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5. From 1967 to 1970 the author spent more than 200 days of fieldwork investigating the leaf-mining insect fauna which occurs on California Fagaceae. He has an intimate knowledge of the mine configuration and host range for each species.
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10. The extant species are undescribed species of *Nepticula* and *Bucculatrix*, as well as *Lithocolletis leucothorax* (Walsingham). Six other species of leaf-mining Microlepidoptera feed on *Quercus chrysolepis* in California.
11. Potassium/argon (K/A) dating gives an age of 18×10^6 years for Buffalo Canyon and 16×10^6 years for Upper Goldyke (D. I. Axelrod, personal communication).
12. Supported by NSF grants GB 4014 and GB 6813X (principal investigator, J. A. Powell). Examination of fossil leaf material in the collections of the University of California was made possible through the courtesy of D. I. Axelrod and H. E. Schorn. The manuscript was reviewed by J. T. Doyen, J. A. Powell, E. I. Schlinger, and H. E. Schorn.

10 October 1972

Bacterial Origin of Sulfuric Acid in Geothermal Habitats

Abstract. *Natural populations of* Sulfolobus, *a new genus of bacteria occurring in sulfur-rich, acid hot springs and soils, were found to oxidize large amounts of sulfur to sulfuric acid at temperatures up to 85°C. These bacteria are important high-temperature geochemical agents in solfatara soils.*

Solfataras are regions in geothermally active areas characterized by hot acid soils and occasional hot springs that discharge limited amounts of acid water. Solfatara soils are heated by steam rising to the ground surface and contain large amounts of elemental sulfur that arises from the spontaneous oxidation of H_2S present in the steam (*1*). The high acidity of solfataras is due to the large quantities of sulfuric acid produced from the biological oxidation of the elemental sulfur (*1*). Bacteria of the *Thiobacillus thiooxidans* type have been thought to be responsible for this acid production because they could be isolated from solfatara soils, but since they occur only at sites with temperature less than 50°C, movement of acid water from low-temperature sites had to be postulated in order to explain the presence of acid at higher-temperature sites (*2*). Recently, a new genus of sulfur-oxidizing bacteria, designated *Sulfolobus*, has been isolated from sites with temperatures of 65° to 90°C in solfatara areas (*3*), and studies with $^{14}CO_2$ have shown that *Sulfolobus* is metabolically active in soils up to 85°C (*4*). The presence of these bacteria in sulfur-rich habitats suggested that they may play an important role in sulfuric acid production. Using ^{35}S-labeled elemental sulfur (S^0), we have measured directly the oxidation of S^0 in sulfur-rich hot springs, and the results indicate that *Sulfolobus* does oxidize large quantities of S^0 to sulfuric acid.

Twelve hot, acid, sulfur-containing pools in Yellowstone National Park, Wyoming, were studied, but data are given here for only two, Sulfur Caldron and Moose Pool, both located in the Mud Volcano area of the park (*5*). Some properties of the two pools are given in Table 1. Large numbers of *Sulfolobus* were present in most of the pools, but other kinds of bacteria could not be detected microscopically. *Sulfolobus* cultures were sought and obtained from seven of the pools. Oxidation of sulfur was measured by using the $^{35}S^0$ technique described for laboratory cultures of *Sulfolobus* (*6*). Samples were transported in thermally insulated containers to the field laboratory where they were distributed into bottles containing $^{35}S^0$ and incubated at several temperatures (*7*). Portions were removed periodically and filtered through 0.45-μm filters, and the $^{35}SO_4^{2-}$ in the filtrates was determined by liquid scintillation counting (*8*). The amount of S^0 oxidized to SO_4^{2-} was calculated on the basis of the specific radioactivity of the $^{35}S^0$ added and the amount of natural S^0 present in the sample. It was assumed that the bacteria oxidized the natural S^0 and added S^0 at the same rates (*9*).

