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Fungal Attack on Rock: Solubilization and Altered Infrared Spectra

Abstract. Penicillium simplicissimum, *isolated from weathering basalt, produced citric acid when grown in a glucose–mineral salts medium with basalt, granite, granodiorite, rhyolite, andesite, peridotite, dunite, or quartzite. After 7 days' growth as much as 31 percent of the silicon, 11 percent of the aluminum, 64 percent of the iron, and 59 percent of the magnesium in some of the rocks were solubilized, and a number of rocks showed altered infrared absorption in the silicon-oxygen vibration region.*

The discovery of fossil microorganisms in ancient sedimentary rocks (*1*) lends strong support to the contention that life has been present on Earth for several billion years. It seems reasonable to expect that during this time at least some microorganisms developed the ability to attack the rocks and minerals of the biosphere, since these substances are the ultimate source of the inorganic elements and compounds required for life. Although participation of microorganisms in the weathering of rocks and minerals has long been acknowledged, very little is known of the specific mechanisms; only a few recent studies (*2*) have been concerned with this problem. As part of our studies on life detection methods we are investigating the possibility that microorganisms, independent of their fossil remains, might leave a record of their metabolic activity in alterations of the rocks and minerals of their environment.

We screened a variety of microorganisms isolated from the surface of weathering basalt for rapid solubilization of unweathered rock. We now report on laboratory studies with the fungal isolate *Penicillium simplicissimum* (Oudemans) Thom, strain WB-28, which solubilized significant quantities of the Si, Al, Fe, and Mg in several examples of igneous and metamorphic rocks and caused changes in the infrared absorption spectra of some of the residual rock material.

Penicillium simplicissimum WB-28 was grown in triplicate in 50 ml of Pope and Skerman's basal mineral salts solution (*3*) supplemented with 4 percent glucose and 0.01 percent yeast extract in the presence of 500 mg of powdered sterilized rock (*4*). Three sets of triplicate controls consisted of sterile medium with rock, inoculated medium without rock, and sterile medium. Incubation was in 250-ml plastic Erlenmeyer flasks at 30°C for 7 days on a rotary shaker (2.5 cm excursion diameter, 320 rev/min). After incubation the contents of all flasks were brought to 100 ml with distilled water and centrifuged in plastic ware to remove the mixture of mycelium and residual rock. The supernatant was filtered through Whatman No. 42 filter paper, followed by filtration through a 0.20-μm membrane filter (Gelman Metricel GA-8) into plastic bottles. This solution was then analyzed for soluble Si, Al, Fe, and Mg by atomic absorption. The mixture of residual

Table 1. Solubilization of the Si, Al, Fe, and Mg of rocks by *Penicillium simplicissimum* after 7 days' incubation at 30°C. Data for the soluble elements in uninoculated controls (in parentheses) and inoculated flasks are the means of triplicate cultures corrected for the elements originally present in the growth medium. Abbreviation: N.D., not determined; < indicates a value below the detection limit given.

Sample	Rock analysis (milligrams per 100 mg of rock)				Soluble rock elements (milligrams per 100 mg of rock)			
	Si	Al	Fe	Mg	Si	Al	Fe	Mg
Dunite								
DTS-1	18.97	0.16	6.07	30.07	(0.13) 5.94	(<0.01) 0.01	(<0.09) 2.09	(0.18) 8.46
Peridotite								
PCC-1	19.61	.41	5.74	26.15	(.17) 2.86	(< .01) .04	(< .09) 1.92	(.16) 11.38
Basalt								
D-100043	21.81	7.89	9.70	5.32	(.28) 1.21	(< .02) .30	(< .03) 2.54	(.02) 2.21
BCR-1	25.30	7.25	9.32	2.09	(.06) 0.33	(< .02) .15	(< .03) 1.19	(.04) 0.24
A	24.13	8.36	6.23	3.62	(.06) .59	(< .02) .58	(< .03) 3.06	(.04) 2.04
Andesite								
AGV-1	27.59	9.05	4.65	0.91	(.06) .28	(< .03) .18	(< .07) 0.35	(< .01) 0.08
Granodiorite								
GSP-1	31.44	8.13	2.97	.59	(.04) .60	(< .03) .63	(< .09) 1.29	(< .01) .35
Granite								
G-2	32.36	8.16	1.86	.46	(.05) .56	(< .03) .41	(< .01) 0.94	(.01) .26
D-100429	34.68	6.77	1.63	.05	(.02) .44	(< .03) .30	(< .01) .91	(.01) < .01
D-100643	35.04	6.63	1.57	.04	(.01) .47	(< .03) .26	(< .01) 1.01	(.01) .01
D-100018	35.36	6.86	0.61	.16	(.02) .18	(< .03) .22	(< .01) 0.14	(.02) .03
D-100012	35.58	7.22	.29	0.00	(.02) .09	(< .03) .12	(< .01) .04	(N.D.) N.D.
Rhyolite								
D-100051	35.65	6.79	.78	.07	(.04) .25	(< .02) .20	(< .01) .10	(<0.01) 0.03
D-100050	35.18	7.17	.60	.04	(.03) .09	(< .02) .05	(< .01) .01	(< .01) .01
Quartzite								
D-100314	34.24	6.24	2.88	1.17	(.02) .89	(< .02) .69	(< .04) 1.05	(< .01) .49
D-100316	37.14	5.09	2.76	0.69	(.02) .86	(< .02) .60	(< .04) 0.98	(< .01) .36

