# ANAEROBIC BACTERIA OF ANTARCTICA —ISOLATION OF CLOSTRIDIA FROM THE SOIL AROUND SYOWA STATION—

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***Abstract:*** From the soil (permafrost, moraine, bottom of lake, coast, etc.) collected in the area around Syowa Station which is located on East Ongul Island in Lützow-Holm Bay of Antarctica, a total of 150 strains of clostridia were isolated. Aerobic sporeforming bacteria were isolated less than clostridia. The soil samples, when heated at 80°C for 10 min or more before the cultivation, proven no *Clostridium*.

One hundred and fifty strains were assigned to 6 species, *i.e.*, *C. perfringens*, *C. bifermentans*, *C. sordellii*, *C. fallax*, *C. sporogenes* and *C. septicum*. The first three species were isolated very frequently and *C. sporogenes* less frequently. All the strains of *C. sordellii* were non-toxigenic and had almost the same biochemical and cultural characteristics as those of *C. bifermentans* except the urease reaction.

It was surprising that many clostridia were found in the soil sampled from places which were considered to be scarcely contaminated by human beings and animals.

The peculiar distribution and taxonomy of clostridia in the soil of Antarctica are discussed in this paper.

## 1. Introduction

As a member of the 13th Japanese Antarctic Research Expedition (January 1972 to February 1973) in charge of medical research, the author had an opportunity to make wintering observations at Syowa Station, Antarctica.

Antarctica, about 38 times larger than Japan, is covered with thick continental glacier and snow all the time, and the climate is very cold and dry. The climatic data recorded at Syowa Station during the author's wintering period were as follows: the highest temperature, 6.2°C; the lowest temperature, −38°C; the average annual temperature, about −10°C; and the average annual humidity, about 65%. Syowa Station is located on East Ongul Island in Lützow-Holm Bay (Figs. 1 and 2).

In this cold Antarctic region, organisms are living under harsh conditions (MATSUDA, 1964; GOTO *et al.*, 1969; SUGIYAMA *et al.*, 1967). Animals

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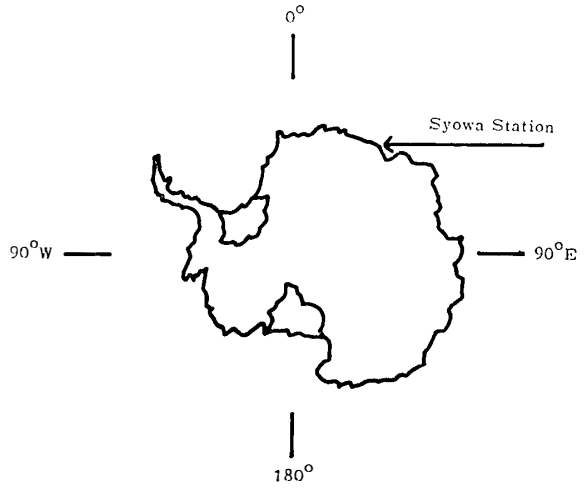


