The Characteristics of Cave Microorganisms

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Ⅰ. Introduction

The plant life of caves is made up of species that can live in total darkness. If any plant in a cave contains chlorophyll, the chlorophyll obviously cannot be energized by light rays from the sun. With a few exceptions noted below, none of the plants in the perpetually dark zone contains chlorophyll. The chief plant life there consists of bacteria, including actinomycetes, and fungi.

With regard to their mode of life within the cave, these micro-organisms are divided into *heterotrophs* and *autotrophs*. Each group requires carbon compounds for its nutrition, but the heterotrophs are unable to synthesize them and must obtain organic material by consuming the remains of other plants found in the cave. The autotrophs, on the other hand, are able to build the organic substances essential for life directly from inorganic raw materials.

Most autotrophs that live on the surface contain chlorophyll and derive the energy for food synthesis from the sun. In the cave environment, however, certain autotrophic bacteria, known as *chemoautotrophs*, are able to obtain all the energy they need from the transformation of certain minerals to different ones.

These chemoautotrophs may play an important role in the food chains of some caves. After synthesizing food from inorganic material, they serve as food for such small animals as protozoans, which in turn feed larger animals. Thus, the chemotrophs theoretically can serve as the basic food supply for the life or the entire cave.

Heterotrophic bacteria and fungi break down waste material deposited by cave-dwelling animals as well as organic material brought in by flowing water or visiting animals. In so doing they perform two functions. First, they act as scavengers; second, they release chemical compounds for further use as nutrient material for other organisms. Heterotrophic bacteria cannot exist in areas devoid of organic material, but autotrophic bacteria can. Life can therefore go on in a cave that is sealed from the surface, provided its flora includes a few autotrophic microorganisms.

II. Characteristics of the Microflora

Since a cave is normally connected by an entrance to the surface, microorganisms found in the dark zone are similar to species found in the surface soil. The spores by means of which they propagate are so small that they can readily be carried deep into the cave by percolating soil water, as well as by currents of air, by streams of water flowing into the cave, or by animals. When they have settled in a suitable environment, the spores germinate and develop into the mature forms of the species.

Demonstrations in English caves have shown that the mere passage of explorers into and out of a cave can cause extreme contamination by certain types of bacteria that had not previously existed in the cave. Sterile petri dishes placed in a virgin cave area and then cultured have proved to be free from bacteria of certain outside species, but when cultured after a party of ten or twelve people had passed through this same area, they contained many of these bacteria.

To determine the extent to which a cave has been invaded by heterotrophic forms brought in from the surface, either by air or by water, we need only take samples at a great many places in a cave and culture them for molds.

The French microbiologist Victor Caumartin has pointed out that molds, and the microorganisms that accompany them, penetrate no farther than tiny fragments of surface plants have been carried in by natural agencies. In passages beyond those containing the innermost molds, the microscopic plant life is chiefly autotrophic.

The presence of molds may therefore define the contaminated areas. At the boundaries between contaminated and uncontaminated areas, the heterotrophic surface bacteria, which break down organic compounds, and the autotrophic underground bacteria, which synthesize these compounds, overlap and compete with one another.

Among the autotrophic microorganisms commonly found in caves are the chemoautotrophic iron bacteria. This is not surprising, since most caves contain everything that these bacteria need in order to live, including an abundance of moisture and of iron compounds, and a sufficient quantity of certain indispensable trace elements. Caumartin has shown how these resources might be utilized by the iron bacterium *Perabacterium spelei.*

This species is anaerobic-that is, it requires no free oxygen-and it can fix nitrogen obtained from the air. It derives the carbon it needs from iron carbonate in the walls of the cave. Decomposition of the iron carbonate supplies the energy required for the bacterium's metabolism. This process liberates ferrous ions that are oxidized to produce the ferric mineral goethite [FeO(OH)], which is the brown pigment of cave silt.

Perabacterium spelei is considered by Caumartin to be the first link in a food chain that requires nothing from outside the cave. Experiments with the cave-dwelling amphipod *Niphargus* have demonstrated that it can subsist in tanks with no food other than clay containing iron minerals and iron bacteria. If no clay is available to young specimens of *Niphargus,* even if organic food is supplied, the young die before achieving their second molt.

