Carbon Metabolism of the Cryptoendolithic Microbiota from the Antarctic Desert

J. ROBIE VESTAL

Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio 45221-0006

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The carbon metabolism of the cryptoendolithic microbiota of sandstones from the Ross Desert of Antarctica was studied in situ and in vitro. Organic and inorganic carbon compounds were metabolized by the microbiota, with bicarbonate incorporation into community lipids occurring primarily in the light. Light intensity affected the photometabolism of carbon with a photosynthesis-intensity response optimum at about 200 to 300 μmol of photons per m² per s. Photosynthesis was also affected by temperature, with a minimum activity at −5°C, an optimum activity at 15°C, and complete inhibition at 35°C, indicating that the cryptoendolithic community was psychrophilic. The primary source of CO₂ for photosynthesis in situ was the atmosphere. CO₂ may also be photometabolized by using the carbon produced from respiration within the endolithic community. Photosynthesis occurred maximally when the microbiota was wet with liquid water and to a lesser extent in a humid atmosphere. This simple microbial community, therefore, exists under extremes of water, light, and temperature stress which affect and control its metabolism.

In the Ross Desert (Dry Valley) region of Antarctica, there exists a microbial ecosystem which lives in the pore spaces of sandstone (3, 5–7). This microbiota, termed cryptoendolithic (hidden within rock; 8), is dominated by lichens of the genera Buellia and Lecidea (3). The primary producers of the ecosystem are the lichen phycobiont, occasionally the green alga Hemichloris antarctica (21), and cyanobacteria. The consumers consist of the lichen mycobiont, filamentous fungi, bacteria, and yeasts (3). This primitive community lives from 1 to 10 mm under the surface rock crust, where light can penetrate and water from occasional snow melt (2) can percolate into the microbial zone. The rocks containing the microbiota are stained on the surface with ferric precipitates which absorb solar heat and warm the inside of the rock to a high of 11°C when conditions permit (4). It has been shown that light, temperature, and moisture conditions favorable for microbial metabolism exist for 375 to 700 h/year at Linnaeus Terrace, Antarctica (4).

The purpose of this study was to determine the ability of the cryptoendolithic microbiota to metabolize organic and inorganic carbon compounds and to determine the effects of light, temperature, and moisture on the metabolic activity of the microbial community.

MATERIALS AND METHODS

Site description. These studies were conducted in the Ross Desert (formerly Dry Valley) region of Antarctica. The specific site was Linnaeus Terrace, Asgard Range, South Victoria Land, Antarctica (77°36’ S, 161°05’ E; altitude, 1,600 m), a 0.5-km² area covered with flat sandstone tables heavily colonized by cryptoendolithic microorganisms. Field experiments were conducted during the summer (December and January 1982 to 1987). During these periods, the ambient air temperature ranged from −22 to +5°C, with a mean of −9°C. For laboratory studies, flat pieces of colonized sandstone ranging from 0.1 to 0.3 m² were collected and returned to the Eklund Biological Laboratory at McMurdo Station for analysis. The rocks were kept frozen (−20°C) until use, which did not affect their subsequent metabolism.

Sample preparation. To prepare the rocks for controlled laboratory studies, colonized rocks were broken into small pieces, and then the colonized zone was aseptically removed with a hammer and chisel, and the zones were crushed to the consistency of sand with a mortar and pestle. These crushed rock samples were used for assays of metabolic activity. Although there was undoubtedly some cell destruction during preparation, the crushed rock samples were always metabolically active. Microscopic examination revealed some breakage of cells. Touvilla and LaRock (20) have recently reported that crushing rocks dry causes some damage to the cells as determined by release of ATP. When rocks had been incubated in the field under natural conditions, the rocks were frozen and returned to the laboratory. The areas which had been exposed to isotopes were excised and crushed to sand, and then their lipids were extracted by using chloroform and methanol (1) so that incorporation of the label into the lipid fraction of the microbial community could be analyzed (see below).