*Fig. 1. Location of Syowa Station in Antarctica.*

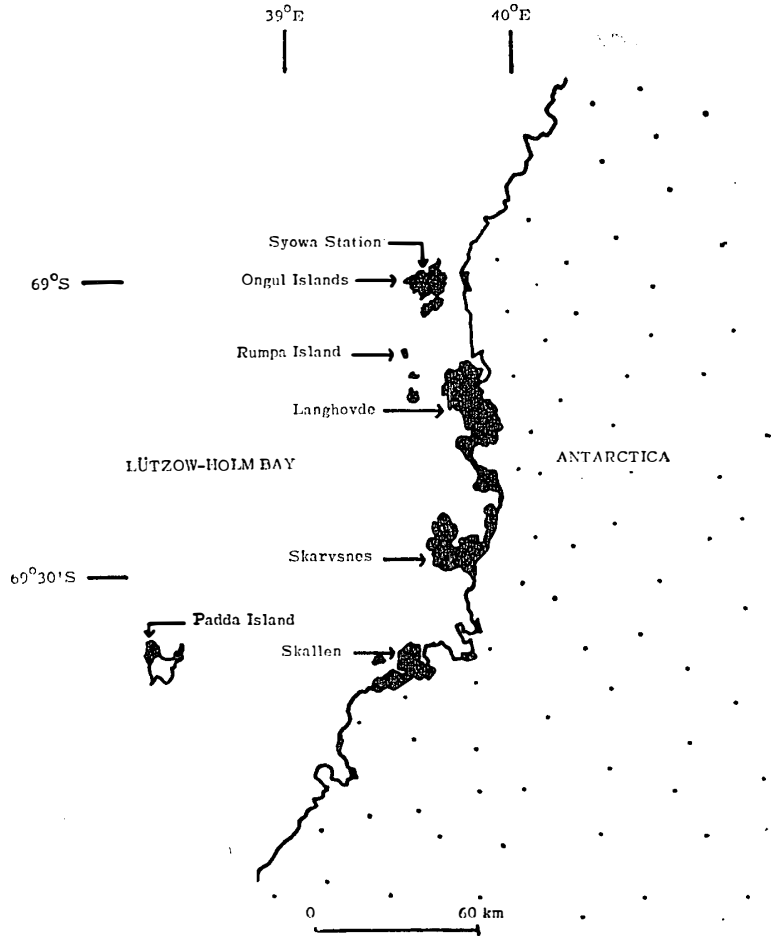


*Fig. 2. Sampling localities in the area around Syowa Station on the east coast of Lützow-Holm Bay.*
*Black areas indicate the grounds exposed only during summer and dotted areas are the places covered with thick ice all the year round.*

generally cannot settle down there, and penguins, seals and seafowls appear on the coast of the continent only during the summer season (December to February). On the other hand, plants such mosses, lichens, yeasts and fungi grow in small swamps on the islands and capes that are exposed only during the summer season when snow and ice melt. The distribution of microorganisms in the Antarctic region was studied by some researchers; EKELÖF (1908) isolated moulds, and DARLING and SIPLE (1941) isolated bacteria (*Bacillus mesentericus*, *B. subtilis*, etc.) for the first time. Studies by the Japanese researchers on the distribution of microorganisms in the Antarctic region gradually progressed since the International Geophysical Year (1957 to 1958); for example, SONEDA (1961) isolated yeasts and TUBAKI (1961) isolated moulds from the soil around Syowa Station.

As mentioned above, moulds, yeasts and bacteria were isolated from the soil and sea mud of Antarctica, and many strains were considered to have been carried by the air current from other continents. However, no study has ever been made on the distribution of anaerobes in the soil around Syowa Station. In this study, sporeforming obligatory anaerobic rods which were relatively easy to isolate were chosen as the target bacteria. Soil samples were collected in the places which seem to be evidently contaminated by human beings and animals, and also in the places where contamination was supposed to be slight or nil.

## 2. Material and Methods

### *2.1. Sampling*

Sampling was carried out in the area about 300 km from East Ongul Island; the area of exposed rocks on the Prince Harald Coast, permafrost that was exposed only during the summer season, moraine zone, and bottom mud of lakes which were covered with ice almost all the year round.

The soil samples were collected by the following methods (MIWA, 1973). In the case of permafrost and moraine zone, a fist-sized sample was taken from 10–20 cm below the ground surface and was put into a sterile Petri's dish or a polyvinyl bag; the container was immediately sealed, and preserved in a frozen condition. In the case of lakes or ponds covered with about 2m thick ice, the surface ice was bored, and the bottom mud (5–15 m below the water level) was taken with water samples.

### *2.2. Methods of isolation of clostridia*

The soil samples preserved in a frozen condition were thawed at room temperature; about 2 g of a sample was mixed with a basal medium (about 10 ml) in a medium-size test tube, and incubated at 37°C for 1–10 days for

enrichment. Bacto Cooked Meat medium and GAM semisolid medium (Nissui) were used as the basal media. After the incubation, test tubes containing the culture were divided into two groups; unheated and heated, and clostridia were isolated with author's laboratory procedures (KOSAKAI and SUZUKI, 1968). Anaerobic culture on a plate medium was made by the steel wool method ($N_2$, 80%; $CO_2$, 20%) (UENO, 1964) at 37°C for 24–48 hours.

*2.3. Identification of clostridia*

The author used the technique that were employed in author's laboratory (KOSAKAI and SUZUKI, 1968) and the VPI manual (HOLDEMAN and MOORE, 1972).

## 3. Results

*3.1. Isolated species*

As presented in Table 1, six species of clostridia were identified. Three species (*C. perfringens, C. bifermentans, C. sordellii*) were isolated from many soil samples from various places. About 17% of all isolated strains remained unidentified.

*3.2. Unidentified strains*

The identification of non-toxigenic clostridia was generally very difficult. Strains which could not be proven to sporulate but were considered to be clostridia because of their thermostability, were tentatively classified into the group of unidentified strains. This group included those strains which resembled *C. felsineum, C. difficile, C. butyricum* and *C. cellobioparum.*

*3.3. Thermostability*

From each gram of the unheated soil samples three to four species were isolated, while virtually no colonies were proven on the plate medium when the soil samples was heated at 80°C for 10 min.

*3.4. Quantitative culture*

The distribution of aerobes in the Antarctic soil was compared with that of anaerobes by the quantitative culture method. The anaerobes were found in the range of $10^{3-4}$/g of soil, but there were only a few aerobes.

*3.5. Pathogenicity to mice*

Strains of *C. perfringens* and *C. septicum* only killed mice mostly within 24 hours. Strains of *C. sordellii* and *C. bifermentans* proved to have no-lethal toxicity to mice.