The *Niphargus* also soon die if the supply of iron minerals in the clay becomes exhausted. *Pembacterium spelei* can therefore serve as food for the *Niphargus* in a cave that is entirely devoid of the chlorophyⅡ-bearing plant materials usually considered essential to animal life on the surface.

Energy is also released by the slow process of transformation from one clay mineral to another in the sediment which partly fills caves. Muscovite, an insoluble mineral derived from beds of shale associated with the limestone, is the principal original constituent of the clay-sized fraction of cave sediment. This muscovite formed in the shale when it was under great pressure in the Earth.

As a consequence, muscovite is unstable in the low-pressure cave environment, and it slowly changes there into calcium-montmorillonite, another clay mineral. We suggest that this and similar transformations of clay minerals may be important energy sources for cave chemoautotrophs.

The peculiar odor, suggesting damp earth or moldiness, so characteristic of some caves, is produced by cave actinomycetes, which are moldlike filamentous bacteria. Some actinomycetes synthesize carotene, a common pigment of certain cave-dwelling insects. The brownish color often found in droplets of water coating cave walls is also caused by this pigment.

Algae occur in caves, and some of them, contrary to a widely held belief, can live heterotrophically in darkness in the presence of organic material. It has usually been assumed that all algae contain chlorophyII, and therefore can synthesize nutrients only in the presence of sunlight. Recently, however, George Claus has found several species of algae that grow in total darkness in Beke Cave and Baradla Cave in northern Hungary. ChlorophyII is present in some of the species, but absent in others.

Sulfur bacteria are found mainly in caves that have developed near shaly rock strata containing such sulfide minerals as pyrite. Since most caves contain some sulfur compounds, almost every cave probably contains sulfur bacteria. Investigators have found that bacteria utilizing sulfur compounds produce vitamins essential for animal growth.

These are the familiar B vitamins, all of which have been isolated from ordinary sulfur bacteria. Most organisms synthesize the B vitamins from amino acids found in chlorophyll-bearing plants that require sunlight. Sulfur bacteria can synthesize them in caves in total darkness.

Microorganisms are themselves digested and utilized by cave insects as a source of amino acids and other materials required for growth Some microorganisms live in the intestines of cave insects. Here they aid digestion by secreting enzymes that help to convert food to a form that can be absorbed.

Cave microorganisms therefore play an important role in enabling cave animals to survive and grow on what would otherwise be an inadequate diet, and to do this in an environment entirely without sunlight.

Ⅲ. Saltpeter

A material that owes its origin in part to microorganisms is cave saltpeter, which was extensively mined to make gunpowder during the War of 1812 and the Civil War. This highly soluble material, consist-ing mainly of nitrocalcite $[Ca(NO_3)_2 \cdot {}_4H_2O]$, impregnates silt in many caves. The saltpeter-bearing silt, or "petre dirt," was processed for the wars by leaching it with water and then bolling the liquor with wood ashes to convert it to niter $[KNO_3]$.

The bacterium *Nitrobacter agilis*, responsible for the final stage of forming the nitrate of saltpeter from nitrogenous organic material, is common in saltpeter caves. A puzzling aspect of the problem, however, is that some of the caves richest in saltpeter contain no obvious accumulations of such organic material as bat guano.

A clue to the puzzle is that during the War of 1812 the principal source of saltpeter other than caves was the soil around houses and under their floors. Before the days of modern plumbing, it was in this soil that nitrogen compounds from human waste accumulated. This points to an hypothesis, originated in the first edition of this book, that the ultimate sources of the raw material for saltpeter in caves may be the urine of the cave rat, *Neotoma magister*.

These animals leave a trail of urine droplets and musk in the dark zones of caves, which helps them to find their way from the surface back to their nests in some dry western caves, where cave rats have been crossing an area of limestone for thousands of years, thick accumulations of dehydrated urine called *amberat* have formed. On silt, however, or in wet caves, bacterial action may break down the urea and convert it to cave saltpeter.

Ⅳ. Manganese Minerals

Microorganisms also probably play a part in the origin of the black manganese deposits commonly present in caves. The manganese minerals form sootlike layers that cover the walls of certain passages or coat cobbles in cave streams. In most limestone caves, as in Weber Cave, Iowa, these layers are composed of the mineral birnessite [$(Na,Ca)Mn_7O_{14} \cdot 3H_2O$].