Microbial nutrient utilization. (i) In situ studies. The ability of the cryptoendolithic microbiota to metabolize ¹⁴C-labeled compounds in situ was measured as incorporation of ¹⁴C into cellular lipids (16, 23). Samples (1 ml) of oligotrophic concentrations of [¹⁴C]glycolate (8.8 mCi/ml; 0.114 μmol/ml), [¹⁴C]acetate (57.9 mCi/ml; 0.017 μmol/ml), and [¹⁴C]bicarbonate (46 mCi/ml; 0.022 μmol/ml) were dissolved in cold (<5°C), CO₂-free (boiled for 10 min), distilled, deionized water and allowed to soak into the surface of the sandstone. After incubation for various periods on Linnaeus Terrace, the rocks were frozen and returned to the laboratory for excision of the biotic zones and lipid extraction to determine label incorporation into lipids as a measure of total community microbial activity, as previously described (16, 23). Radioactivity was determined by scintillation counting with quench correction by an external standard.

(ii) In vitro studies. For analysis of the ability of the microbiota to metabolize carbon compounds in the laboratory, 1.5 g of crushed rock was placed into scintillation vials (glass; 20 ml) containing 1.9 ml of CO₂-free, distilled, deionized water, and 0.1 ml of ¹⁴C isotope was added. In addition to the isotopes described above, [¹⁴C]glutamate (275 mCi/ml; 0.0080 μmol/ml) and [³¹C]glucose (283 mCi/ml; 0.0088 μmol/ml) were used to measure metabolism of or-
ganic carbon sources. Samples were incubated under various regimes of temperature and light for various times. Incubation was stopped by addition of 2.5 ml of chloroform and 5.0 ml of methanol, and the lipids were extracted and analyzed as previously described (16).

Effect of light. (i) In situ studies. In experiments to determine the effect of light intensity on the metabolism of the microbiota in situ, [14C]bicarbonate was added to intact rocks, and the rocks were covered with one, two, or four layers of black window screen (1-mm aperture size). This did not affect the spectral quality of the light but reduced the ambient light intensity to 50, 25, and 6.25% as determined by a LiCor model LI-185B quantum radiometer photometer. Dark controls were covered with aluminum foil. After incubation, the areas exposed to the isolate were crushed to sand, and the lipids were extracted and analyzed for 14C incorporation into lipids.

(ii) In vitro studies. Crushed rock samples were put into a small-volume, short-incubation device (photosynthron) as previously described (13). After incubation with [14C]bicarbonate at various light intensities for various times, the lipids were extracted and analyzed for incorporation of label.

Effect of temperature. Crushed rock samples were placed in a water bath and incubated at various temperatures under constant light (130 μmol of photons per m² per s; photosynthetically active region). Triplicate samples were randomly chosen at 1, 2, 4, 8, and 12 h, and [14C]bicarbonate incorporation into lipids was determined as described above. Dark controls were covered with aluminum foil.

Effect of exogenous carbon in situ. [12C]acetate and [14C]glucose (1 ml each) were added to the rock surface, followed by addition of 1 ml of [14C]bicarbonate. After incubation, incorporation of 14C into community lipids was determined.

Chemolithotrophic metabolism. Various log concentrations of thiosulfate (Na2S2O3), ferrous sulfate (FeSO4 - 7H2O; H2SO4 [pH 2.5]), sodium nitrite (NaNO2), and ammonium sulfate [(NH4)2SO4] were incubated with crushed rock samples and [14C]bicarbonate in the dark for 48 h, after which the lipids were extracted and incorporation was determined.

