NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

Technical Report 32-1524

Survival of Antarctic Desert Soil Bacteria Exposed to Various Temperatures and to Three Years of Continuous Medium-High Vacuum

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Preface

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Abstract

Samples of cold desert soil containing viable bacteria from McKelvey dry valley, Southern Victoria Land, Antarctica were subjected to 3 years of continuous medium-high vacuum of 10^{-3} to 10^{-4} torr at room temperature and storage for 4 years at -30, -5, $+5$, $+20$°C. Dependent upon storage temperatures, the survivability of bacteria decreased with increase in temperatures, with only 3 bacteria/g of soil surviving at room temperature in vacuum, and 500 bacteria/g of soil surviving storage at -30°C. *Corynebacterium* sp., a soil diphtheroid, constituted approximately 90% of the surviving populations. *Arthrobacter* spp. and a *Micrococcus* sp. also survived, but no *Bacillus* spp. survived in any of the samples, although they were present in the soil when it was cultured soon after collection. The reduction in abundance and kinds of bacteria from this naturally harsh terrestrial environment is relevant to the importance of storage conditions for return of Martian soil samples. Based upon Antarctic soil microbial ecology as a Mars model, the most likely life forms for a Martian cold desert soil ecosystem are diphtheroid-like microorganisms.

Survival of Antarctic Desert Soil Bacteria Exposed to Various Temperatures and to Three Years of Continuous Medium-High Vacuum

I. Introduction

In preparation for microbiological studies of returned samples of Martian soils, a soil sample from the Antarctic cold desert was selected for long-term testing in the range of medium-to-high vacuum. This study was undertaken subsequent to a continuous vacuum experiment that demonstrated the rate of survivability of indigenous mixed populations of microorganisms in a temperate desert algal soil crust (Ref. 1). This report presents the results of another phase of a soil storage study undertaken at JPL at the suggestion of the NASA Bioscience Subcommittee.

This sample was selected because of the relevance of the naturally harsh Antarctic environment to the detection and characterization of possible extraterrestrial microorganisms and the Mars quarantine problem. The Antarctic climate is more harsh than any other terrestrial desert region (Ref. 2) and it is one of the important factors for the weak development of soils (Refs. 3 and 4), the low level of terrestrial biota and biotic activity (Refs. 5 through 7), and paucity of microorganisms in the Antarctic ecosystem (Refs. 8 through 10).

II. Site, Soil, and Microorganisms

The soil sample chosen for this study was collected from the surface, 2 cm beneath a layer of approximately 2 to 3 cm of varnished desert pavement in McKelvey dry valley, 77°26′S and 161°15′E, in southern Victoria Land, about 130 km west of McMurdo Sound Station.

The sample was a dry, sandy, brownish, saline, moderately oxidized, dioritic frigic soil of slight cohesion, with a pH > 7, moderate salt content, a low buffer capacity and low exchange capacity, dominated primarily by NO_3^- , $> Cl^- > Na^+ > SO_4^= >> CA^{++} > Mg^{++}$. It also had a low organic (Kjeldahl) nitrogen and organic carbon content and very narrow C/N ratio indicating the highly decomposed, colloidal or microbial nature of the organic constituents.

Depending on the method of analysis, bacterial abundance in the original sample ranged from approximately 10 to <1000/g of soil. A mold, *Penicillium* sp., a blue-green alga, *Schizothrix calcicola*, and anaerobic bacteria were previously reported as recovered from this soil when tests were first made at the NSF McMurdo Biology

Laboratory (Ref. 11). However, the mold was traced to a source of laboratory contamination, the alga was not recovered from five successive tests with duplicate 10-g samples of soil. Although it is a common alga in Antarctica (Ref. 12), it has been recovered in low abundance from other samples obtained from McKelvey Valley in austral summer 1969–1970. No anaerobes could be recovered when the soil was subsequently incubated in a CO_2 chamber at JPL instead of a reduced atmosphere obtained with pyrogallic acid and NaOH in the McMurdo Biology Laboratory (Ref. 11). Additional detailed and illustrated accounts of area, site, soil profile, aseptic sampling procedures, storage, processing, and analyses of soils and microorganisms and their comparison with other desert soils have been given previously for this sample (Refs. 13 through 15).