rock and fungal mycelium was pooled, washed three times with distilled water, dried, and the mycelium and other organic matter removed from the rock by low-temperature combustion in an oxygen plasma (*5*). All controls were carried through the same procedures. Infrared absorption spectra of the residual rock (0.15 percent in KBr) were determined according to the method of Lyon (*6*) for sample preparation.

Fungal attack on igneous and metamorphic rocks in most cases markedly increased the quantity of Si, Al, Fe, and Mg in solution when compared with uninoculated controls (Table 1). The Si solubilized by ***P. simplicissimum*** ranged from 0.3 to 31 percent of the total Si in the rocks. The range for Al was 0.7 to 11.8 percent. Iron and Mg were the most extensively solubilized of the elements studied, provided they were present in amounts of at least 0.30 mg per 100 mg of rock. Biogenic solubilization equivalent to 25 to 60 percent of the total Fe and 25 to 56 percent of the total Mg in the rocks commonly occurred.

Among the rocks tested not all were equally susceptible to fungal attack (Table 1). Dunite DTS-1 and peridotite PCC-1 were the most susceptible. For dunite the quantities of Si, Al, Fe, and Mg solubilized represented 31.3, 6.3, 34.4, and 37.8 percent, respectively, of the total content of these elements in the rock. For peridotite the values were Si, 14.6; Al, 9.8; Fe, 33.4; and Mg, 32.4 percent. At the other extreme, ryholite D-100050 and granite D-100012 were the most resistant to fungal attack. The remaining rocks tested were intermediate in their susceptibility to attack. There were differences also in the susceptibility of individual rock samples within a group of rocks of the same type; for example, among the basalts sample BCR-1 was more resistant to fungal attack than basalts A or D-100043. Among the granites, rocks D-100012 and D-100018 were more refractory to fungal attack than the remaining three granites. A similar situation prevailed between the two rhyolites. However, the two quartzites showed equivalent susceptibility to fungal attack. Even though all the rocks tested except basalt A were < 200 mesh sieve size (*4*), it is possible that an unequal distribution of particles was responsible for the differences in susceptibility to fungal attack. It seems more likely, however, that the explanation lies in differences in the chemical composition and grain size of the individual rock-forming minerals.

