

# NOTES ON THE MICROBIOLOGY OF SOIL FROM NORTHERN GREENLAND

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## Introduction.

Our knowledge of the microorganisms in soils from Greenland is still very incomplete. An important contribution to this question has been given by BARTHEL (1922) who examined fourteen soil samples from Disko, two from the Kap York District, and two from the North Coast of Greenland (I. P. Koch's Fjord), besides samples of feces from various polar animals. BARTHEL's results showed that the identifiable species of bacteria were largely the same as those found in soils from other geographic regions, and that many common species of soil bacteria are ubiquitous. Among specific groups, the aerobic nitrogen-fixing bacteria (*Azotobacter*) were not encountered except possibly in one sample from I. P. Koch's Fjord, but anaerobic types of nitrogen-fixing bacteria (*Bacillus saccharobutyricus* syn. *Clostridium butyricum*) were regularly found. All the soil samples contained nitrate, but the cultivation of nitrifying bacteria (*Nitrosomonas* and *Nitrobacter*) succeeded in only a few cases. In the same samples, NIELSEN (1927) identified 5 species of *Actinomyces* (*Streptomyces*), 8 Mucoraceae, (*Mucor*, *Absidia*, *Mortierella*) 9 species of *Penicillium*, and one *Aspergillus*; all these genera are common soil inhabitants. PETERSEN, quoted by MÜLLER (1924), found nitrification in 7 among 10 soil samples from Disko. Root nodule bacteria of leguminous plants (*Rhizobium*) seemed to be absent in Disko but were found at Julianehaab (NIELSEN, 1928).

In September 1950 nine soil samples from the environment of Jørgen Brønlands Fjord, Peary Land, were received from Mr. KJELD HOLMEN, M. Sc., who had collected the samples in sterile test tubes in early August before the return of the wintering party of the Danish Pearyland Expedition (WINTHER et al., 1950). These samples were subjected to various tests, particularly regarding the presence of microorganisms taking part in the nitrogen cycle. Owing to the small size of the samples (12—15 gm), only a limited number of chemical and microbiological tests could be made.

## Results.

Table 1 shows the general character of the sampled areas, according to information received from Mr. HOLMEN, together with a few chemical data. The samples, which all except one (no. 6) represent surface soil at a depth of 5 cm, were of a sandy character and contained variable

Table 1. General Character of Soils from Peary Land.

Sample No.	Origin	pH	Humus <sup>1)</sup> %	Total-N <sup>1)</sup> %	NO <sub>3</sub> -N <sup>2)</sup>
1	Wet ground, snow-free. Frost-free period <i>ca.</i> 85 days. Predominant vegetation mosses ( <i>Tortella tortuosa</i> ).	7.6	20.6	0.92	+++
2	Wet ground, snow-free. Frost-free period <i>ca.</i> 85 days. Predominant vegetation <i>Eriophorum polystachium</i> , <i>Carex aquatilis</i> , mosses.....	7.7	8.4	0.47	+
3	Somewhat dry ground, stonefield at 460 m altitude. Frost-free period <i>ca.</i> 65 days. Predominant vegetation <i>Cassiope tetragona</i> .....	8.0	45.0	1.71	+
4	Like No. 3 .....	7.5	35.6	1.05	++
5	Wet ground near border of Klaresø, snow-covered in winter. Frost-free period <i>ca.</i> 55 days. Predominant vegetation <i>Eriophorum scheuchzeri</i> , <i>Juncus biglumis</i> , <i>Cinclidium arcticum</i> , <i>Philonotis tomentella</i> .....	6.8	32.8	1.11	+
6	Same, from a depth of 25 cm .....	5.6	15.0	0.53	(+)
7	Wet ground, near duck's nest, at border of Klaresø, snow-covered in winter. Frost-free period <i>ca.</i> 70 days. Predominant vegetation <i>Puccinellia angustata</i> , <i>Carex maritima</i> .....	7.5	21.9	1.12	+
8	Somewhat dry ground, snow-covered in winter. Frost-free period <i>ca.</i> 50 days. Predominant vegetation mosses ( <i>Timmia austriaca</i> ) .....	8.2	18.2	0.63	++
9	Moist ground, deep snow-cover in winter. Predominant vegetation <i>Luzula arctica</i> , <i>Papaver arcticum</i> , <i>Saxifraga setigera</i> .....	7.2	15.5	0.58	++

<sup>1)</sup> in soil dried at 95° C.