Elemental sulfur was readily oxidized by samples from most of the sources tested. In Moose Pool, the optimum temperature for oxidation was 80°C, but oxidation occurred at a substantial rate at 85°C also (Fig. 1). At 90°C, some oxidation occurred but eventually ceased, probably due to thermal injury or death of *Sulfolobus*. Moose Pool samples were incubated at 95°C in a separate experiment, and no oxidation occurred at this temperature. No oxidation occurred in bottles to which 4 percent formaldehyde had been added at the start of incubation (Fig. 1); this, together with the lack of oxidation at 95°C, indicated that spontaneous, or nonbiological, oxidation of S^0 did not occur. The absence of a lag in oxidation at the beginning of incubation suggests that the bacteria were actively oxidizing S^0 when the samples were taken from nature. The optimum temperature for S^0 oxidation was reasonably close to measured environmental temperatures (Table 1). Similar results were obtained with samples from Sulfur Caldron, but in this case the optimum temperature for S^0 oxidation, also 80°C, was about 13 degrees higher than the mean environmental temperature (Table 1). These results directly confirm that sulfur oxidation in solfataras is a biological process, a conclusion previously based on an analysis of $^{34}S/^{32}S$ isotopic ratios (*1*).

Table 1. Properties of Moose Pool and Sulfur Caldron and rates of S^0 oxidation. Data on sulfuric acid production (*10*) were used to compute S^0 oxidation rates in the Yellowstone solfataras and the Japanese crater lakes.

Site	Temperature (°C)	*p*H	S^0 (mg/ml)	*Sulfolobus* (cell/ml)	Surface area (m^2)	Volume (m^3)	S^0 oxidation	
							g/m^2/day	kg/day
Moose Pool	72–80.5	1.59	1.02	2.5×10^7	300	300	67	20
Sulfur Caldron	65–67.5	1.53	1.09	7.4×10^7	100	400	190	19
Solfatara areas								
Roaring Mountain					128,000		3.1	
Ampitheater Springs					72,000		5.0	
Norris Ranger Station					6,700		3.5	
Norris Junction					11,000		2.5	
Crater lakes								
Okunoyu	80				1,000		16	
Ōyumuma	50				16,000		25	

Rates of S^0 oxidation in nature by *Sulfolobus* were estimated so that a comparison could be made with previous measurements of sulfuric acid production in Yellowstone solfatara areas and in two lakes in a Japanese thermal explosion crater (*10*). Oxidation rates at mean environmental temperatures were estimated by interpolation of 24-hour and 48-hour temperature optimum plots, such as that shown for Moose Pool at 48 hours in Fig. 1. The rates of oxidation of S^0 for the *Sulfolobus* sites were somewhat greater than those calculated for the Japanese thermal lakes and considerably greater than those estimated for the solfataras (Table 1). The amounts oxidized per day represent 1/15 and 1/23 of the total amounts of S^0 assayed in Moose Pool and Sulfur Caldron, respectively. In the previous work, acid production was estimated by measuring sulfate concentrations in runoff waters. The solfataras contain sources of thermal water, but most of their areas consist of soil habitat. A possible explanation for the low rates of oxidation in the solfatara areas may therefore be that *Sulfolobus*, and other S^0-oxidizing bacteria, are more active in aquatic habitats. In addition, it is likely that the estimation of sulfuric acid production based on surface water runoff may give erroneously low results because of loss of acid-rich water by subsurface flow (*10*). Direct measurement of $^{35}S^0$ oxidation probably provides a more reliable means of measuring sulfuric acid production.

No attempt was made to control the amounts of O_2 and CO_2 available to the bacteria during the oxidation assays. Incubation conditions may have deviated in this respect from those in nature, but in view of the linear rates of S^0 oxidation obtained and the relatively short incubation periods employed, our results probably provide a good estimate of oxidation rates in nature. Other pools containing similar numbers of *Sulfolobus* exhibited S^0 oxidation rates comparable to those reported here for Moose Pool and Sulfur Caldron, but sites containing fewer bacteria oxidized S^0 more slowly. The extent to which the observed optimum temperature coincided with the environmental temperature also influenced the rate of S^0 oxidation at a particular site. Temperature optima for S^0 oxidation of 60°, 70°, and 80°C were obtained at other sites, and these optima were frequently higher than the corresponding environmental temperatures. The frequent lack of correspondence between optimum and environmental temperatures suggests that there are different temperature strains of *Sulfolobus* that are incapable of adapting to a wide variety of environmental temperatures.