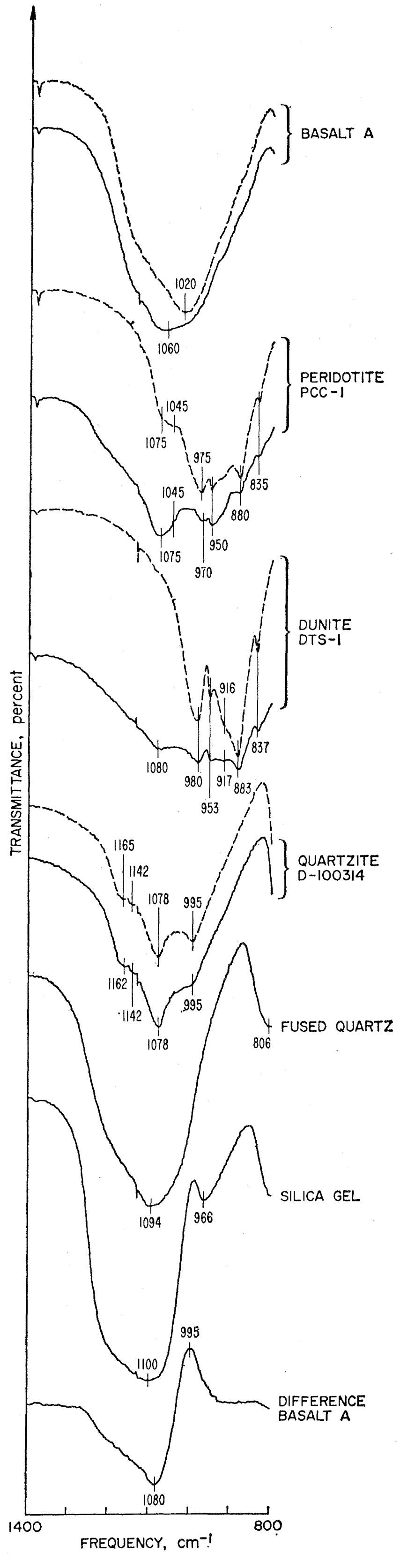

Fig. 1. Altered infrared absorption spectra of rocks subjected to fungal attack for 7 days. Broken line spectra are of rocks from sterile controls; solid line spectra are of rocks incubated with *Penicillium simplicissimum.* The spectra of fused quartz and silica gel are of original material. The difference spectrum of basalt A was made with the control rock in the reference beam and the inoculated rock in the sample beam of the spectrophotometer.

Linear correlation coefficients (r) were calculated from the data in Table 1 to determine if there was a positive relation between the quantity of an element solubilized and its total content in the rocks. There was a very strong positive correlation for Mg ($r = .97$), less strong for Fe ($r = .75$), poor for Al ($r = .39$), and a negative correlation for Si ($r = -.69$). However, soluble Si correlated very strongly with the Mg content of the rocks ($r = .94$) but not with the Fe content ($r = .38$). Soluble Al showed no correlation with the Mg or Fe content of all the rocks, but there was a very strong positive correlation between soluble Al and the Mg ($r = .91$) and Fe ($r = .96$) content of the rocks when only the granodiorite, five granites, two rhyolites, and two quartzites were considered. These findings suggest that the minerals most susceptible to fungal attack probably are the iron- and magnesium-bearing minerals such as biotite, olivine, pyroxene, hornblende, serpentine, and others, whereas the feldspars and quartz are more resistant.

The mechanism of fungal attack appears to be one in which the organism produces acid which then attacks the rock. This is supported by the following observations. During growth the medium (initial *p*H 6.8) became progressively more acid, reaching *p*H values of less than 3.5 in 7 days. The rate of solubilization of the Si, Al, Fe, and Mg of basalt A correlated well with increases in titratable acidity. Spent acid growth medium (derived from cultures grown in the absence of rock and filter-sterilized to remove the mycelium) solubilized basalt A when placed in contact with the rock, whereas spent medium neutralized with NaOH showed no such effect. The major acid produced was identified as citric acid (*7*). Comparable solubilization of basalt A was achieved in spent acid medium and in fresh uninoculated medium containing an equivalent concentration of citric acid. Finally, extracellular enzymes did not appear to be involved, for heated (80°C, 1 hour) and unheated spent acid mediums were equally effective in solubilizing basalt A.

Many of the rocks susceptible to fungal attack showed alterations in their infrared absorption spectra in the Si-O stretching vibration region. The changes generally observed were either the appearance of an absorption band in the region between 1060 and 1100 cm^{-1}, or intensification of a band in this region relative to neighboring bands at lower frequencies (Fig. 1). Basalt A showed a shift in absorption from a lower to a higher frequency; this was most pronounced in the difference spectrum (sterile rock versus inoculated rock). Dunite DTS-1 showed the appearance of a new band at 1080 cm^{-1} and a general diminution in intensity of the other bands. Intensifications similar to the latter were observed in basalts D-100043 and BCR-1 and in quartzite D-100316 (not illustrated in Fig. 1). None of the granites or other rocks, whether or not susceptible to fungal attack, showed altered infrared absorption spectra in this region.

