Enumeration of Thermophilic Heterotrophs in Geothermally Heated Soils from Mount Erebos, Ross Island, Antarctica

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Soil samples with temperatures up to 64°C were collected from Mount Erebos, an active volcano located on Ross Island, Antarctica. Acidine orange direct counts and most probable number counts of soil samples stored at 4°C for 2 months showed a wide variation in the number of thermophilic microorganisms in different soils. Organisms similar to Clostridium thermohydrodsulfuricum, Bacillus schlegelii, and Bacillus acidocaldarius, as well as neutrophilic Bacillus strains, were isolated.

Hot soils are present on the slopes of Mount Erebos, Ross Island, Antarctica, and provide suitable habitats for the growth of algae and mosses (1). The presence of these organisms strongly indicated that bacteria would also be present, and the fact that some areas of soil were reported to have temperatures approaching 60°C (1) suggested the likely occurrence of thermophilic bacteria. Ugolini and Starkey (8) reported high numbers of bacteria in soil from Mount Erebos and another soil to be sterile. However, the temperature of incubation was not reported.

Described here are the results of acidine orange direct (AOD) and most probable number (MPN) counts and preliminary enrichment studies of the thermophiles in these soils.

Location of sample sites. Sample sites were located above 3,400 m on the upper slopes of Mount Erebos, Ross Island, Antarctica (77°32’S, 167°10’E), which is an active volcano (3). Most sites (Table 1) were located at or near Tramway Ridge and in the inactive side crater. A map showing thermal areas of Mount Erebos is given by Broady (1). Samples were scooped into sterile plastic containers which were placed into a well-insulated bag kept warm with a hot-water bottle to prevent freezing of the samples. At Scott Base (New Zealand’s permanent antarctic base) and in New Zealand, soil samples were stored at 4°C.

Soil temperature, pH, and moisture contents. Soil temperature was measured with a Cole-Farmer 8522-10 Digisense temperature meter. The pH values were measured from a soil suspension consisting of 5 g of soil suspended in 10 ml of distilled water. Moisture content was determined by drying soils to constant weight. The values for these parameters are shown in Table 1. Only soil from sites E23 and E25 had visible algal growth on the soil surface. At none of the sites were there visible sulfur deposits or the smell of hydrogen sulfide, which are normal characteristics of fumaroles in New Zealand. Also, the pH values of some of the soils were much higher than would be obtained for New Zealand fumarolic soils. It may be of significance that soil at site E22, which was at 55°C on the first occasion the site was visited, had completely frozen after a blizzard (temperature, −38°C; wind speed, ca. 50 knots) of 2 days in duration. Site E23 (60°C), however, remained unfrozen.

MPN and AOD counts. Anaerobic MPN counts were carried out according to the method of Pankhurst (6) on soil samples that had been prevented from freezing and otherwise maintained at 4°C for 2 months before analysis. A similar set of dilutions was prepared under aerobic conditions. The diluent used was 0.1 M phosphate buffer (pH 7.6), and anaerobic tubes were reduced by the addition of 0.1% (wt/vol) cysteine hydrochloride. Anaerobic dilution series were inoculated into medium TYEG (11), and aerobic dilution series were inoculated into Castenholz medium D (7). Five tubes of medium were inoculated at each dilution in the series. Incubation of both sets of tubes was for 3 days at 60°C. The total numbers of bacteria in each of the soils were estimated by the method of Ghiorse and Balkwill (2). Results for the five tube MPN and AOD counts are shown in Table 2. These counts are, however, probably underestimates of true viable numbers because of the short incubation period. During direct microscopic examination of soils, branching mycelia were only observed in soil from E27. Algae were observed in soils from all sites except for E29 and E30.

There is a considerable difference between the total and viable thermophile counts. There are many possible reasons for this. There is no reason to expect that all of the bacteria present are thermophiles, because not only is there a wide range of soil temperatures present, but these soils also contain steep thermal gradients near their surfaces. Ugolini and Starkey (8) reported the isolation of bacteria from Mount Erebos fumaroles, and these were presumably mesophiles. Only two media have been used to carry out viable counts, and a more extensive survey would probably find other suitable media which would demonstrate the presence of populations of other thermophilic bacteria. Samples were never allowed to freeze, but even so, it is not known by how much the viable counts decreased between the time of collection and processing (<2 months). In general, the Tramway Ridge sites yielded the largest numbers of organisms, and this may be at least in part due to the abundance of algae in this area, since nutrients for heterotrophs would be available. Although only two sites had visible surface algal growth, all but two soil samples contained algae which were observed during AOD counting. The observation that a 55°C site free of visible algae froze after a two-day blizzard might indicate that summer surface algal growth is restricted to areas where the soil does not freeze.

Enrichment cultures. To enrich for sulfate-reducing bacteria, 1 g of each soil was aseptically added to 100 ml of lactate medium (5). None of the soils contained organisms capable of growth under these conditions. A similar enrichment was carried out for thiosulfate-utilizing autotrophs, by using a medium which comprised the following (in grams per liter):

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TABLE 1. Locations, temperatures, pHs, and percent moisture values for Mount Erebus hot soils

