proportion of endospores in the case of aerobic or anaerobic sporeforming bacteria. The resulting soil culture is then allowed to dry at room temperature in the case of molds, actinomycetes, and nonsporeforming bacteria.

When the culture is composed of sporeforming bacteria, it may be dried in a vacuum oven at about 45° C. This procedure is preferred for clostridia intended for use in the production of solvents, because it has been found that yields are maintained best by means of spore inocula. The combination of drying and heat largely destroys vegetative growth. Soil cultures may be refrigerated as are other types of stock cultures. An active culture may be obtained from a soil culture by transferring a loopful of soil to a tube of appropriate medium and incubating it under suitable conditions.

Soil cultures are not satisfactory for preserving either yeasts or most nonsporeforming bacteria, but where appropriate they have certain advantages over the other methods described. Because of the dryness of soil cultures, the environment is essentially nonnutritive and the cells are practically dormant. Under these circumstances there is less likelihood of variation. Also, soil cultures seem to have a longevity that varies with the type of micro-organism, but is appreciably longer than that for cultures kept as agar or broth cultures. Aerobic sporeformers seem to remain viable and unchanged for several years, but some anaerobic sporeformers die in less than 2 years.

The best method for maintaining cultures of most bacteria, yeasts, and molds is lyophilization or freeze drying (*13*). It has the following advantages. First, a culture preserved in lyophil is safe from

contamination as long as the tube is unopened. Second, lyophilized cultures are essentially dormant and therefore changes associated with growth are absent. Strains removed from lyophil have the same physiological, cultural, and morphological characteristics as before lyophilization. The value of retaining these characteristics is obvious in industrial fermentations and in taxonomical investigations. Third, the longevity of lyophilized cultures exceeds that of cultures maintained by any other method. For example, *Lactobacillus leichmannii*, which often fails to survive more than a week in refrigerated stab or deep cultures, has been recovered repeatedly from lyophil after as long as 3½ years. Probably it would survive for at least 5 to 10 years.

Our experience with lyophilization has entailed the processing of more than 10,000 strains in about 60 genera of bacteria, all available species of actinomycetes, and most yeasts and other fungi, especially in the genera *Aspergillus* and *Penicillium* and in the order Mucorales. The ARS Culture Collection of micro-organisms has been maintained for about 20 years. Among the bacteria, yeasts, and molds in this collection, no large or important group has failed to survive lyophilization.

Contrary to general opinion, lyophilization does not require expensive equipment, lengthy processing, or highly trained technicians. The most expensive piece of equipment needed is a vacuum pump, like that found in most microbiological laboratories. The remainder of the apparatus consists of a manifold and a cold trap, which can be made by a glassblower, and assorted pieces of glass and rubber tubing. The manifold is generally made to hold two to three dozen tubes, so that many tubes of one culture or one or a few tubes of several cultures may be processed simultaneously.

By the methods used successfully at the ARS Culture Collection, 30 tubes may be completely processed in 2 hours. During this period the cells or spores are scraped from slants or plates or centrifuged out of broth culture, suspended in bovine serum, dispensed to lyophil tubes, frozen, dried, and sealed with an oxygen-gas crossfire torch. The resulting lyophilized cultures are stored in vials in a refrigerator. An ordinary 9-cubic foot household refrigerator fitted with trays holds about 20,000 lyophil tubes. In a similar space the number of test-tube cultures that could be accommodated would not exceed 2,500. Cultures lyophilized by this method are viable for years. Those put up in 1940, when the ARS Culture Collection was established, are viable and typical after more than 20 years.

Lyophilization of cultures for industrial fermentations is especially useful. When a run is unusually successful, it is a good practice to reisolate the fermentative agent and to make multiple lyophil cultures for future reference. Thereafter, whenever the current culture is suspected of becoming unsatisfactory for any reason, it is best to discard it and to begin succeeding runs with a culture freshly removed from lyophil. Such procedures will save much time and tedious work.

NOMENCLATURE AND TAXONOMY

Nomenclature and taxonomy, two subdivisions of the sciences, are of special concern and applicability to the biological scientist. No-

menclature deals with names. In microbiology it deals chiefly with the names of micro-organisms and groups of micro-organisms. Taxonomy is the science of classification. Microbial taxonomy pertains to the relationships among micro-organisms and their groups.