In Jasper Cave, South Dakoda, however, where the ground water contains a higher than average amount of barium, the mineral is romanechite [$BaMn_9O_{16}(OH)_4$]. The deposits tend to be layered like stalactites.

They consist of crystals so extremely minute that they are almost beyond the limit of resolution of the X-ray diffraction technique normally used for identifying such minerals.

Limestone and cave water ordinarily contain enough manganese to provide a source for the manganese in the cave deposits. The common occurrence of the black material on stream cobbles a short distance into caves from the surface suggests that an increase in alkalinity caused by a greater bicarbonate concentration creates a favorable chemical environment for manganese deposition.

The dissolved manganese is held in solution in the cave water in a chemically reduced form, either as Mn^{2+} ions or as part of a soluble complex organic molecule. Specialized bacteria such as *Clonothrix fusca* and *Leptothrix discophora,* which are known to precipitate manganese in domestic water-supply systems, may live in the caves and cause the deposits to form there.

The bacteria may derive energy from the oxidation of the manganese from Mn^{2+} to Mn^{4+} and free it from the complex molecules, thus causing the water to become more supersaturated with manganese near the bacterial colonies. This causes the birnessite or romanechite to be deposited.

Ⅴ. Moonmilk

Laboratory cultures made from the water found on certain cave deposits reveal the presence of bacteria and other microorganisms that may play a part in the construction of certain calcareous mineral deposits and in the disintegration of the limestone wall rock.

Microorganisms have been shown, for example, to play a major role in the origin of a curious cave material known as moonmilk. This is a soft, white, claylike substance present on the walls of many caves. Its name comes from Switzerland, where in the 15th century it was called Monmilch (gnome's milk), because the people there believed that the caves were inhabited by gnomes. *Mon*(sometimes written *Moon*) meant *gnome*, but the word has become mistaken for *Mond*, which is German for moon.

The mineralogy of moonmilk has been intensively studied in Europe and North America during the past fifteen years. We now know that the microscopic grains in the moonmilk of limestone caves consist mainly of calcite [$CaCO_3$]. But in relatively warm caves where the wall rock contains appreciable quantities of magnesium as a constituent of the mineral dolomite, the grains may consist of any of the following magnesium minerals.

When the mineral constituents of moonmilk are removed by dissolving it in a weak acid, an abundant organic residue remains, which consists chiefly of such bacteria as *Macromonas bipunctata*, along with actinomycetes and algae.

This microflora probably assists in breaking down the minerals of the wall rock and aids in their conversion to the solids contained in the moonmilk.

The larger mineral bodies in calcite moonmilk have a distinctive surface sculpture that can be seen under the scanning electron microscope. The bodies consist of rods of calcite with an average size of 1×8 micrometers. A diagonal grain is impressed on the surfaces of the rods, and parallel ridges commonly trend along the lengths of the rods superimposed on the diagonal grain. The combined effect produces bodies somewhat resembling ears of corn.

The diagonal grain is aligned with the crystal structure, as can be seen through an optical microscope with polarized light. Because the crystal structure of calcite normally parallels the long dimension of calcite crystals, the grains in calcite moonmilk were once erroneously identified and named as a separate new mineral, "lublinite."

In many samples, the rods are enmeshed in a net of calcite filamenrs about 0.1 micrometers in width. These filaments are believed to have been associated with actinomycetal filaments that served as nuclei for growth of the calcite bodies.

According to this hypothesis, an inclined crystal face of a seed crystal becomes aligned with the filament and subsequent growth leads to the unusual crystal orientation and surface sculpture of the moonmilk grains.

Most moonmilk occurs where water may reasonably be inferred to move through the substance to its surface where deposition takes place by loss of carbon dioxide. The life processes of individual microorganisms cause a microvariation of the chemical environment that leads to deposition of discrete mineral grains rather than to a more solid speleothem such as cave coral.

More research is needed to determine the energy source of the microorganisms in moonmilk. The snow-white rather than brown color of most moonmilk suggests that oxidation of iron is not the source. We tentatively suggest that soluble organic compounds from the soil provide the energy for the microorganisms that control the growth of this strange substance.