Metabolism of CO2 gas. Samples (1.5 g) of crushed rock

<p>| TABLE 1. Incorporation of 14C-labeled carbon sources into the lipids of the cryptoendolithic microbiota incubated in situ at Linnaeus Terrace, Antarctica |
|---------------------------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Carbon source</th>
<th>14C incorporation (dpm/μmol PO4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bicarbonate</td>
<td>5,982 ± 1,000</td>
</tr>
<tr>
<td>Acetate</td>
<td>23,250 ± 1,446</td>
</tr>
<tr>
<td>Glucose</td>
<td>1,464 ± 696</td>
</tr>
<tr>
<td><strong>a</strong></td>
<td></td>
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</tbody>
</table>

* Incubation was for 23 days, starting on 20 December 1984 (n = 5).

were placed under an inverted evaporation dish (50 by 35 mm) as shown in Fig. 1. [14C]bicarbonate (0.5 ml containing 2.5 μCi) was added to one of the small wells and acidified with 0.1 ml of 3.6 N H2SO4 through the clay seal to generate 14CO2 in the closed atmosphere. A 2-ml volume of pH 2.0 buffer was added to the other well to saturate the atmosphere with water vapor. In another set of samples, no buffer was added to saturate the atmosphere. The ambient relative humidity (RH) in these chambers was about 15%. In a third set of samples, 0.4 ml of CO2-free, distilled, deionized water was added directly to the crushed rock. Dark controls were covered with aluminum foil. All of the samples were preincubated in the dark at 15°C for 24 h before generation of CO2.

Biomass of the microbiota. The biomass of the cryptoendolithic microbiota was measured as the amount of lipid phosphate (24) extracted from the rock samples. Intact rock was crushed before lipid extraction as previously described (22).

RESULTS

Various carbon sources (bicarbonate, acetate, and glycolate) dissolved in water and allowed to soak into the rocks were readily metabolized in situ by the cryptoendolithic microbiota (Table 1). In dark controls, there was a 20-fold reduction in bicarbonate incorporation, but incorporation of organic carbon sources was not affected. Similar results were obtained in the laboratory with crushed rock under constant environmental conditions (Table 2). There was a slight enhancement of glutamate and glucose incorporation in the dark.

The effect of light on bicarbonate metabolism was further studied in situ and in vitro. In the field, intact rocks were incubated under natural conditions under various light regimes. A 50% reduction in ambient light (Table 3) caused a fivefold reduction in the activity of the microbiota, while a 75% or more reduction reduced bicarbonate incorporation to dark control levels. This was further confirmed in the

<p>| TABLE 2. Incorporation of 14C-labeled carbon sources into lipids of the cryptoendolithic microbiota of crushed rocks |
|---------------------------------------------------------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Carbon source</th>
<th>14C incorporation (dpm/μg of rock)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bicarbonate</td>
<td>6,103 ± 365</td>
</tr>
<tr>
<td>Acetate</td>
<td>25,720 ± 1,056</td>
</tr>
<tr>
<td>Glucose</td>
<td>1,561 ± 24</td>
</tr>
<tr>
<td>Glutamate</td>
<td>772 ± 72</td>
</tr>
<tr>
<td><strong>a</strong></td>
<td></td>
</tr>
</tbody>
</table>

* The rocks were incubated at 9°C with 30 μmol of photons per m² per s for 8 h (n = 3).

a Dark control vials were covered with aluminum foil.
TABLE 3. Effect of light intensity on [14C]bicarbonate incorporation into lipids on the cryptoendolithic microbiota incubated in situ on Linnaeus Terrace, Antarctica

<table>
<thead>
<tr>
<th>Light intensity (% of ambient light)</th>
<th>14C incorporation (dpm/µmol of PO4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>5.982 ± 1.000</td>
</tr>
<tr>
<td>50</td>
<td>1.214 ± 18</td>
</tr>
<tr>
<td>25</td>
<td>232 ± 89</td>
</tr>
<tr>
<td>6.25</td>
<td>304 ± 36</td>
</tr>
<tr>
<td>0</td>
<td>286 ± 18</td>
</tr>
</tbody>
</table>

* Incubation was for 22 days, starting on 21 December 1984 (n = 5).