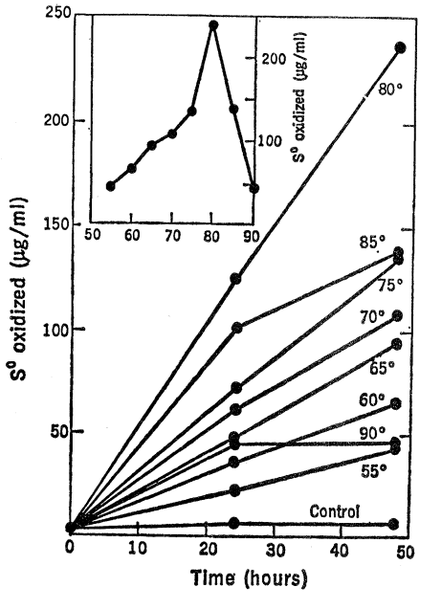

Fig. 1. Oxidation of S^0 by *Sulfolobus* from Moose Pool at different incubation temperatures. Inset shows the amount of S^0 oxidized in 48 hours plotted against incubation temperature.

The data presented here show that *Sulfolobus* is capable of oxidizing large amounts of S^0 to H_2SO_4 at temperatures up to 85°C. Because of the technical problems involved in distributing $^{35}S^0$ throughout soil samples, our study was confined to aquatic samples, but the presence of *Sulfolobus* in soils as hot as 85°C and its ability to fix $^{14}CO_2$ there at this temperature strongly suggest that S^0 oxidation occurs also in soils. *Sulfolobus* is therefore an important geochemical agent in solfatara areas, and its S^0-oxidizing activity at high temperatures obviates the need to postulate movement of sulfuric acid from lower-temperature sites containing *Thiobacillus thiooxidans* to higher temperature sites. Similar radioisotopic approaches could probably be applied to the study of bacterial action in other geochemical processes.

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References and Notes

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3. T. D. Brock, K. M. Brock, R. T. Belly, R. L. Weiss, *Arch. Mikrobiol.* **84**, 54 (1972).
4. C. B. Fliermans and T. D. Brock, *J. Bacteriol.* **111**, 343 (1972).
5. "Moose Pool" is our unofficial designation for an apparently unnamed sulfur-containing pool located about 230 m southwest of Black Dragon's Caldron.
6. D. W. Shivvers and T. D. Brock, *J. Bacteriol.*, in press.
7. Ten-milliliter portions were added to 50-ml bottles containing 2 to 8 mg of $^{35}S^0$ (1 μc/mg). Three bottles were incubated at each of the following temperatures: 55°, 60°, 65°, 70°, 75°, 80°, 85°, and 90°C. Control bottles were incubated at 75°C after the addition of 4 percent (weight to volume) formaldehyde.
8. The ^{35}S-labeled material in the filtrates was insoluble in CS_2 and was precipitable with excess $BaCl_2$, indicating that unoxidized S^0 did not pass through the filters and that oxidized sulfur compounds of oxidation state less than that of SO_4^{2-} were not formed in detectable amounts.
9. Elemental sulfur was measured spectrophotometrically by the method of F. Pachmayr [dissertation, Ludwig-Maximilians University, Munich, Germany (1960), pp. 21–27]; the S^0 was extracted from samples into trichloroethylene and quantitated by determining absorbance at 276 nm, with solutions of known concentration for standardization. Cell counts were made microscopically by using a Petroff-Hausser counting chamber. Temperatures were determined with a telethermometer (model 42SC, Yellow Springs Instrument Co.) equipped with a banjo or Teflon probe, and with maximum-reading mercury thermometers. The surface dimensions of Moose Pool and Sulfur Caldron were measured with a steel tape, and their depths were estimated with a weighted, calibrated rope.
10. R. Schoen, *Geol. Soc. Amer. Bull.* **80**, 643 (1969).

11. Chemical assays were done by K. Boylen. Supported by NSF grant GB-35046 and by the Wisconsin Alumni Research Foundation.

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Toxin from Fusarium moniliforme: Effects on Plants and Animals

Abstract. *A mycotoxin-producing strain of* Fusarium moniliforme *was isolated from southern leaf blight–damaged corn seed. A water-soluble toxin, subsequently purified from the fungus, had an oral median lethal dose of 4.0 milligrams per kilogram in 1-day-old cockerels. The toxin also produced plant growth-regulating and phytotoxic effects on plant systems. Physical and chemical data presented for the toxin suggest a structurally new toxin. The trivial name "moniliformin" has been assigned to the toxin.*

The southern corn leaf blight epidemic in the United States in 1970 seriously reduced yields in the Southeast and Midwest. Additional concern at that time was the possible danger to animals fed blight-damaged corn from toxins produced by either the causal agent, *Helminthosporium maydis* Nisiki