Such common names of micro-organisms as viruses, rickettsiae, bacteria, actinomycetes, algae, protozoa, and fungi are used by English-speaking people. Those who speak other languages use equivalent although often quite different names. A scientific name is properly derived from either Latin or Greek or else is Latinized; i.e., made to conform to Latin rules. It is used without change by scientists all over the world, except for possible transliteration to Russian, Hindi, Chinese, and other languages with different systems of writing.

Often scientific names are so constructed as to describe a distinctive characteristic of the micro-organism. For example, the scientific name for bacteria is Schizomycetes, which means literally fission fungi, a reference to their mode of reproduction. However, a name is fundamentally a device and need not be at all descriptive. Names have been derived from those of famous men and women, mythical characters, and cities and rivers. *Salmonella* is a genus of bacteria named to commemorate D. E. Salmon, an American bacteriologist. *Proteus* is a generic name, which is also the name of a Greek polymorphic sea god. *Nevskia*, derived from the Neva River in Russia, is also a generic name.

Names of micro-organisms, like those of larger forms of life, always appear in two parts, e.g., *Hansenula anomala.* The first part is the genus name and is equivalent to the surname of a person. It is always capitalized. The second part is the species name and might be likened to our Christian names. Generally the species name is not capitalized. In keeping with the convention that foreign words and phrases are often written in italics, genus and species names are italicized or in some other way made to stand out from the rest of a printed passage.

Names are also applied to ranks below species and above genus in the taxonomical hierarchy. To indicate a variety the abbreviation "var." should be placed after the species named and then the varietal name is appended, e.g., *Bacillus subtitlis* var. *niger.* Sometimes the word or abbreviation for the subspecific rank is omitted, e.g., *Bacillus cereus mycoides.* This method of designating subspecific forms is not recommended, because the resulting name resembles an illegal trinomial and the rank is not specified.

Higher ranks, such as family, order, and class, are also given Latin or Latinized names. At these taxonomic levels the rules of nomenclature differ according to which code is followed. For proper endings and other information, one should consult whichever of the following references is appropriate: International Code of Nomenclature of Bacteria and Viruses (*17*), International Code of Botanical Nomenclature (*16*), and International Rules of Zoological Nomenclature (*26*).

Theoretically each micro-organism has one name that takes precedence over all others applied to it. Because no one system of classification has gained universal acceptance, micro-organisms often have several names. Microbiologists of one country commonly adhere

to the same system; frequently one system will gain acceptance in several countries. In any event, the system and the code of nomenclature under which a microbiologist operates will determine which name is correct. All other names then become synonyms. Thus, under the system for classification of bacteria used most widely in the United States, as given in Bergey's manual (*4*), *Pseudomonas pseudomallei* (Whitmore) Haynes is the correct name for the micro-organism that has also been known as *Malleomyces pseudomallei*, *Pfeifferella pseudomallei*, *Bacillus pseudomallei*, *Flavobacterium pseudomallei*, *Actinobacillus pseudomallei*, *Bacillus whitmori*, *Sclerothrix whitmori*, and *Loefflerella whitmori*.

An American microbiologist who wishes to know the correct name of a bacterium or an actinomycete should refer to the seventh edition of Bergey's manual (*4*), unless he knows of a more recent authoritative treatise about the group under consideration that supersedes it. Until the Index Bergeyana is published, the sixth edition of Bergey's manual must be relied upon for the most complete listing of synonyms and other historical data about viruses, bacteria, and rickettsiae. References are given for other categories of micro-organisms where they are discussed in this bulletin.

As defined previously, taxonomy is the science of classification. In biology, classification is the systematic arrangement of living things into groups. Living things can be divided into the plant and animal kingdoms. Each kingdom may be divided into two or more smaller segments, which themselves may be fragmented. This procedure is repeated down to the single cell. In botanical sciences, these resulting groups in descending rank below kingdom are division, class, order, family, genus, species, and various subspecific and infrasubspecific categories. Every species belongs to a genus, every genus to a family, every family to an order, and so on up to one of the kingdoms.

