Reports

Microcolonial Fungi: Common Inhabitants on Desert Rocks?

Abstract. *Microcolonial structures have been harvested from desert rock samples for cultivation and ultrastructural examination. The results indicate that these microcolonial structures are fungi previously unrecognized as inhabitants of desert rocks.*

The scarcity of moisture and nutrients and the high temperatures encountered combine to make desert rocks one of the most inhospitable habitats on earth. Nonetheless, lichens inhabit rock surfaces in some desert regions where they produce macroscopic colonies (*1*), and algae colonize crevices in desert rocks (*2*) or grow endolithically in a zone beneath the surface of the rock (*3*).

We have observed microcolonial structures on desert rocks, even those that have no detectable lichen or algal growth and are exposed well above soil level. Even when examination of the rock surface with the unaided eye shows no evidence of colonization (Fig. 1, top), a hand lens or dissecting microscope reveals the microcolonial structures (Fig. 1, bottom).

These microcolonial structures appear to be very common rocks in the Mojave Desert and in the Sonoran Desert in the vicinity of Phoenix, Arizona, where most of our fieldwork has been conducted. We have found them on the air-rock interfaces of most of the rocks we have examined from these areas, including samples of basalt, granite, chert, and caliche. Samples sent to us from other arid areas, including the Simpson Desert and the Great Victoria Desert, Australia, and the Gobi Desert, China, also have surface microcolonies.

The microcolonies are generally less than 100 μm in diameter and usually consist of spheroidal subunits approximately 5 μm in diameter (Fig. 2, A to D). Almost all of the microcolonies appear black or brown. Microcolonies are soft but can be removed with fine forceps.

The density of the microcolonial structures varies considerably from rock to rock and often from one area on a rock to another. For example, the microcolonies from the Mojave rock sample (Fig. 1A, top) are confined mostly to small fissures and crevices, where they are quite dense (Fig. 2A). In contrast, there is a relatively even distribution on the rock from the Victoria Desert (Figs. 1B, top and bottom, and 2B) in which there are approximately 200 microcolonies per square centimeter, primarily occupying recessed pits on the surface. In the Ormiston Gorge sample from the Simpson Desert the dark areas on the rock (Fig. 1C, top) are actually due to dense areas of black microcolonies (Figs. 1C, bottom, and 2C). The highest concentration of these occur in recesses where densities are as high as 10,000 per square centimeter. The Sonoran desert sample (Fig. 1D, top) was broken with a hammer and exposed to the environment 45 months before being examined. The newly exposed surface (Figs. 1D, bottom, and 2D) contained approximately 250 microcolonies per square centimeter, whereas the older surfaces contained about 2000 per square centimeter.

Samples from several different rocks from the western United States and Australia were harvested, fixed, and embedded for electron microscopy (*4*). Thin sections in each instance revealed that the 5-μm subunit structures were cellular (Fig. 2E). The ultrastructure was typical of heterotrophic eukaryotic microorganisms; that is, mitochondria and membrane-bound nuclei were observed, but chloroplasts were not. In addition, concentric bodies indicative of the Ascomycetes have been observed in many of the cells.

Several of these microcolonial structures were grown by transferring them to Czapek's medium or to a soil extract medium (*5*). In all instances, those that grew developed as slow-growing fungal colonies, frequently as pure cultures. Most strains have not yet been identified. One strain was tentatively identified as a member of the *Capnodiales*, and most of the others are dematiaceous Hyphomycetes (Fungi Imperfecti). *Taeniolella subsessilis* (Ell. and Everh.) Hughes are included, and *Humicola*-like