III. Vacuum System

An apparatus was constructed of Pyrex glass, which enabled its 30 chambers to be exposed to vacuum simultaneously (Fig. 1).[1] Each chamber contained a 1-g sample of sieved, ≤ 2 mm soil at the *in situ* moisture content of 1.4 wt % (determined by oven-drying at 105 $\pm$ 5°C). The control samples were stored at ambient (room temperature) and in sterilized, plastic snap-cap test tubes. Other aliquots were stored at −5, +5, and +20°C. The bulk soil sample was stored with >5 tons of other Antarctic soils in a walk-in freezer at −30°C (Ref. 15), which has subsequently received additional external insulation.

A Veeco vacuum gauge tube, Type DV-4AM, was mounted in the system and connected to a Veeco vacuum gauge, Type RG-31X, to provide pressure readings. The vacuum was obtained with a Welch Duo-Seal Vacuum purge (Type 1405B). The pressure stabilized at 10^{-3} to 10^{-4} torr, and remained there throughout the 3-year period of the experiment. The system was kept at room temperature, and the temperature measured periodically with a mercury thermometer suspended adjacent to the apparatus. Room temperature was monitored continuously on a long-term basis with a recording thermometer (Tempscribe); it varied between 18 and 27°C, but usually was between 21 and 23°C.

IV. Cultural Methods

Five samples were sealed off and removed from the distal end of the glass apparatus on July 28, 1970 at a pressure of $<10^{-3}$ torr. All cultural determinations were performed on July 28, 1970, and the samples stored at other temperatures were cultured between August 4 and 13, 1970 and incubated at +20 and +5°C for 3 months.

A washing technique was used to remove the samples from their chambers into appropriate dilution tubes for subsequent microbiological examinations. Microbiological analyses were performed on samples diluted serially from 10^{-1} through 10^{-4} for bacterial growth in trypticase soy broth, fluid thioglycollate medium, lactose broth, and

[1]Apparatus constructed by H. A. Heyn and R. L. Condon of JPL.