It is evident that such a scheme as outlined expresses relationships of the organisms fitted into it. The primary objective of taxonomy is to establish the phylogenetic or evolutionary relationships of living things. The phylogenetic relationships of some micro-organisms, notably bacteria, actinomycetes, and viruses, are not so readily determined as they are among higher forms of life. Because of this deficiency in knowledge, schemes for their classification have at best been only quasi-phylogenetic. Year by year taxonomic investigations provide greater insight to taxonomists, so that the true relationships of micro-organisms of all types are becoming better known.

Once a system of classification for a group of micro-organisms is devised, it can be used to identify an unknown. After making certain that the unknown is pure, or uncontaminated, it must be characterized. This is generally done by determining its morphological, cultural, and, if appropriate, physiological properties. In the classification and identification of the more highly developed micro-organisms, such as molds, morphological characterization is relied upon to a large extent. An important part of the morphological characterization of the higher forms pertains to sexual and asexual spores. Such cultural characteristics as growth patterns and temperature responses may also be helpful. Among the lower forms, such as bacteria, morphological and cultural distinctions are inadequate and physiological character-

ization supplements them. Identification at the species level and often even at the genus level is sometimes almost entirely biochemical.

Among the common physiological tests are those for determining the ability to liquefy gelatin, to reduce potassium nitrate to nitrite or even to ammonia or gaseous nitrogen, to change the reaction of milk, and to digest the curd produced in milk by some types of micro-organisms. Many other examples are given in the Manual of Microbiological Methods (*6*) and in textbooks of bacteriology.

It must not be supposed that an unknown must be completely characterized before identification by classification keys is attempted. In fact, keys will suggest which tests are necessary at each step in the identification process.

Nomenclature and taxonomy are complex subjects. Only the fundamentals have been presented here. Two more principles might be emphasized however. The first is that in taxonomic work only characteristics that are constantly reproducible can be relied upon, and the second is that to be really of value a characteristic should correlate with one or more other properties. Obviously every characteristic will not be of taxonomic value. Merely because two otherwise similar micro-organisms differ, for example, in ability to reduce nitrates, it does not follow that they are different species. The proper conclusion might rather be that the ability to reduce nitrates is inconstant and not suitable for use in the classification of these micro-organisms.

SYSTEMS OF CLASSIFICATION

Most groups of micro-organisms have been studied taxonomically by several investigators. It must be realized that no classification or treatment, no matter how complete and thorough, can ever be considered perfect. Generic and specific concepts are subjective and vary with the monographer. In some instances a species is clearly distinct from all its relatives and therefore its limits do not in any way overlap those of other species in the same genus. For example, *Absidia glauca* Hagem is always found in the soil and has globose spores and a green aerial mycelium. Never has a culture been found to have other colors or other shaped spores, or to come from any place but soil. This organism is clearly a distinct taxon. However, one expert might consider it a species and the next a color variety of another soil-inhabiting species with globose spores and colored mycelium.

A species is actually a filing system, in which individuals in nature may be conveniently grouped. Several related groups of micro-organisms then may be considered as distinct entities; but since they are closely related, they are placed together in a genus. The best classification of a particular group will be determined by the usefulness of the system; the one most usable on the natural population will be the best. Often a taxonomic work quite adequate for a given geographical location will be completely useless in another region.

A second factor is that increased study of more and more individuals will cause the expert to alter his species concept: Species A and B were created on the basis of two isolates, one of A and one of B. Later when a hundred or more forms have been found, A and B cannot be distinguished because of all the degrees of intergradation between the

two. Therefore, it is necessary to combine them in one large species with the limits defined to include forms found originally in both A and B.

Thus far only individual micro-organisms as found in nature have been considered. A further problem arises as to manmade forms—mutants and hybrids. Mutants have been produced in many micro-organisms used industrially; for example, in the production of penicillin by *Penicillium chrysogenum*. Some *P. chrysogenum* mutants are so unlike their wild-type ancestors that one would never recognize them as *P. chrysogenum*. Such highly mutated forms probably could not survive in nature if they had to compete with the myriads of other micro-organisms for food. It appears to us that there is really no problem. The mutants should just be given numbers and carried as *P. chrysogenum*, for we know what their ancestral form was in nature. If, however, the mutant were to be made in nature or to escape into nature and become established, then it would be treated in the same way as any new taxon found in nature. Undoubtedly mutants have been produced in nature and are now recognized as distinct species.