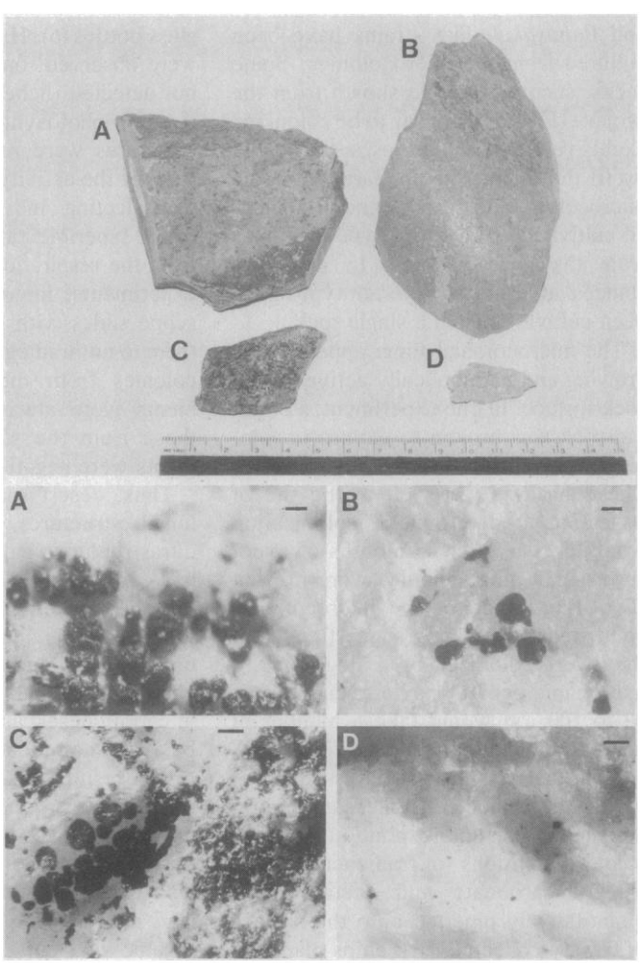

Fig. 1. (Top) Rocks collected from various desert regions: (A) an andesite from the Mojave Desert near Stoddard Wells; (B) a quartzite rock from the Great Victoria Desert of eastern Western Australia; (C) a quartz chlorite vein from the Ormiston Gorge near Alice Springs in the Northern Territory of Australia; (D) a felsite chip from the Sonoran Desert near Phoenix, Arizona. (Bottom) Photomicrographs showing the appearance of the microcolonial structures on the four rocks above, under low magnification with a light microscope. The structures appear dark. Scale bar, 100 μm.

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Fig. 2. (A–D) Scanning electron micrographs of the microcolonial structures from rocks shown in Fig. 1. The appearance of structures in (A) differs from those of the others. The subunits on (B), (C), and (D) are about 5 μm in diameter. Scale bar for (A), (B), and (C), 50 μm, and for (D), 10 μm. (E) Thin section of a microcolonial structure from a granite specimen from the Sonoran Desert. Mitochondria and nuclear membranes can be observed. The dark circular structures appear to be concentric bodies (*8*). Dense granular material is present in the areas between cells and especially on the external region of the cells. Scale bar, 1.0 μm.

and *Bahusakala*-like strains have been cultured from the microcolonies. Some rocks, such as the one shown from the Mojave (Fig. 1A), appear to be colonized exclusively by one species, as indicated by (i) the distinctive appearance of the microcolonies on the rock and (ii) repeated cultivation of a single type of fungus from the microcolonies. In other instances, at least two different types have been cultivated from a single rock.

The microcolonial fungi appear to be growing and metabolically active on the rock surface. In one experiment, a Sonoran rock was broken to expose uncolonized rock surfaces to the environment. These surfaces were left at the site of collection to determine if colonization would occur. After 45 months of exposure, small microcolonies were present (Fig. 1D). Some of the microcolonies contained several cells, suggesting that if they were colonized by a single cell, a mature microcolony having a diameter of 50 to 100 μm would take a number of years to develop.

Microbial activity was assessed in experiments conducted to measure carbon dioxide fixation and respiration. In these studies, solutions of radioactively labeled bicarbonate and acetate were quantitatively pipetted onto the surface of freshly collected rock chips placed in glass bottles (*6*). High rates of respiration were observed, but photosynthesis was not detected (lichen controls were positive for photosynthesis). Microcolonial structures were responsible at least in part for the activity. This was confirmed by selecting individual microcolonies from experimental and fixed controls from the respiration and photosynthesis experiments, mounting them on microscope slides with gelatin, and exposing them to autoradiographic film (*7*); microcolonies from the respiration experiments were always positive, whereas those from the photosynthesis experiments were negative.

Thus, desert rocks contain microcolonial structures whose morphology, ultrastructure, cultural characteristics, and carbon assimilative activity indicate that they are fungi. These microcolonial fungi are alive and metabolically active on the rock surfaces. The widespread occurrence and abundance of these fungi in certain desert areas suggests this may be a common mode of desert life.

Our results raise the question of the source of carbon and energy for the growth of these organisms. Unlike lichens and algae normally associated with desert rock surfaces, these organisms must rely on an external source of organic nutrients for growth, possibly fine, windblown material brought to the rock surface from the surrounding soil and vegetation; during wet periods this material, or leachates from it, might be used as nutrients for the growth of the fungi.