The effects of different temperatures (0, 7.5, and 15°C) and light intensities were studied (Fig. 3). As expected, photosynthesis was reduced by temperatures below the optimum. In another series of experiments, crushed rock samples were incubated at temperatures from −10 to +35°C for at least 12 h (Fig. 4). As can be seen, longer incubation times resulted in more activity. The optimum temperature for photosynthetic activity was 15°C, with a secondary peak at 5°C. At temperatures above 15°C, photosynthesis declined, and at 35°C there was no activity above that of the dark controls. At the low end of the temperature curve, measurable (light − dark > 0) photosynthetic activity was determined at −8°C after 8 h but not after 4 h. At −5°C, measurable activity was found after 1 h. No measurable light-driven activity was determined at −10°C after 12 h. If just the heterotrophic (dark) incorporation of bicarbonate into community lipids (Fig. 5) is considered, it is evident that the activities were low compared with light incorporation and there was a temperature and time response. The peaks of activity were much more broad around 5 and 15°C, but the optimum activity was still around 15 to 20°C, making the heterotrophic community psychrophilic in its response to temperature.

To determine whether dark bicarbonate and CO2 incorporation was caused by chemolithothrophic bacteria versus incorporation by heterotrophic bacteria, samples were mixed with various possible chemolithothropic energy sources, and bicarbonate incorporation was determined (Table 4). There was no stimulation of carbon incorporation in the presence of thiosulfate, ferrous sulfate, ammonia, or sodium nitrite during the 48-h incubation period.

To test whether incorporation of organic compounds depresses CO2 uptake from the atmosphere, rocks were soaked with water containing various concentrations of acetate or glucose (pH 7.0), and incorporation of [14C] bicarbonate was measured. The results (Table 5) showed that addition of organic carbon at 10 µmol indeed caused a depression in photosynthetic incorporation. The pH of the rock interstices is about 5.0 to 5.5 (Johnston and Vastel, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, 1-102, p. 189). It is unlikely that the pH of the added dilute solutions caused the depression in CO2 incorporation.

To determine the effect of liquid water versus water vapor in the photosynthetic metabolism of CO2, crushed rock samples were placed in closed jars (Fig. 1), and an atmosphere of 14CO2 was generated. The cryptoendolithic microbiota was wetted with CO2-free water, placed in an atmosphere saturated with water vapor (100% RH), or left at

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**FIG. 2.** Effect of light intensity on the photosynthesis of the cryptoendolithic microbiota of crushed rock from Linnaeus Terrace, Antarctica.

**FIG. 3.** Effect of light intensity and temperature on the photosynthesis of the cryptoendolithic microbiota of crushed rock from Linnaeus Terrace, Antarctica.

**FIG. 4.** Effect of temperature on the photosynthesis (light − dark) of the cryptoendolithic microbiota of crushed rock from Linnaeus Terrace, Antarctica.
metabolism of CO₂. By using these measures of community photometabolism, it was found that the optimum temperature of photosynthesis was 2 to 7°C, the maximum was about 15°C, and the minimum was estimated to be about −6 to −8°C. When water was sprayed either to moisten or to saturate the rock interstices, no major change in CO₂ metabolism occurred. The present study extends these initial observations.

On Linnaeus Terrace, when intact rocks were allowed to soak up [%] labeled carbon compounds as they would during a snow melt (2), it was found that metabolic incorporation of bicarbonate into cellular material was photosynthetically driven (Tables 1 and 2). Organic compounds, however, were not photosynthetically incorporated into the microbiota, either in intact rocks or under laboratory conditions. This suggested that CO₂ dissolved as bicarbonate, was the source of carbon for photosynthetic metabolism by this microbial community. There was a rich heterotrophic community which could easily metabolize the products of photosynthetic metabolism. It is known that Trebouxia sp., the phycobiont often found in these cryptoendolithic lichens (3), can take up glucose heterotrophically (19). However, it apparently does not do this to any great extent in this cryptoendolithic association (Table 2).