The second special category comprises fertile hybrids produced in nature that have become established and are known to have been produced by species A mating with species B to form a fertile hybrid C. Hybrid C has reproduced by itself and become thoroughly established. The correct manner of citing such an organism is to designate A × B. For example, if *Rhizopus arrhizus* and *Rhizopus stolonifer* formed such a hybrid, then the designation would be *Rhizopus arrhizus* × *Rhizopus stolonifer*.

In general, two distinct types or forms of classification will be encountered. In the first, a purely arbitrary system is developed without any regard to natural relationships. It is purely mechanical. For example, F. E. Clements and C. L. Shear in their book, The Genera of Fungi, separated the families of the order Moniliales as to whether the fungi had either light-colored spores (hyaline) and mycelium or dark spores and mycelium. The light-colored forms were placed in families divided on the basis of whether the conidia were one-celled, two-celled, and so on. Obviously, very closely related forms might have both one- and two-celled spores; yet in this type of system they are not in the same genus or even in the same family. A similar mechanical key to the genera of bacteria is given in Bergey's manual (*4*).

The second type of classification is based on natural relationships. Thus, the families of the order Mucorales (*14*) are erected on the basis of genera having exactly the same type of sexual reproductive organs and similar types of asexual reproduction. The genera *Zygorhynchus* and *Mucor* have sporangia exactly alike and the sexual stage, zygospore, is represented by a roughened thick-walled spore. The distinction between the two genera is based on the homothallic condition and on the fact that one of the progametangia is larger than its mate in *Zygorhynchus*. In contrast, most species of *Mucor* are heterothallic, and its few homothallic species have progametangia alike in size and appearance. In some taxonomic treatments both types of keys for identification are incorporated, so that one can use one key to check against the other.

To identify an unknown micro-organism, the following procedure is given. First, at the time of isolation or collection, keep as complete a record as possible. Too often one thinks he will remember the circumstances when he first sees the organism, but later he cannot recall accurately the information he needs. If the organism is going to be studied in a living condition and not as a herbarium specimen for only taxonomical purposes, the source of the isolate should be recorded immediately. This record should include the location of the material from which the isolate was made, for example soil sample, 1 inch below soil surface, oak woods, Bradley Park, Peoria, Ill. The data should indicate whether the soil was dry or moist, the exact collection and isolation dates, the medium used for isolation, and the temperature of incubation. It is often well to give a brief description of the organism at the time it was isolated, and if possible to photograph it.

Sometimes organisms will change greatly after a period of time in culture or be changed in the absence of other micro-organisms. With organisms that have a complex morphology, such as lichens and many fungi, the original plate or specimen should be dried and kept as reference material. Often identification may be impossible unless this is done. The isolation record should also show who collected the material and who made the isolation. If the organism occurs as a parasite or in association with another organism, the host should be identified, and if this is not possible, the entire host, for instance a plant, should be collected for a plant taxonomist to identify. Any unusual observation at the time of collection should be noted; for example, if the organisms sporulated only in the region of growth next to a certain bacterium. At this time the culture should be assigned a number for record keeping.

Before making a complete study of the isolate for purposes of identification, one should make absolutely certain that the culture has been purified of other micro-organisms. If this is not possible, one should at least know what the other associated micro-organisms were. For example, in studying the genus *Syncephalis*, a fungus that is an obligate parasite, it should be transferred onto a previously identified host. In some cases with protozoa and algae, the culture may still contain many species of bacteria.

In the ARS Culture Collection, regardless of the source of the material, we check the purity of the culture by isolating single spores or cells, or if none are produced, then hyphal tips from the margin of the colony. This procedure is followed whether the culture came to us from an untrained person or from a laboratory with the highest standards of pure-culture techniques. For Mucorales, in addition to selecting spores from a single aerially borne sporophore, we always inoculate the purified culture on four different media, including one that almost completely suppresses growth. This procedure will allow a contaminant, especially bacteria, to appear that would not ordinarily be detected on the customary media used to identify the organism.