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References and Notes

1. A. W. Herre, *Bot. Gaz. (Chicago)* **51**, 286 (1911); F. G. Follmann, *Naturwissenschaften* **14**, 434 (1965); E. I. Friedmann and M. Galun, in *Desert Biology*, G. W. Brown, Ed. (Academic Press, New York, 1974), vol. 2, p. 187.
2. S. V. Odintsova, *Dokl. Akad. Nauk SSSR Ser. Biol.* **32**, 578 (1941); M. A. Glazovskaya, *Tr. Pochv. Inst. Akad. Nauk SSSR* **34**, 28 (1950); I. Friedmann, *Int. Bot. Congr., 19th* **1964**, 290 (1964) (Abstr.); E. I. Friedmann and M. Galun, *ibid.*, p. 1 (Abstr.).
3. E. I. Friedmann, *Am. Zool.* **8**, 79A (1968); ——— and R. Ocampo, *Science* **193**, 1247 (1976).
4. Specimens were fixed in 2 percent glutaraldehyde in 0.1*M* cacodylate buffer, *p*H 7.0, then in 2 percent osmium tetroxide before being dehydrated in acetone and embedded in Epon 812 and 815. Sections were cut with a diamond knife and observed in an electron microscope (JEOL JEM 100B).
5. The soil extract medium was prepared by autoclaving, for 20 minutes, 1 kg of Sonoran Desert soil in 1 liter of distilled water. The liquid was filtered through paper (Whatman No. 1), and the volume was increased to 1 liter. Manganese sulfate was added to a final concentration of 0.02 percent. Agar was used as the solidifying agent

at 15 g per liter. The medium was autoclaved again before use.

6. In the photosynthesis experiments, 0.2 ml (1.0 μCi) of radioactively labeled bicarbonate in 0.05*M* tris-HCl was dropped on the rock chip in a 2-ounce bottle. The bottles were closed with Bakelite screw caps. Dark controls were covered with aluminum foil. Lichens collected from the same vicinity were used for positive controls. Samples were exposed to ambient light for 24 hours and then fixed with Lugol's iodine. When they were returned to the laboratory the rock chips were washed in 1*N* HCl, then soaked in tap water for 30 minutes and rinsed with tap water several times to remove unassociated label. The chips were then transferred to scintillation vials with Aquasol (New England Nuclear) scintillation fluid and counted (Beckman LS-230 counter). For respiration experiments, 0.5 ml of sodium acetate (0.5 μCi) was added to each rock chip placed in a 16-ounce bottle which also contained a vial with 2 ml of 0.1*N* sodium hydroxide. Control specimens were fixed with Lugol's iodine. After 24 hours, the experimental rocks were fixed with Lugol's iodine, and the base was removed for scintillation counting in Aquasol. Control values for respiration averaged 14,436 count/min, whereas experimental values were 191,995 count/min.
7. For autoradiography, microcolonies were individually picked from rocks used in the labeling experiments. These were mounted on glass slides with gelatin. Medical x-ray film (GAFMED SR-2) was fastened to the slide surface so that the microcolonies were in direct contact with the emulsion. Controls included lichens for the photosynthesis experiments and the fixed specimens for the respiration experiments. The film was exposed for 14 days before development.
8. H. B. Griffiths and A. D. Greenwood, *Arch. Mikrobiol.* **87**, 285 (1972); D. A. Samuelson and J. Bezerra, *Can. J. Microbiol.* **23**, 1485 (1977).
9. We thank Drs. Joe J. Ammirati, Department of Botany, University of Washington, and Robert Samson, Centraal Bureau voor Schimmekultures, Baarn, Netherlands, for their assistance with the identification. Most specimens could not be attributed to a species. None that were identified are species associated with lichens. We thank D. Borns, S. Taylor-George, B. Curtiss, J. Askey, and R. Stewart-Perry for assistance and E. I. Friedmann for comments on the manuscript. Australian rock specimens were provided by J. Bauld, D. Carmony, and M. Jackson. Supported in part by NASA subcontract 955520 with the Jet Propulsion Laboratory of the California Institute of Technology.

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Nitrogen Oxide Reactions in the Urban Plume of Boston

Abstract. *The rate of removal or conversion of nitrogen oxides has been determined from airborne measurements in the urban plume of Boston. The average pseudo–first-order rate constant for removal was 0.18 per hour, with a range of 0.14 to 0.24 per hour under daylight conditions for four study days. The removal process is dominated by chemical conversion to nitric acid and organic nitrates. The removal rate suggests an atmospheric lifetime for nitrogen oxides of about 5 to 6 hours in urban air.*

The nitrogen oxides, NO_x (*1*), play a pivotal role in the chemistry of the atmosphere. In the upper troposphere, NO_x may be the major source of O_3 (*2*). In polluted atmospheres, NO_x are precursors to O_3 and other manifestations of photochemical smog. The major products of NO_x reactions are inorganic and organic nitrates, principally nitric acid (HNO_3), peroxyacetyl nitrate (PAN), and particulate nitrate (NO_3^-) (*3*). The effects of PAN on vegetation and human health are well documented (*4*), and the role of HNO_3 in precipitation chemistry has been the focus of much attention recently (*5*). The behavior and effects of particulate NO_3^- in the atmosphere are not well understood at this time.