<sup>2)</sup> +++: reaction with diphenylamine very strong; ++: strong; +: weak; (+): very weak, or doubtful.

amounts of plant roots that were removed as completely as possible before the analysis. The reaction (pH) was determined by means of the quinhydrone electrode, humus by oxidation with chromic acid (BONDORFF, 1946), total nitrogen by the Kjeldahl method (OLSEN, 1927). Qualitative tests for nitrate (with diphenylamine in sulphuric acid), nitrite (with GRIES' reagent) and ammonia (with NESSLER'S reagent) were made in a soil extract with approx. 0.5 molar KCl; the results of the last two tests were in all cases very weak or negative.

All the soils are seen to be rich in humus and total nitrogen; this agrees perfectly with the general rule of JENNY (1941) that the content of organic matter and nitrogen in the soil increases exponentially with decreasing mean annual temperature. In fact, the present soils are comparable to Alpine soils from high altitudes at mean annual temperatures below 0° C, in which 20—30 % organic matter accumulates in spite of a scanty vegetation (JENNY, l. c.). Nitrate was found in all samples with the possible exception of the acid subsoil No. 6.

The following microbiological tests (all in duplicate) were made in various selective media (WAKSMAN, 1932):

**Aerobic nitrogen-fixing bacteria (particularly *Azotobacter*):** 1.0 ml of soil suspension 1:10 in sterile water was streaked on the surface of nitrogen-free glucose-agar in Petri dishes which were incubated for 7 days at 25° C. No *Azotobacter*-colonies developed from any of the soils, and no other organisms made any conspicuous growth that would suggest nitrogen-fixing ability. *Azotobacter* thus seems generally lacking in Greenland soils (cf. BARTHEL, 1922) although all the present soils except one (No. 6) have a reaction favourable for the growth of this organism.

**Anaerobic nitrogen-fixing bacteria (*Clostridium butyricum* and related organisms):** 1.0 ml soil suspension in dilutions from 1:10 to 1:2000 was inoculated into test-tubes with potato medium (small potato-cubes to a depth of about 5 cm, with 10 ml tap water and some calcium carbonate, boiled and cooled immediately before inoculation). The cultures were incubated at 25° C, and when gas formation appeared the cultures was examined microscopically for the presence of clostridia staining blue with iodine. The following figures show the limits of dilution at which growth of clostridia appeared:

No.	Highest dilution	No.	Highest dilution
1	1:2000	6	1:2000
2	1:200	7	1:2000
3	1:200	8	1:10
4	1:10	9	1:2000
5	1:200		

All the soils, like those examined by BARTHEL (1922), thus contain bacteria of the anaerobic nitrogen-fixing group, some of them in quite appreciable numbers.

**Nitrifying bacteria:** Test tubes with 5-ml portions of WINOGRADSKY'S ammonium sulphate solution plus calcium carbonate were inoculated with 0.5 ml soil suspension dil. 1:10. After incubation for 4 to 6 weeks at 25° C all the cultures, except those from soil No. 6, showed a vigorous nitrite reaction. Subcultures were then made in (a) the same medium + 0.001 mol potassium chlorate which specifically inhibits the nitrate-forming bacteria and thus renders the medium selective for *Nitrosomonas* (LEES and QUASTEL, 1945) — and (b) WINOGRADSKY'S sodium nitrite solution for *Nitrobacter*.

The following amounts of nitrite (determined colorimetrically by the Gries-Ilosvay method) were found in medium (a) after 20 days:

No.	NO <sub>2</sub> -N, $\gamma$ /ml.	No.	NO <sub>2</sub> -N, $\gamma$ /ml.
1	31	6	0.05
2	38	7	16
3	44	8	35
4	9	9	6
5	16	Sterile	0.05

Organisms producing nitrite from ammonia (*Nitrosomonas*) are thus present in all soil except No. 6. The cultures in medium (b) showed no consumption of nitrite after incubation for 8 weeks. The presence of *Nitrobacter* thus could not be directly demonstrated, but may be inferred from the fact that most soils contained appreciable quantities of nitrate but very little or no nitrite. The nitrate found in the samples may certainly to some extent have been formed during the time between sampling and testing (approx. 6 weeks), but conditions for nitrification in these soils in their natural surroundings also seem to exist. According to Mr. HOLMEN, the soil temperature at a depth of 5 cm often approaches 10° C during the summer months in Peary Land, and nitrification may proceed, although slowly, at temperatures well below this limit (SCHÖNBRUNN, 1922; JENSEN, 1939).