We shall assume that by now the micro-organism has been isolated in pure culture and that one knows to which of the major groups it belongs. Let us assume that it is a fungus. One first examines the culture and determines that it produces abundant mycelia with no cross walls and that it produces spores inside saclike structures. Fur-

thermore, when the spores germinate, they form germ tubes rather than motile spores. If one now turns to a general treatment of all the fungi, such as the key by Martin (*22*), one sees that the fungus must belong to the class Phycomycetes, and that because many spores are borne in sporangia, it belongs to the order Mucorales. Further study of the culture indicates that in the original isolation plate zygospores are present, and after isolating several individuals, zygospores are formed when the proper strains are put together. It is obvious now that the material under study belongs in the family Mucoraceae.

In order to proceed further, one must now refer to one or more monographic treatments on this family to get detailed descriptions of the genera and the species. Often one may be sufficiently familiar with the organism to tell at a glance that it belongs in a certain class, order, family, and even genus, so that he can start immediately with a detailed treatment. Assuming that a complete treatment of the genus is available, such as *Rhizopus*, one must then determine from this treatment the conditions required to duplicate that of the monograph. For instance, one finds that among the first characteristics to be determined is whether the fungus will grow at 37° C. and whether the spores are marked in a peculiar fashion when grown on malt agar. The same temperature, media, and other conditions should be used regardless of whether they are best for growth or not. Of course, if at a later time one may want to monograph a particular genus, then he can introduce various other conditions. But, for identification only, one should always try to compare the organism under as nearly as possible the same set of conditions as those in the monographic treatment.

Next, step by step each characteristic should be checked and compared to those found in the descriptions. If the identification is still in doubt, an attempt then should be made to obtain a type culture or a type specimen in the form of dried material, or a slide, to make an actual comparison. Even when this type material is obtained, especially a culture, it should be examined critically and compared with the published description. This check should preferably be made with the original published description prepared when the organism was first described, provided it is recent. Many species descriptions published 75 or 100 years ago are so incomplete that they are of little use, except to the specialist. Some individuals assume that if the type culture is obtained, it is above question. However, sometimes this type culture has degenerated, so it is no longer typical of the natural occurring strains, or it has been completely replaced by a contaminant. It is, therefore, always necessary to check a reference strain against the published description.

After a comparative study has been made, if one is still uncertain as to the identity of the organism, he should then send it to an expert on the group for study. The specialist has many other duties and should not be burdened with frequent and repeated identifications. The specialist should be supplied with all the pertinent information needed for the identification. It is also understood that he may, if he chooses, describe the organism as a new species or use it in his studies. It is not ethical to send the same culture to two individuals for identification unless both are told that you have done so.

Once the organism is conclusively identified, the record should be completed with the information one has amassed, such as growth conditions and measurements. The entry should give the generic name, the species name, and the authority for the binomial. Thus, if one cites *Phycomyces blakesleeanus* Burgeff, this name indicates that the genus is *Phycomyces*, the species is *blakesleeanus*, and Burgeff first described the species.

NAMING NEW GENERA OR SPECIES

The naming of new genera or species is generally best left to the taxonomic specialist, who is familiar both with the organisms closely related to the new one and with the problems and necessary procedures involved in the naming of new organisms. First, it is necessary to know the related organisms in order to ascertain that an organism represents a new taxon. Second, since published names are practically irrevocable, the name must be checked against the literature to see that there is no duplication. Third, for a new name to be accepted, its publication should conform to the pertinent code of international nomenclature.

Checking a proposed name is no small task. There are some 150,000 names of fungi, in addition to approximately 50,000 that are currently accepted. A new species name must not duplicate any name of currently accepted species nor any name of any species, valid or not, that has been ascribed to the genus since the nomenclatural starting date of the particular group of fungi. Checking the name for a new fungus genus is even more complex; it must not duplicate any generic name in the entire plant kingdom, including a large number of synonymous or otherwise invalid names dating back to 1753.

However difficult the assignment of names to particular strains of organisms may be, correct assignment is necessary, and mention should always be made of the specific organism in the publication of microbiological data. Experiments performed on a particular strain or group of strains are meaningless in many instances where they were reported as performed on a mold or bacterium. Even reference to strains as *Bacillus* sp. or *Fusarium* sp. does not permit confirmation of results in many cases or assignment of the data to the information known about specific organisms. In order that such data can be confirmed, assigned to specific organisms, and correlated with other data if the species is not known or cannot be determined, the strain should be deposited in an accessible culture collection, where it can be permanently maintained. The number assigned to the organism in that culture collection should be reported along with any data that are published.

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