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# Heterotrophic Dominance Observed Among the Microorganisms Of the Schermerhorn Park Cave In Cherokee County, Kansas

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## ABSTRACT

Although the Schermerhorn Park Cave, in Cherokee County, is considered the most biologically diverse cave in Kansas, the microfauna of the cave does not diverge significantly from the nearby soil, and the cave's inhabitants rely primarily on the surface for their supply of organic nutrients. Analysis of water samples through bacteria density plate counts, metabolic and morphologic tests on isolated bacteria, and a quantitative water analysis provided by the Kansas Department of Health and Environment gave a preliminary survey of the underground bacteriology of the cave. The bacteriological tests revealed an abundance of heterotrophic bacteria in samples collected from the stream insurgence and an isolated pool. The water analysis showed levels of essential nutrients and toxins for iron oxidizing bacteria that diverge significantly from optimum conditions. Therefore, it seems that the Schermerhorn Park Cave is dominated by heterotrophic organisms, that the cave's microenvironment closely resembles the soil, and that large regions containing an abundance of chemolithoautotrophs do not exist.

## INTRODUCTION

Analysis of water samples through bacteria density plate counts, metabolic and morphologic tests on isolated bacteria, and a quantitative water analysis provided by the Kansas Department of Health and Environment gave a preliminary survey of the underground bacteriology of the Schermerhorn Park Cave in Cherokee County, Kansas. The Kansas Speleological Society identified the Schermerhorn Park Cave as an ideal candidate for a bacteriological investigation because it is known to have a diverse fauna including several salamander species listed as threatened or endangered in Kansas.

> [The Schermerhorn Park Cave] is the only known significant Kansas habitat for four Ozark species: the cave salamander (Eurycea lucifuga), the dark-sided salamander (Eurycea longicauda melanupleura), the graybelly salamander (Eurycea multiplicata griseogaster), and the grotto salamander (Typhlortriton spelaeus).... Other animals living in this cave include troglobitic aquatic isopods (crustaceans), numerous leopard frogs, eastern pipistrelle bats, and occasional migratory gray bats. Because of the presence of these species, some officials consider this cave the most faunistically important cave in Kansas and thus in need of protection. (Young and Beard, 1993, p.13)

The interior of the cave is quite forbidding, guarded by low crawls in cold water and mud. Those who enter the back corners of the cave must be protected with special equipment and navigate a short passage with only three inches of air (Young, 1986). Consequently, the cave remains in near pristine conditions past the 200 foot mark. The caretaker of the park estimates that only 30-35 people have entered the cave in the seven years of his employment. When Jon Beard and Jim Young, along with other members of the KSS, mapped the cave in 1985-86, they found no traces of human traffic in the back three-quarters of the 2,566 foot cave (Young, 1986).

The cave is the longest of the 14 known caves in Cherokee County (Young and Beard, 1993), and the 6th longest in the state (Bain, 1994). However, compared with other Ozark region caves, the Schermerhorn Park Cave is relatively short and close to the surface. Numerous tree roots are found penetrating the cave through the roof in the first 500 feet providing direct channels for the flow of water containing organic nutrients and soil bacteria.

In 1963, Victor Caumartin published a paper reviewing cave microbiology in the National Speleological Society Bulletin. Much of the following description comes from that article.

The microflora of caves contains bacteria, fungi, actinomycetes, and poorly characterized, ultra-microscopic forms. Primarily, caves contain the same organisms found in the soil (Caumartin, 1963), although recent discoveries of previously isolated and unknown caves have revealed new species of bacteria (Gain, 1994). However, due to the rigorous levels of selection through total darkness, filtration, variation in chemical concentrations, and lack of organic nutrients, few organisms reach the deeper and more isolated regions within caves and compete effectively for relative dominance. Therefore, the biological characteristics of cave sediments can vary greatly from that on the surface (Caumartin, 1963).

When the flow of water from the surface is blocked by impermeable bedrock or because the previous filtration channels have become impeded by clay or silt, the regular supply of organic nutrients ceases. Chemolithoautotrophs, utilizing sulfur and iron compounds in the ground water, are then particularly advantaged and become the dominant form. While the iron and sulfur oxidizing bacteria represent only a small fraction of the surface soil bacteria, pristine regions within caves may contain little else (Caumartin, 1963).