<table>
<thead>
<tr>
<th>Site no.</th>
<th>Location</th>
<th>Temp (°C)</th>
<th>pH</th>
<th>% Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>E22</td>
<td>Tramway Ridge</td>
<td>55</td>
<td>7.3</td>
<td>26</td>
</tr>
<tr>
<td>E23</td>
<td>Tramway Ridge</td>
<td>60</td>
<td>7.8</td>
<td>42</td>
</tr>
<tr>
<td>E24</td>
<td>Tramway Ridge</td>
<td>40</td>
<td>3.1</td>
<td>28</td>
</tr>
<tr>
<td>E25</td>
<td>Tramway Ridge</td>
<td>64</td>
<td>6.1</td>
<td>40</td>
</tr>
<tr>
<td>E26</td>
<td>Near Tramway Ridge</td>
<td>47</td>
<td>4.7</td>
<td>13</td>
</tr>
<tr>
<td>E27</td>
<td>Near Tramway Ridge</td>
<td>37</td>
<td>4.8</td>
<td>19</td>
</tr>
<tr>
<td>E28</td>
<td>Side crater</td>
<td>56</td>
<td>5.6</td>
<td>28</td>
</tr>
<tr>
<td>E29</td>
<td>Side crater</td>
<td>52</td>
<td>6.9</td>
<td>13</td>
</tr>
<tr>
<td>E30</td>
<td>Side crater</td>
<td>62</td>
<td>7.4</td>
<td>17</td>
</tr>
<tr>
<td>E33</td>
<td>West crater</td>
<td>44</td>
<td>8.1</td>
<td>31</td>
</tr>
</tbody>
</table>

nitrilotriacetic acid, 0.1; NaCl, 0.008; MgSO₄ · 7H₂O, 0.1; CaSO₄ · 2H₂O, 0.06; KNO₃, 0.103; NaNO₃, 0.689; Na₂HPO₄, 0.111; NH₄Cl, 0.4; Na₂S₂O₃, 10; and NaHCO₃, 2. Trace elements and ferric chloride were added as described by Ramaley and Hixson (7), and vitamins were also added (10). One soil (Table 1) contained the organism which grew under these conditions, and preliminary characterization suggests that this isolate is a strain of Bacillus schlegelii (data not shown). Inocula of 1 g of soil from three of the more acidic soils were added to 100-ml volumes of media for the cultivation of Sulfobolus and Thermoplasma spp. (4). Positive enrichments were obtained for all three soils, but the isolates appear to be strains of Bacillus acidocaldarius (data not shown). Inocula of 0.1 g of soil were added to media for the cultivation of Thermus spp. (medium CMD [7]), flexibacteria (American Type Culture Collection, Rockville, Md., medium 284), glycolytic anaerobes (medium TYEG [11]), and Thermoproteus spp. (medium Db [12]). Strains growing on CMD and medium 284 were all neutrophilic Bacillus isolates, whereas anaerobes growing on TYEG were similar to Clostridium thermohydrosulfuricum (data not shown). No positive enrichments were obtained on medium Db. Table 3 shows those soils for which positive enrichments were obtained.

The isolation of Bacillus strains from all but three soils demonstrates that thermophilic bacteria are common in Mount Erebus steam-warmed soil. It is not known whether these organisms are actively metabolizing or are in a resting state. If the heterotrophic Bacillus strains and glycolytic anaerobes are actively metabolizing, then they could obtain nutrients released by algae or other cells. It is speculated

that other autotrophic organisms must be present to support heterotrophic growth in areas where algae do not occur. Only one soil yielded a strain capable of autotrophic growth.

The isolates obtained from Mount Erebus soils were all sporeformers, being members of either Clostridium or Bacillus. Although sporeforming anaerobes are ubiquitous (9), they are at higher numbers in geothermal environments than in mesophilic ones, and the MPN data obtained for Mount Erebus soils are comparable with those obtained from other geothermal soils (9). The fact that bacteria were evident in AOD counting suggests that organisms were not present in the soils merely as spores.

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LITERATURE CITED


TABLE 2. MPN and AOD counts for Mount Erebus hot soils

<table>
<thead>
<tr>
<th>Site no.</th>
<th>MPN</th>
<th>AOD (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aerobic</td>
<td>Anaerobic</td>
</tr>
<tr>
<td>E22</td>
<td>8.9 x 10⁴</td>
<td>1.2 x 10³</td>
</tr>
<tr>
<td>E23</td>
<td>2.8 x 10⁴</td>
<td>1.3 x 10³</td>
</tr>
<tr>
<td>E24</td>
<td>2.6 x 10⁴</td>
<td>1.2 x 10⁴</td>
</tr>
<tr>
<td>E25</td>
<td>4.2 x 10²</td>
<td>4.2 x 10²</td>
</tr>
<tr>
<td>E26</td>
<td>1.2 x 10³</td>
<td>4.5</td>
</tr>
<tr>
<td>E27</td>
<td>&lt;1.2</td>
<td>1.0 (0.38) x 10⁸</td>
</tr>
<tr>
<td>E28</td>
<td>1.7 x 10²</td>
<td>&lt;1.3</td>
</tr>
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<td>E29</td>
<td>&lt;1.1</td>
<td>1.4</td>
</tr>
<tr>
<td>E30</td>
<td>&lt;1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>E33</td>
<td>6.6</td>
<td>1.3</td>
</tr>
</tbody>
</table>

* Dilution series were incubated for 3 days at 60°C. Numbers in parentheses show standard deviations (SD) for the AOD counts.

TABLE 3. Results for enrichment cultures of Mount Erebus soils

<table>
<thead>
<tr>
<th>Site no.</th>
<th>Aerobic heterotrophs</th>
<th>Glycolytic anaerobes</th>
<th>Thiosulfate-utilizing autotrophs</th>
</tr>
</thead>
<tbody>
<tr>
<td>E22</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>E23</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>E24</td>
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<td>+</td>
<td>-</td>
</tr>
<tr>
<td>E33</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* Media formulated to grow sulfate reducers and Thermoproteus spp. did not yield enrichment cultures. Media formulated to grow Thermoplasma and Sulfobolus spp. were only used with the most acidic soils (E24, E26, and E27) and in both cases all enrichments yielded acidophilic bacilli. Soils from sites E29 and E30 did not give positive enrichments with any of these media. All enrichments were at 60°C.