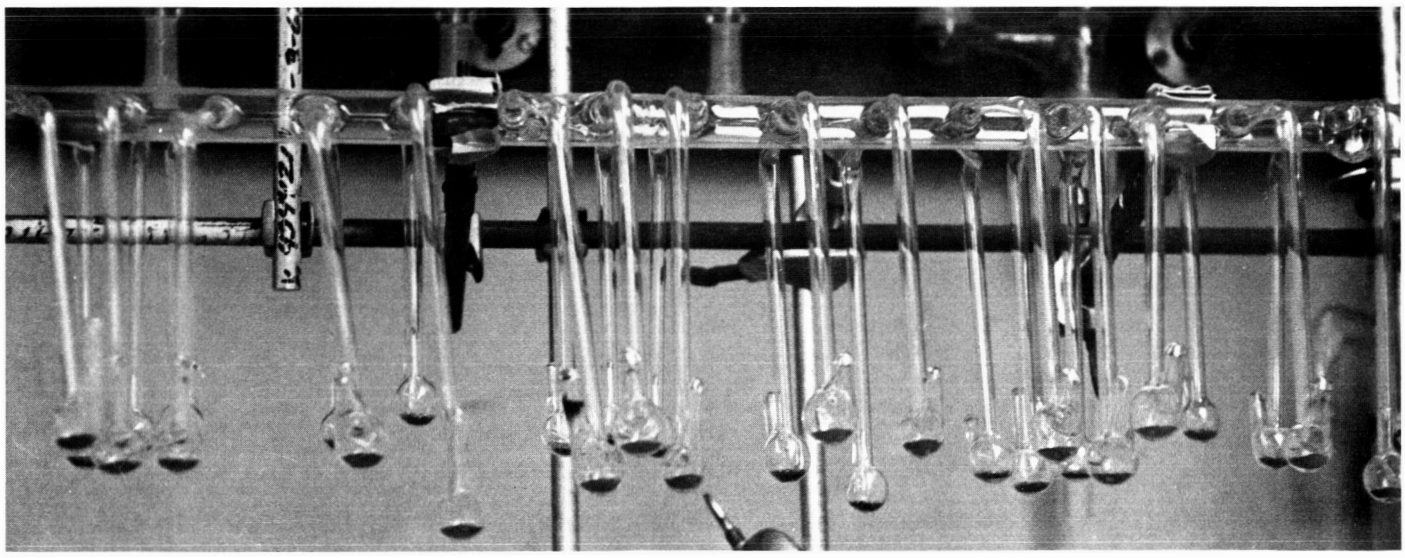

Fig. 1. Vacuum system glass manifold and attached soil sample chambers

nitrate reduction broth. Dilutions for trypticase soy agar plates were 10^{-1}, 0.5×10^{-2} and 0.5×10^{-3}; soil also was sprinkled on the surface of agar plates. All agar plates were pretempered at the required incubation temperature before the inoculum was spread on the plates with a loop. Bacterial colonies were counted at 1-week intervals with an illuminated Quebec Colony Counter.

V. Results and Discussion

Survival of bacteria varied from <10 to 500/g of soil, depending on storage conditions. Abundance of survival of bacteria cultured at +20°C on trypticase soy agar is shown in Fig. 2. A relatively straight curve was obtained for survivable bacteria, primarily dependent on storage temperature, and regardless of vacuum exposure. An average bacterial count of only 3/g of soil was obtained following vacuum exposure, but 500 bacteria were cultured from the soil stored at −30°C. A less smooth curve, but similar to that obtained for the culture of bacteria at +20°C was obtained for abundance of bacteria cultured at −5°C, except that the number of bacteria recovered from −30°C storage was only 250/g of soil. However, a longer incubation period, up to 6 months, may show a greater recovery of bacteria (R. E. Benoit, Table 4, quoted in Ref. 10). Recovery of bacteria following incubations at +20°C in liquid culture media showed 10 or $<10^3$ survivable bacteria in some tubes of fluid thioglycollate, nitrate broth, and lactose fermentation broth. Nevertheless, at least 100, but $<10^3$, survivable bacteria were recovered in trypticase soy broth from the −30°C storage condition, and $\leq$10-100 bacteria were recovered in this medium when they were cultured from other storage conditions.

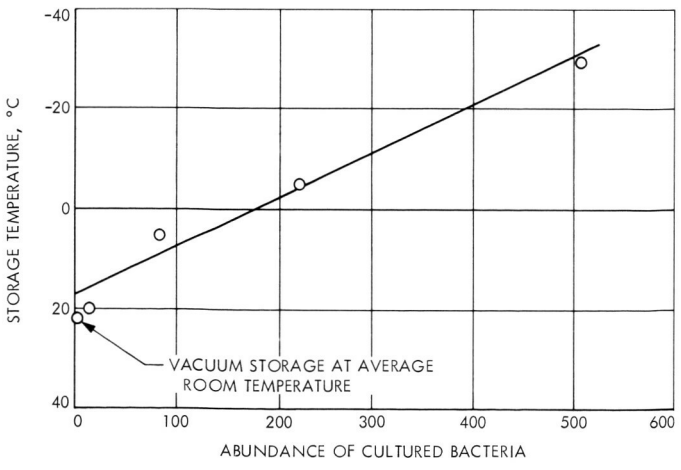

Fig. 2. Survival of Antarctic soil bacteria at various storage conditions following culture in trypticase soy agar at +20°C

The predominate bacteria which survived, regardless of culture conditions, appeared on agar plates as slightly raised, convex, shiny, smooth and entire, light red (2.5 YR 6/8) colonies of medium size. These have been identified as *Corynebacterium* sp. (Fig. 3),[2] one of the members of the diphtheroid group. This species constituted approximately 90% of the recoverable bacteria. Other surviving bacteria were two *Arthrobacter* spp. (Fig. 4) which also are diphtheroids, and a *Micrococcus* sp. When all three of these isolants were subcultured, it was found that they grew best at 25°C, rather than at higher temperatures or at 5°C. Only the *Micrococcus* sp. (Fig. 5) grew on Burk's N-free Ion-agar medium, but the *Arthrobacter* spp. tolerated salt in nutrient broth with 5% added NaCl.[3]