Some dispute how frequently this occurs and whether the chemolithoautotrophs make a significant

contribution to the food chain. Christiansen (1970) reported on troglobitic collembola which apparently survived on chemolithoautrotrophic bacteia in the cave clay in the absence of other food sources. Barr and Kuehne (1971), from the department of Zoology and Institute of Speleology in the Universtiy of Kentucky, later stated that any primary production within caves is negligible.

Chemolithoautotrophs grow slowly and require precise concentrations of certain growth factors. They can be easily over-taken by the more rapidly growing heterotrophs. Heterotrophic processes like denitrification and ammonification establish concentrations of $NO_3^-$ and $NH_4^+$ which are toxic to iron oxidizing bacteria. In addition, heterotrophs cause the calcium carbonate speleothems to dissolve and disintegrate through catabolic release of carbon dioxide and organic acids. Stalactites that develop a pasty, white coating, or the development of curdled, white deposits in shallow waters, known as moonmilk, indicate heterotrophic speleothem destruction (Caumartin, 1963).

Under conditions which allow a regular supply of organic nutrients, such as flooding, percolation through the soil and bedrock, capillary action along the walls of the cave, and air currents, heterotrophic organisms gain dominance and the cave microenvironment comes to resemble the soil above (Caumartin, 1963). Human traffic can also support new regions of heterotrophic dominance (Dickson, 1975; Barr and Kuehne, 1971).

Actinomycetes are common to areas within caves which are dominated by heterotrophic bacteria. Heterotrophs themselves, they play a part in the soil and within caves in the complete utilization and decomposition of organic substances (Caumartin, 1963).

The presence of fungal spores is an additional hallmark of cave regions which are in contact with the outside environment. Upon contact with a chemically reducing medium such as clay, fungus spores form a cyst until eventually falling prey to some other organism. Therefore, propagation does not continue and fungi are only found in regions that are in direct contact with the outside (Caumartin, 1963).

## MATERIALS AND METHODS

Water samples for bacteria isolation were collected on January 11, 1997. One sample was collected from an isolated pool in the rear of the cave approximately 100 feet from the stream insurgence. The small pool, approximately 6x9 inches wide, rested precariously eight inches from the stream edges and two inches above the water level. However, in the low and tight Schermerhorn Park Cave, it represented the greatest possible isolation of water. Two additional water samples were taken from the stream insurgence.

The samples contained 10 ml of cave water, were collected in sterile pipettes and transferred to sterile screw-cap test tubes, and were placed on ice while being transported to the lab. Approximately six hours after collection, the samples were distributed in a laminar flow hood to various sterile media.

Four 1 ml aliquots from the mainstream water samples and four 0.5 ml aliquots from the isolated pool samples were used to inoculate separate petri dishes containing Sabouraud's Dextrose agar to survey for the presence or absence of fungal spores. The plates were incubated at 23°C and observed six and ten days after inoculation.

One milliliter aliquots from the samples were passed through a series of one-tenth dilutions and used to inoculate petri dishes of Bacto plate count agar for bacteria density plate count. The plates were incubated at 23°C for 36 hours. Plates containing less than 100 colonies, usually 1/10 or 1/100 dilutions, were then counted under a magnifying glass. The raw score was converted to the number of colony-forming-units per milliliter.

Several more 1 ml aliquots from the mainstream sample and 0.5 ml aliquots from the isolated pool sample were spread over large diameter petri dishes containing nutrient agar to initially isolate pure colonies. The plates were incubated at 18°C. Eleven days after inoculation, the 20 fastest growing, pure colonies from a sample of the isolated pool were tested for motility, oxygen requirements, fermentative capability, denitrification, catalase production, and anaerobic release of $H_2S$.

Motility was determined through inspection of a wet mount with phase contrast microscopy. Facultative anaerobism was tested through incubation of a sealed container with catalytically removed oxygen at 18°C for 11 days. Fermentation was determined through Durham tubes containing either glucose or lactose enhanced nutrient agar and again with the Methyl Red-Vogues Proskauer series. Denitrification was tested through inoculating Durham tubes containing nitrate broth and incubating them at 18°C until suitable growth was obtained. The tubes were then tested for nitrite, ammonia, and gas. Catalase production was determined by spreading a loop containing the colony through two drops of hydrogen peroxide. Peptone iron agar tubes were used to identify the release of $H_2S$ from anaerobic attack on proteins.