Ⅵ. Medical Use of Cave Actinomycetes

Cave actinomycetes have been the subject of much research as a possible source of antibiotics. Several expeditions into caves in Central America, South America, and the United States have been conducted to collect cave silt that contains actinomycetes and molds. From these, scientists hope to obtain new and powerful antibiotics.

In the 16th and 17th centuries, long before modern "miracle drugs" were dreamed of, physicians used dried moonmilk from European caves as a dressing for wounds. They did so primarily because this substance would stop bleeding and act as a dehydrating agent, but they also believed it had curative qualities.

Now that we know that moonmilk contains actinomycetes, and that some actinomycetes possess antibiotic properties, we see that the early use of this cave material in medicine may have had a valid scientific basis, even though the early physicians did not know what it was.

It is of passing interest to note here that speleologists testify that sometimes when they enter a cave while suffering from a cold, they find that after they have been underground for several hours their symptoms largely disappear. A probable explanation for most of these cases is that deep inside caves the air is almost free from pollen, and the clean air would alleviate symptoms caused by some allergies. It is possible, however, that in some cases the cold victim obtains relief by inhaling an unknown cave product that may someday be used medically in treating common colds.

Ⅶ. Harmful Microorganisms

Not all species of cave microflora can be considered beneficial to people. As noted above, certain bacteria cause the breakdown of organic material-a welcome sanitary process when the material broken down is organic waste. But the same bacteria also disintegrate what might have been especially interesting remains.

Countless vertebrates, including humans, have been buried in caves. yet it is rare to find anything more than their bones, because the cave bacteria have usually caused complete decay of all other parts. The only exceptions to this are in certain caves with extremely low humidity; in these the microflora is nearly inactive, and in especially dry areas it is sometimes possible to find desiccated bodies.

Thus, in recent years a sequence of extinct marsupial species with skin and fur intact has been studied from the caves under the Nullarbor Desert of Australia. Also, in drier parts of caves in Mammoth Cave National Park, Kentucky, desiccated human bodies over 2,000 years old have been found.

Several pathogenic microorganisms are also known to exist in caves. One of these, *Histoplasma capsulatum*, is responsible for a disease known as histoplasmosis, whose symptoms and effects are similar to those of tuberculosis. In recent years, cave explorers in the United States, Central America, Australia, and South Africa have contracted histoplasmosis by inhaling the spores of *Histoplasma capsulalum*. Outbreaks of histoplasmosis from noncave sources have been reported from the Ohio and Mississippi Valleys.

The spores have been detected in chicken coops and in roosting areas of other birds where dried droppings accumulate, as well as in caves. Symptoms of histoplasmosis include loss of weight, anemia, fever, coughs, and sever chest pain. The usual therapeutic agent administered is Amphotexicin-B.

Ⅶ. Relation of Microorganisms to Cave Food Chains

Microorganisms are an extremely important constituent of the cave environment. They are involved in the development of cave deposits such as moonmilk, in the production of food for cave animals, and in the breakdown of organic material in the cave.

Ideally, a nearly closed ecologic system could exist in the dark zone of a cave, where the energy required for metabolism is derived from minerals in the wall rock and in sediment on the floor. The basic food cycle in such a cave setting would depend on chemo-autotrophic bacteria. These bacteria could serve as nutrient material for cave-dwelling animals, with no organic input from outside the cave.

Most caves that contain established communities of cave organisms, however, are ecologically similar to abyssal depths in the ocean, where the sunlight required for supporting the green plants on which the lives of the surface fauna depend is completely absent. In each case, bacterial decomposers rely on a flow of organic material from sunlit areas.

In caves, this can be from dissolved organic matter in drip water, from plant remains in cave streams, or from organic substances in cave silt. Organic material is also obtained from the fecal matter of animals that periodically spend time outside the cave, such as bats, cave rats, and crickets.

Thus, the primary energy sources exploited by cave organisms are either minerals broken down by autotrophic bacteria, or surface-derived organic material utilized by heterotrophic bacteria. In the quantitatively more important latter case, the decomposer bacteria are eaten by protozoans which are eaten by such aquatic cave dwelling animals as flatworms, isopods, and amphipods, which are eaten in turn by larger animals such as crayfish, salamanders, and fish. Finally, these aquatic forms release waste material that supports the heterotrophic bacteria that helped to initiate the chain, and the cycle is complete.

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