Although *Bacillus* spp. were originally reported in this soil (Ref. 11 and footnote 4), none were recovered in any of the stored samples. It is of significance that bacterial isolants from cold and hot temperate desert soils show variations with latitude and that more *Bacillus* spp. are found in hot temperate deserts, but the abundance of soil diphtheroids increases with latitude and in cold deserts (Ref. 16). The survivability rate of bacteria from non-Antarctic soils when subjected to extended simulated drought has shown that *Bacillus* sp. outlive *Arthrobacter*

[2, 3]Cameron, R. E., personal communication with Johnson, R. M., Arizona State University, Tempe, Ariz.

[4]Cameron, R. E., personal communication with Kemper, K. Byers, Oregon State University, Corvallis, Ore., JPL Contract No. 950783.

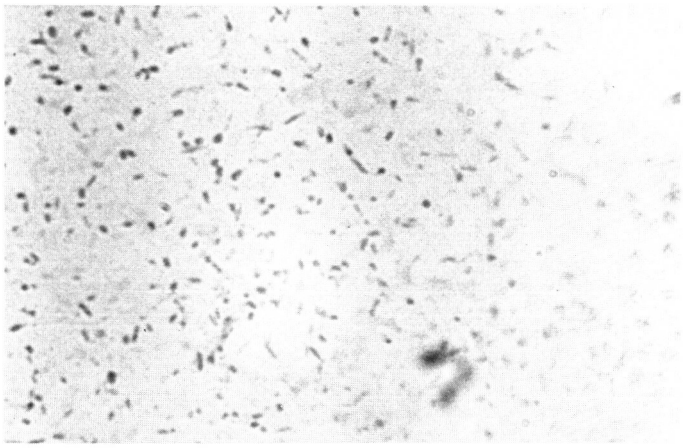

Fig. 3. Photomicrograph (1250 ×) of stained Antarctic soil diphtheroid, *Corynebacterium* sp

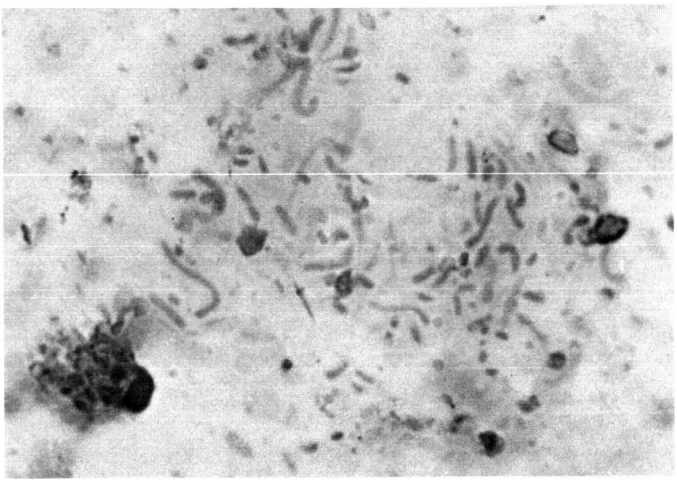

Fig. 4. Photomicrograph (1250 ×) of stained Antarctic soil diphtheroid, *Arthrobacter* sp