An additional water sample was collected at the cave resurgence downstream from the gate on October 9, 1996, by Bryan Bain, an executive committee member of the Kansas Speleological Society, as part of the Kansas Ground Water Quality Monitoring Network. A thorough quantitative analysis of the inorganic, organic, and radioactive components of the sample was done by the Environmental Laboratories of the Kansas Department of Health and Environment.

## RESULTS

Plate counts of the water samples revealed an average of 700 colony-forming units per milliliter in the main stream water and 50 colony-forming units per milliliter

**Table 1.** Select Ionic Biocatalysts for Iron Bacteria

| Biocatalyst | Optimum Concentration gram-ions/L | Observed Concentration gram-ions/L | Effect |
|---|---|---|---|
| $Mn^{++}$ | $10^{-8}$ | $800x10^{-8}$ | poisonously high concentration |
| $Ni^{++}$ | $10^{-6}$ | $3x10^{-6}$ | near optimum |
| $Cu^{++}$ | $10^{-6}$ | $2x10^{-6}$ | near optimum |
| $Al^{+++}$ | $10^{-5}$ | $11.3x10^{-5}$ | high but acceptable |
| $Br^{-}$ | $10^{-5}$ | $10x10^{-5}$ | high but acceptable |

**Table 2.** Select Ionic Toxins for Iron Bacteria

| Toxin | Minimum Toxic Concentration gram-ions/L | Observed Concentration gram-ions/L | Effect |
|---|---|---|---|
| $NO_3^{-}$ | $10^{-5}$ | $100x10^{-5}$ | poisonously high concentration |
| $NH_4^{+}$ | $10^{-5}$ | $4x10^{-5}$ | poisonously high concentration |

**Table 3.** Ratios of Nutritional Elements

| Components | Optimum Value* | Observed Value** | Effect |
|---|---|---|---|
| $Cl^{-}/SO_4^{--}$ | 0.1 | .52 | decline in growth rate |
| $Ca^{++}/Mg^{++}$ | 2.5 | 15.2 | ??? |

* Optimum values obtained from Caumartin, Victor. 1963. "Review of the Microbiology of Underground Environments." The National Speleological Society Bulletin. Vol. 1. Part 1.

** Observed values obtained from a water analysis provided by the Kansas Department of Health and Environment. Lab #700606PT.

in the isolated pool.

The plates of Sabouraud Dextrose agar inoculated with the main-stream water samples revealed one fungus colony and numerous bacteria colonies 6 days after inoculation. The plates of Sabouraud Dextrose agar inoculated with the isolated pool water samples revealed neither fungus nor bacterial growth 6 days after inoculation. After 10 days, one of the four plates inoculated with isolated pool water showed a 1 mm diameter bacteria colony with several other pinpoint colonies.

Several plates of nutrient agar inoculated with the sample from the isolated pool that were placed in the refrigerated incubator developed fungus colonies. However, it was confirmed that at least a portion of these colonies was caused by contamination from fungus growing on the dried grasses which were previously stored in the refrigerated incubator.

One of the 20 colonies from the isolated pool sample selected for additional tests was identified as an actinomycete and not studied further. Motility was observed in 68% of the remaining bacteria. Ninety-two percent had strictly aerobic forms of metabolism, while eight percent showed the capacity for facultative anaerobism. All isolates tested negative for fermentation in the Methyl Red-Vogues Proskauer series and Lactose fermentation tubes. Eighty-nine percent did not produce acid with glucose. While 11% produced acid with glucose, none formed gas. Seventy-one percent tested negative for denitrification. Twenty-nine percent reduced nitrate to nitrite, but none produced ammonia or gas. Eighty-nine percent tested catalase positive.

Selected results of the water analysis done by the Kansas Department of Health and Environment are listed in Tables 1-3. None of the tests for organic or radioactive pollutants revealed concentrations greater than or equal to the minimum reporting level.