The optimum light intensity for photometabolism was about 200 to 300 μmol of photons per m² per s (Fig. 2), and a great degree of variation in light-driven incorporation occurred above this range. This variation was probably due to the fact that the microbial community was mixed in these crushed rock samples, whereas it was in a definite stratification in an intact rock. Therefore, the organisms at different levels in a rock would be adapted to the light at various intensities. When mixing this community in these experiments, a greater variation in response would therefore be expected. Natural light intensities during the summer on

TABLE 6. Effects of water vapor and liquid water on the ability of the cryptoendolithic microbiota to incorporate gaseous

\[ {^{14}}\text{C}} \text{CO}_2 \text{ into cellular lipids}^a

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>[^{14}\text{C}} \text{ incorporation (dpm/1.5 g of rock) at:}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>Light, liquid water</td>
<td>2,385 ± 202</td>
</tr>
<tr>
<td>Light, water vapor</td>
<td>ND (^a)</td>
</tr>
<tr>
<td>Light, dry air</td>
<td>128 ± 35</td>
</tr>
<tr>
<td>Dark, liquid water</td>
<td>76 ± 47</td>
</tr>
<tr>
<td>Dark, water vapor</td>
<td>ND</td>
</tr>
<tr>
<td>Dark, dry air</td>
<td>42 ± 1</td>
</tr>
</tbody>
</table>

\(^a\) Crushed rock was kept at 15°C with 22 μmol of photons per m² per s (\(n = 4\)).

\(^a\) ND. Not determined.
Linnaeus Terrace reach as high as 1,860 μmol of photons per m² per s (4; J. Nienow, C. P. McKay, and E. I. Friedmann, 1988a, Microb. Ecol., in press), which would dramatically affect photosynthesis. It has been shown (Nienow et al., 1988a, in press) that the lichen zone in intact rocks receives light intensities on the order of 0.1 to 100 μmol of photons per m² per s. Light penetration increases about an order of magnitude when the rock is wet (J. Nienow, C. P. McKay, and E. I. Friedmann, 1988b, Microb. Ecol., in press). This suggested that the microbes, even in the bottom layers of the lichen zone, can receive light intensities of about 1 to 10 μmol of photons per m² per s, which are suitable for photosynthesis (Fig. 2).

The photosynthetic response was also regulated by temperature (Fig. 3 and 4). The optimum temperature was 15°C, with a measured minimum between −5 and −8°C. These minimum values correspond to those estimated by Kappen and Friedmann (12), who used an infrared gas analyzer to measure net community photosynthesis. The fact that there was measurable photosynthesis (light > dark) at −8°C after 8 h but not at 4 h and that there was photosynthesis after 1 h at −5°C indicates that the minimum temperature occurred between these two temperatures but not between the two temperatures but not between the two temperatures (i.e., −10°C). These may be the temperatures at which the contents of the cryotrap freeze because of solute concentration. Lichens, with green algae phycobionts, are known to produce polyols (9), such as ribitol, sorbitol, and erythritol (14), which could lower the freezing point of cellular water and thus allow the metabolic temperature to be lowered to this range. Many epilithic lichens have temperature minima in this range and are quite freeze-thaw resistant (9, 11). The temperatures inside the rock have been reported to be as high as 11°C (4, 12, 15). This elevated temperature was due to a cloudless day with no wind, which allowed the iron-stained surface to absorb solar energy and heat the inside of the rock. The normal temperatures of the rock surface during December and January range from −22 to +5°C (4), Friedmann et al. (4) have calculated the response of the nanoclimate in the rocks on the basis of continuous satellite data monitoring and have postulated that, if the rock was wet (>75% RH) and warm (>−5°C) and had enough light (>100 μmol of photons per m² per s), community metabolic activity could occur for 705 h/year for a rock sloped in a northerly direction and for 375 h/year in a horizontally positioned rock table. The microbial activity would also be decreased by the hourly to daily changes in temperature, light, and moisture. The cryptoendolithic microbiota also face frequent freezing and thawing, which would also tend to lessen the amount of time per year for metabolic activity. This maximum of 29 days of metabolism per year indicates the harsh environmental extremes to which this community is exposed.