In earlier studies our group investigated the nitrogen balance and the distribution of NO_x reaction products in several urban atmospheres (*3*, *6*) and in smog chamber simulations (*7*). Using inert tracer data and our measurements of the oxidized nitrogen (all the oxides of nitrogen) distribution in the Los Angeles area, Chang *et al.* (*8*) derived a value of 0.04 hour^{-1} as a lower limit for the yearly average NO_x removal rate during daylight hours. They calculated an average NO_x residence time of 2.1 days or less. Calvert (*9*) used tracer and NO_x measurements from the Los Angeles Reactive Pollutant Program to estimate the NO_x removal rate at approximately 0.09 hour^{-1} during the midmorning to early afternoon. Since the major reaction products PAN and HNO_3 interfere with the NO_x measurement technique used in that program, this value represents only a portion of the true removal rate. During an airborne study of the Phoenix urban plume (*10*), we observed an NO_x upper-limit removal rate of 0.05 hour^{-1}. This rate is lower than one would expect from computer modeling and smog chamber simulations. A computer model of the kinetics of polluted atmospheres (*11*) suggests an NO_x removal rate of 0.10 to 0.12 hour^{-1} for the conditions of our Los Angeles and Phoenix studies. Smog chamber experiments suggest a removal rate of 0.2 to 0.4 hour^{-1} when simulated urban air is irradiated at realistic hydrocarbon/NO_x ratios (*7*).

The uncertainty in the NO_x removal rate and the need to study NO_x reactions under conditions other than the hot and dry environments of Los Angeles and Phoenix led to this investigation of the NO_x removal rate in the urban plume of Boston. Boston was selected because of its higher summertime relative humidity and because the prevailing westerly winds frequently carry the Boston urban plume over the ocean, effectively isolating it from the confounding effects of fresh emissions of NO_x and other pollutants. The experiments were performed in a Lagrangian manner (*12*) with the use of an instrumented research aircraft (*13*) with mobile laboratory ground support. The study was conducted between 27 July and 30 August 1978. The goal was to follow the polluted air parcel (0800 to 0900 EDT) from Boston as it reacted during transport downwind. Flight altitude was generally 150 to 300 m above sea level in order to sample the highest pollutant concentrations. The experiments of 14, 18, 23, and 30 August 1978 are the most appropriate for an analysis of NO_x reaction rate and are reported here. As an example of the experiments, the flights of 18 August are shown at the right in Fig. 1. The position of the air parcel leaving Boston at 0800 EDT is shown for each hour to 1600 EDT. Three flights, 20 through 22, were conducted during this day. The sample collection intervals used in data analysis are shown as solid lines. These specific locations were chosen after initial "scouting" traverses through the plume because they

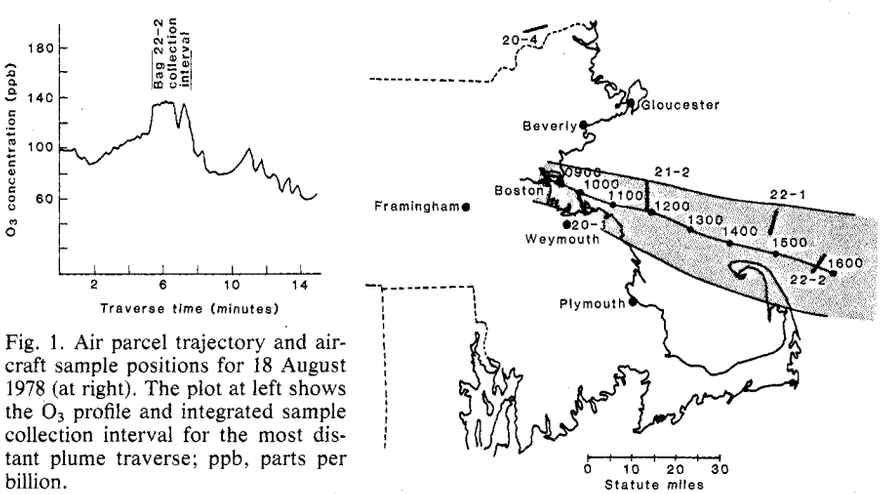

Fig. 1. Air parcel trajectory and aircraft sample positions for 18 August 1978 (at right). The plot at left shows the O_3 profile and integrated sample collection interval for the most distant plume traverse; ppb, parts per billion.

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