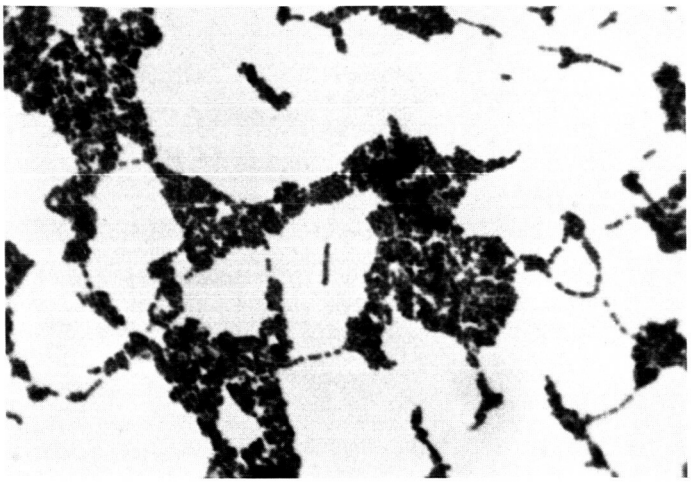

Fig. 5. Photomicrograph (1250 ×) of stained Antarctic soil coccoid bacterium, *Micrococcus* sp

sp. (Ref. 17). In stored, air-dry arid and semiarid California museum soils at least 70 years old, but not more than 90 years old, 75% of the bacterial survivors were *Bacillus* spp. and only 15% were soil diphtheroids (Ref. 18). However, *Arthrobacter* spp. are typical soil inhabitants and show little or no growth at 37°C. *Bacillus* spp. are commonly found in soil, as well as in other habitats, and some species can grow at temperatures up to 65°C (Ref. 19). Most of the soil *Cornybacteria* isolated from Antarctica cold desert soils show optimal growth[5] at 25°C rather than 37°C. *Corynebacterium* spp. are widely distributed in nature (Ref. 19), but soil forms have not been well-defined.

VI. Concluding Remarks

Although only one Antarctic soil has been studied in detail for survival of bacteria following storage at various conditions, others have been tested intermittently and also show some reduction of bacterial abundance and populations depending upon whether samples were stored at −5, −30°C, or higher temperatures. It is obviously important to maintain storage of bulk samples of Antarctic soil at −30°C, which approaches an average Antarctic dry valley temperature (Ref. 15), if it is desired to retain the *original* soil microbial community. Further testing is needed, not only of this soil but of others as well, to determine the survivability of microbial populations with time at various storage temperatures.

The vacuum study will be continued until it has been determined that there are no survivable bacteria. A long-term, continuous, very high vacuum study of a temperate desert soil has shown some similarities in reduction of various groups of microorganisms similar to microbial groups occurring in Antarctic cold desert soils (Ref. 1).

Based upon Antarctic soil microbial ecology as a Mars model, as well as the survival tendencies of bacteria in a hot desert soil, the microorganisms most likely to be found in a Martian cold desert ecosystem could have diphtheroid-like characteristics. Some Antarctic soil diphtheroids also have the added advantage of surviving incubation in a CO_2 atmosphere; however, no strict anaerobes have been obtained from the least-favorable soil habitats. For any return of Martian soil samples and subsequent investigations for microorganisms, the storage conditions of the sample as well as the advisability of low temperature storage should be considered, as demonstrated by the survivability of Antarctic cold desert bacteria.

[5]Cameron, R. E., personal communication with Johnson, R. M., Arizona State University; Kemper, K. Byers, and Bollen, W. B., Oregon State University.

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15. Supplementary Notes

16. Abstract

Samples of cold desert soil containing viable bacteria from McKelvey dry valley, Southern Victoria Land, Antarctica were subjected to 3 years of continuous medium-high vacuum of 10^{-3} to 10^{-4} torr at room temperature and storage for 4 years at -30, -5, +5, +20°C. Dependent upon storage temperatures, the survivability of bacteria decreased with increase in temperatures, with only 3 bacteria/g of soil surviving at room temperature in vacuum, and 500 bacteria/g of soil surviving storage at -30°C. Corynebacterium sp., a soil diphtheroid, constituted approximately 90% of the surviving populations. Arthrobacter spp. and a Micrococcus sp. also survived, but no Bacillus spp. survived in any of the samples, although they were present in the soil when it was cultured soon after collection. The reduction in abundance and kinds of bacteria from this naturally harsh terrestrial environment is relevant to the importance of storage conditions for return of Martian soil samples. Based upon Antarctic soil microbial ecology as a Mars model, the most likely life forms for a Martian cold desert soil ecosystem are diphtheroid-like microorganisms.

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