One explanation for the spectral changes observed is that fungal attack disrupted the ordered crystalline structures of one or more silicate minerals with subsequent production of amorphous silicates with major absorption bands between 1060 and 1100 cm^{-1}. Figure 1 shows that amorphous silicates, such as fused quartz and silica gel, have major absorption bands in this region. On the other hand, it is equally plausible that silicate minerals absorbing between 1060 and 1100 cm^{-1} were already present but were masked or made to appear less intense by overlapping broad intense neighboring bands of other silicates. Complete or partial destruction of the latter would eliminate or diminish the intensity of the corresponding absorption bands and would unmask or appear to intensify the 1060 to 1100 cm^{-1} silicate bands.

Additional experiments with basalt A revealed that spent acid medium as well as equivalent concentrations of citric acid, oxalic acid, HCl, H_2SO_4, or H_3PO_4 solubilized comparable amounts of Si, Al, Fe, and Mg and caused similar alterations in the infrared absorption spectra. Thus, solubilization of basalt A and the concomitant spectral changes were not unique to biogenic acid but were the result of acid attack in general. A more detailed description of these and other experiments will be published elsewhere.

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5. Low-temperature combustion was carried out with the use of a Tracerlab PA 3000 plasma activator, PR 3000 plasma reactor, and RFG 3000 radio frequency generator. All rock samples were exposed to the oxygen plasma for 18 to 24 hours.
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7. Gas chromatography of the trimethyl esters of authentic citric acid and the unknown gave identical retention times and peak shapes, as did a mixture of the two; their mass spectrometric fragmentation patterns were also identical.
8. We thank R. Janda for guidance in the field, R. J. P. Lyon for valuable discussions, Dorothy I. Fennell for identifying *Penicillium simplicissimum* at the species level, and Patricia J. Kirk for technical assistance.

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Enzyme Replacement in Fabry's Disease, an Inborn Error of Metabolism

Abstract. *Two patients with Fabry's disease were infused with normal plasma to provide active enzyme (ceramide trihexosidase) for hydrolysis of the plasma substrate, galactosylgalactosylglucosylceramide. Maximum ceramide trihexosidase activity occurred 6 hours after infusion of the plasma, attaining a level approximately 150 percent of that in normal plasma; enzymatic activity was detectable for 7 days. The amount of accumulated substrate in the plasma of these recipients decreased about 50 percent on day 10 after infusion. Thus, periodic replacement of ceramide trihexosidase activity in the plasma of patients with Fabry's disease might lead to consistently lower amounts of substrate in the plasma and a decrease in its rate of accumulation in tissues.*

Fabry's disease is an inborn error of glycosphingolipid catabolism transmitted by an X-linked gene. It is characterized biochemically by the systemic accumulation of galactosylgalactosylglucosylceramide (gal-gal-glc-cer) (*1–3*) and by the deficiency of ceramide trihexosidase (galactosylgalactosylglucosylceramide : galactosyl hydrolase) in the tissues (*4*) and plasma (*5*) of hemizygous male patients. Increased amounts of gal-gal-glc-cer also occur in plasma (*2*), urinary sediment (*3, 6*), and cultured skin fibroblasts (*7*) from patients with this disease. It has been assumed that a major source of the accumulated glycosphingolipid is from the metabolism of globoside [2-acetamido-2-deoxygalactosyl-(β1 → 3)-galactosyl-(β1 → 4)-galactosyl-(β1 → 4)-glucosylceramide (*8*)] which occurs in the erythrocyte membrane and in lesser amounts in other tissues. This possibility is supported by studies of gal-gal-glc-cer, which accumulates in kidney, showing that the glycosidic linkages have the β configuration, that is, galactosyl-(β1 → 4)-galactosyl-(β1 → 4)-glucosyl-(β1 → 1′)-ceramide (*9*). This glycosphingolipid is accompanied by abnormal amounts of an incompletely characterized galactosyl-(1 → 4)-galactosyl-(1 → 1′)-ceramide in the kidney and pancreas, but not other organs (*1*).

The ceramide trihexosidase of normal plasma has greatest activity at *p*H