The community responds in a typical psychrophilic manner (17), having a temperature optimum below 20°C (Fig. 4). The two optima at 5 and 15°C were similar to that observed by Schofield and Ahmadjian (18), who studied the growth (as dry weight) of two Antarctic epilithic lichens, Lecanora tephroreta and Lecidea sp. They found that Lecidea sp. had growth temperature optima at 7 and 15°C and L. tephroreta had optima at 3 and 19°C. Lecidea sp. was the type of lichen found in the Linnaeus Terrace cryptoendolithic community (E. I. Friedmann, personal communication). Schofield and Ahmadjian (18) further speculated that (i) the two temperature optima could be the result of the higher temperature optimum evolving outside the Antarctic and the lower one evolving after colonization of the Antarctic by the lichens or (ii) the higher optimum evolved when the Antarctic was a warmer continent. In this study, photosynthetic metabolism at 5, 10, and 15°C was repeated with the same results, as shown. Certainly, comparative studies with many different Antarctic lichens are needed to help explain this apparent anomaly. However, the two optima appear to be a physiological trait of the lichenized community. This was further suggested by the response of the heterotrophic community (dark incorporation) to temperature (Fig. 5). There was a broad peak between 0 and 10°C and then another at 15 to 20°C before temperature inhibition occurred. This was similar to the data shown in Fig. 4 but at a much reduced level. These data indicate that the heterotrophic community was closely tied to the photosynthetic community for its supply of nutrients.

One of the interesting questions concerning the inorganic metabolism of the cryptoendolithic microbiota is how much of the primary production is photosynthetic and how much is chemosynthetic. To try to answer that question, various chemolithotrophic energy sources were added to crushed rock to attempt to stimulate bicarbonate incorporation (Table 4). Of the four substrates tested, at concentrations ranging over 3 orders of magnitude, none showed stimulation of activity above background. In contrast to this, there was no appreciable chemolithotrophic biomass which could contribute to primary production in the community. The dark CO₂ fixation found was due to the heterotrophic microbes present in the community. Although chemolithotrophic hydrogen metabolism was not studied, it was unlikely that these microorganisms would occur in these rock microbial communities.

It has been suggested (4, 6, 12) that, because the CO₂ gas exchange with the atmosphere is slow throughout the porous rock surface, the microbes could recycle some of the respired CO₂ from within the community. In an attempt to investigate this question further, organic compounds (glucose and acetate) were added to rocks, and the effect on the community photosynthetic response was measured (Table 5). Addition of simple organic compounds did depress the incorporation of added [¹⁴C]bicarbonate, indicating that the pool of CO₂ in the rock was greater because of respiration of the organic substrates by the heterotrophic microbes. It has been reported (19) that Trebouxia sp. can take up glucose heterotrophically in some lichen symbioses. This could explain the decreased CO₂ incorporation. However, [¹⁴C]glucose incorporation was not exaggerated in this study, either in the light or in the dark (Table 2). Thus, internal cycling of carbon can occur within the cryptoendolithic community.

The metabolic activity of the cryptoendolithic microbiota was studied with regard to whether water vapor or liquid water would allow metabolism, since it was thought (4) that, even after the rock was wet from snow melt, the pore spaces contained a high relative humidity for 1 or 2 weeks. Table 6 clearly shows that water, either as liquid or as vapor (100% RH), was necessary for photometabolism. There was a 19-fold reduction in metabolism in dry air (ca. 15% RH) compared with wetted rock but only a 6-fold reduction between the water vapor and liquid water treatments. Kappen (10, 11) has shown that Antarctic epilithic lichens from Birthday Ridge, North Victoria Land (70°46' S), can take up water quickly and can carry out photosynthesis above 20% water content. Optimal activity occurred between 80 and 100% water content, however. Therefore, even though intact rocks may retain water as vapor after a snow melt, the metabolic activity can be greatly reduced compared with the time when liquid water was present. This suggests that the
annual period when metabolic activity is theoretically possible (4) may actually be much shorter than 29 days.

It was concluded from this study that the carbon metabolism of the Antarctic endolithic microbiota was primarily by photosynthesis, which could occur over a wide range of psychrophilic temperatures and with various light intensities and moisture regimes. These metabolic studies can therefore be used in conjunction with data and models (Nienow et al., 1988a and 1988b, in press) of this ecosystem to better understand the effects of this extreme environment on this simple microbial ecosystem.

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LITERATURE CITED