**Denitrifying bacteria;** Giltay's solution, 10 ml in test tubes, was inoculated with 0.5 ml soil suspension dil. 1:10. Active denitrification occurred in all cases, as shown by gas evolution and disappearance of the nitrate within a week at 25° C.

**Aerobic cellulose-decomposing bacteria:** Test tubes with a strip of filter paper and 10 ml of Dubos' solution were inoculated with

1 ml soil suspension dil. 1 : 10. The acid sub-soil No. 6 showed no growth or attack of the paper after 6 weeks at 25°C. In all other cases the paper showed visible destruction after a few weeks. These cultures were plated on cellulose-agar where transparent plaques (*Cytophaga*) or definite colonies surrounded by clear haloes appeared after various lengths of incubation. Strains of *Cytophaga* (BERGEY, 1948) were found in soils No. 1, 2, and 5; small curved or straight rods resembling organisms of the genera "*Cellvibrio*" and "*Cellulomonas*" (BERGEY, 1948) were found in the other soils. The general types of aerobic cellulose-decomposing bacteria thus seem to be the same as in soils from other regions.

Nitrogen-fixing blue-green algae: 250-cc milk bottles with 25 ml of ALLISON, HOOVER and MORRIS' (1937) nitrogen-free solution + 5 p.p.m. sodium molybdate were inoculated with 1 ml soil suspension dil. 1 : 10. After incubation for two months in a cool greenhouse (12—16°C) only the cultures from soil No. 1 showed a feeble growth of cyanophyceae, among which *Nostoc* sp., probably *N. sphaericum*, and *Anabaena* sp. (kindly determined by Dr. J. BOYE PETERSEN, Botanical Museum, Copenhagen). In all the other cultures only a few specks of purplish or yellowish-green growth appeared. Nitrogen-fixing cyanophyceae, if occurring at all, thus do not seem to be prevalent, at least at a depth of 5 cm.

Finally, counts of the general bacterial flora were made on glucose-casein-agar in dilutions of 1 : 20,000 and 1 : 200,000; the colonies were counted after 14 days at 25°C. The numbers of bacteria varied from 2.2 mill. (in No. 6) to 14.1 mill. (in No. 4) per gm of moist soil, a rather low density in comparison with Danish field soils where counts on the same medium usually show some 10 to 80 mill. per gm. The figures are admittedly of little significance because the numbers of bacteria may have altered greatly since the samples were taken, and the counts were chiefly made in order to get an idea of the prevailing types of bacteria. Microscopic examination of 20 or 25 representative colonies from each soil showed that the predominant organisms were of the types that are characteristic of the general soil microflora (CONN, 1948): small or middlesized, non-spore-forming, rod-shaped bacteria, often of an irregular shape suggesting the genus *Corynebacterium* (called *Arthrobacter* by CONN, l. c.). Spore-forming bacteria were rare, but colonies of typical cocci, often in tetrads, were seen in several soils, and in No. 5 this type was entirely predominant. Upon isolation it appeared to be *Micrococcus flavus* Trevisan (BERGEY 1948). Besides the true bacteria, various actinomycetes were found in numbers from 0.1 to 2.2 mill. per gm. Most of these belonged to the genus *Streptomyces*; a few colonies of *Micromonospora* sp. were seen in soil No. 4.

In addition to the bacteria and actinomycetes, the agar plates showed some colonies of filamentous fungi that were not studied any further; a species of *Rhodotorula* was found in soil No. 4.

### Conclusions.

The soils from Peary Land seem to contain the same general groups of bacteria as soils from other regions, except for the apparent lack of *Azotobacter* which otherwise is commonly found in neutral and alkaline soils. Organisms of the *Clostridium butyricum*-group certainly occur, but the question whether their nitrogen-fixing activity is of importance in the nitrogen-economy of the soils can hardly be answered, because we know virtually nothing about the correlation between the density of the *Clostridium*-population and the resulting gains of nitrogen. The prevailing low soil temperature during the short frost-free period would suggest that these gains can only be estimated at a low figure. Auto-trophic nitrogen-fixing microorganism (blue-green algae) seem to be sparsely represented at a depth of 5 cm. A study of their abundance on the very soil surface would be necessary in order to form a idea about their importance as nitrogen-fixers.

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