*Table 1. Isolation of clostridia from the Antarctic soil.*

| Locality | Species | *C. perfringens* | *C. bifermentans* | *C. sordellii* | *C. fallax* | *C. sporogenes* | *C. septicum* | Unidentified strains |
|---|---|---|---|---|---|---|---|---|
| East Ongul Island | Bottom of Lake Mizukumi* | + | + | + | | | | + |
| | Around Lake Midori | + | + | + | | | | + |
| | Bottom of Lake Taratine | + | | | | + | | + |
| West Ongul Island | Bottom of Lake Ô-ike | | + | | | | | |
| | Around Lake Higasi* | + | + | + | | | | + |
| | Hill | | | | + | | | + |
| Rumpa Island | Penguins' rookery, south | + | + | + | | | | + |
| | Penguins' rookery, north | + | + | + | + | | | + |
| | Lake edge, permafrost | + | | | + | | | |
| Padda Island | Lake edge | + | | | | | | + |
| Ongulkalven Island | Hill | + | + | + | | | | + |
| Langhovde | Bottom of Lake Yuki* | + | + | + | | | | |
| | Around Lake Yuki* | | + | + | | | | + |
| | Around Lake Yukidori | + | + | | | | | + |
| | Bottom of Kami-kama | | + | | | | | + |
| | Hill | + | | + | | | | |
| | Moss, Heitô Glacier | + | + | + | + | | | + |
| | Ground, Heitô Glacier | + | | | + | | | |
| | Moraine, Heitô Glacier | + | | | | | | + |
| | Moraine, Hamna | + | + | | | | | |
| | Moraine, Langhovde | | + | | | | | + |
| Skarvsnes | Around Lake Suribati | + | + | + | | | | + |
| | Bottom of Lake Kizahasi* | | | + | | + | | |
| | Around Lake Kaminoike* | + | + | | | | + | + |
| | Hill, permafrost | | | | | | | + |
| Skallen | Bottom of Lake Skallen Ôike | | | | | | | + |
| | Bottom of Lake Dairi | | + | | | + | | |
| | Coast, permafrost | + | + | + | | | | + |
| Continental coast | Cape Hinode | + | | | | | | |
| | F0 point, permafrost | + | | | | | | + |
| | F0 point, moraine | | | | | + | | + |

* : Provisional name.
\+ : Positive isolation from one or more portion of a sampling places.

## 4. Discussion

There are a few studies (EKELÖF, 1908 ; DARLING and SIPLE, 1941; GOTO, 1969) on the distribution of bacteria in Antarctica, but no study was made on the distribution of anaerobes including clostridia. Studies in Japan and other countries on the distribution of clostridia in soil have aimed chiefly at the isolation of some special species of the genus, *i.e., C. perfringens* (YAMAGISHI *et al.,* 1964) and *C. tetani* (SANADA and NISHIDA, 1965).

The distribution of clostridia isolated by the author from the Antarctic soil was compared with the distribution of clostridia in the soil of Japan reported by other authors (NOTOMI, 1959; TAKAGI, 1960). It is of particular interest that, while *C. sordellii* has never been isolated from the soil of Japan. In other continents, isoltion of this species is very difficult. The author, however, isolated *C. sordellii* from the Antarctic soil in many places. Eighty-five percent of the strains of *C. sordellii* were isolated from the same soil samples from which *C. bifermentans* were isolated. *C. perfringens* was the most widely distributed in the Antarctic soil as well as in the soil of Japan. On the other hand, *C. sporogenes* was frequently isolated from the soil of Japan, while only six strains of this species were isolated from the Antarctic soil.

There were thus some marked differences between the findings in the author's present study and the reports hitherto published (NOTOMI, 1959; TAKAGI, 1960). Moreover, it is interesting that many clostridia were found in the places which were considered to be hardly contaminated by human beings and animals (MIWA *et al.,* 1974).

Thermostable strains of Antarctic clostridia which could resist the heating at 80°C for 10 minutes or more were not found in this study. MORINAGA (1962a, b) showed that the strains of *C. perfringens* isolated from the soil of the area scarcely contaminated by human beings and animals, or the soil in old ages. The similarity between the author's and MORINAGA's finding is interesting.

Biological properties of the isolated clostridia from Antarctica revealed that there were many mutants of each species. These mutants may have resulted from degeneration in the special natural enviroment of the Antarctic region. Identification of *C. sordellii* and *C. bifermentans* has long been a subject of controversy from the taxonomic standpoint (BROOKS and EPPS, 1959; NISHIDA *et al.,* 1964). In this study, many strains (85%) of *C. sordellii* and also many strains of *C. bifermentans* were simultaneously isolated from the same soil samples of Antarctica. It may be reasonable to interpret this fact as an identification that *C. sordellii* and *C. bifermentans* of Antarctica are not two different species but one and the same species with mutation, occurring

only in urease and toxinogenicity. Then, it is necessary to study further on saccharides composition of cell membrane (NOVOTONY, 1968) and DNA homology which are very important taxonomically.

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