## DISCUSSION

The results from the bacterial density measurements and survey for the presence of fungi indicate that the isolated pool contains a less dense collection of bacteria

which are more sensitive to the 4.5 pH of Sabouraud's Dextrose Agar. The fragile, water-saturated, clay walls bounding the isolated pool showed no signs of damage which would indicate contamination from outside sources. Any vector for cross contamination would have certainly carried the acid tolerant bacteria into the isolated pool. The ten-fold difference in relative abundance of bacteria again suggests that unlike the mainstream, the isolated pool has not received the great influx of organic nutrients and foreign bacteria clinging to the coveralls of human cave explorers. For these reasons, I propose that the isolated pool sample provided a representative selection of the bacteria native to the cave before human exploration and contamination.

The complete absence of fungal spores has traditionally identified pure cave sediments uncontaminated by outside sources (Caumartin, 1963). Although the Sabouraud Dextrose agar plates from the isolated pool did not grow fungus and the plates for the mainstream water did, other nutrient agar plates inoculated with the isolated pool water grew fungus colonies. The origin of these fungus colonies must be questioned due to confirmed contamination from air within the refrigerated incubator. Unfortunately, conclusive evidence of their origin is unavailable. Therefore, verifying the presence or absence of fungal spores in the sample from the isolated pool remains a question unanswered by this investigation.

These findings confirm a long held understanding that vastly different microbial communities exist within close proximity and that regions can be found in caves, even in areas of human traffic, which remain relatively uncompromised with respect to the natural fauna.

Of the bacteria studied more closely, insufficient evidence was collected to provide reliable genus or species level differentiation. However, there was a tendency toward motility, strictly aerobic respiratory metabolism, and catalase production which suggests a fauna typical of a shallow, freshwater stream minus the phototrophic organisms (Brock, Madigan, Martinko, and Parker, 1994). While only 29% of the isolates were capable of denitrification, they followed Caumartin's (1963) characterization of cave heterotrophs by going no further than the reduction of nitrate to nitrite.

Because this research focused on those bacteria that grew sufficiently well on nutrient agar, the studied bacteria were predominately heterotrophic. No attempts were made to establish enrichment cultures which favored the chemolithoautotrophic iron or sulfur oxidizers. However, heterotrophic bacteria establish equilibriums of essential nutrients which diverge from the concentrations for optimum growth of iron oxidizers (Caumartin, 1963). Tables 1 and 2 show how this was observed in the cave water. Also, certain waste products from heterotrophism are toxic to chemolithoautotrophs even in low concentrations (Caumartin, 1963). Table 3 shows that nitrate and ammonia were detected in the cave water at levels toxic to iron oxidizers.

The abundance of heterotrophic bacteria and the presence of actinomyces indicate that sufficient organic nutrients are available in the far corners of the Schermerhorn Park Cave and that large habitats supporting chemolithoautotrophic dominance probably do not exist. Although the Schermerhorn Park Cave is considered the most biologically diverse cave in Kansas, the microfauna of the cave does not diverge significantly from the nearby soil, and the cave's inhabitants rely primarily on the surface for their supply of organic nutrients.

Further study to identify the presence and relative abundance of sulfur oxidizing or reducing bacteria as well as their relative contributions to the food chain are warranted. Additionally warranted are investigations into the changing availability of organic nutrients with respect to seasonal weather changes and how this may affect the populations of the other cave inhabitants.

Because the Schermerhorn Park Cave relies heavily on receiving nutrients from the outside, it may be especially sensitive to various forms of pollution. Although the lack of reportable levels of organic and radioactive pollutants suggests that the Schermerhorn Park Cave is not in immediate danger, a continued emphasis on conservation is needed to protect the cave's rich biological diversity.

This study examined water samples from the Schermerhorn Park Cave, in Cherokee County, Kansas for the presence of chemolithoautotrophic and heterotrophic bacteria because the relative dominance of either type leads to important understandings of how the cave interacts with the surface. Metabolic and morphologic tests on isolated bacteria collected from deep within the cave and a water analysis provided by the Kansas Department of Health and Environment identified heterotrophic dominance and ruled out large populations of chemolithoautotrophic bacteria. This suggests that the cave's microfauna resembles the soil above and that the cave inhabitants rely on the outside for their supply of nutrients.

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