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Bacterial flora in soils of Western Spitsbergen

ABSTRACT: 53 soil samples collected in the Bellsund Region in Western Spitsbergen were examined. An acid-resistant strain difficult to identify was isolated and recognized as *Mycobacterium friburgensis*. 54.7% of isolated strains were acid-resistant and growing at 25°C only. They were microorganisms at borderline of *Mycobacteria* and *Actinomycetes*. Other microorganisms isolated in the studied soil samples were bacilli (55.7%) and coccaceae (15.4%).

Key words: Arctic, Spitsbergen, soil bacteria, *Mycobacterium*.

Studies on isolation of soil bacteria from soil samples collected in Bellsund Region in Western Spitsbergen have been carried out lately (Janowiec *et al.* 1991, Jóźwik 1992). The soil samples examined came from the area which has not been occupied by a man permanently. They were taken in different tundra sites.

During the expedition to Spitsbergen in 1991 samples were collected from sites where people (whale hunters) used to live, and also from sites where people live at present (settlements Barentsburg and Longyearbyen). Presence of acid-resistant microorganisms seems to be associated with a man in this region. This problem was also of interest of a research group headed by Professor Janowiec (Institute for Tuberculosis and Lung Diseases, Warsaw), studying non-tuberculous *Mycobacteriosis* and mycobacteria (Janowiec 1975, Janowiec and Sobiech 1975, Janowiec *et al.* 1977, 1986, 1987).

Material and methods

53 soil samples collected by Jóźwik in the Bellsund Region in Western Spitsbergen and also at Barentsburg and Longyearbyen in July and August 1991 were microbiologicaly controlled. 20 soil samples were collected in the Norwegian miners' settlement Longyearbyen, 10 samples in the Russian miners' settlement Barentsburg, 5 samples from outwash of the Renard Glacier in the Bellsund Region, 13 samples — in the archeological site "Viking" from under a till of the Renard Glacier — probably from the Viking period (Dzierżek *et al.* 1990a, Jasiński and Starkhov 1992) and 5 samples from Tomtodden where whalers lived.

Technique to isolation of mycobacteria

From each soil sample an amount of 1 g was suspended in 100 ml of *chlorhexidinum gluconicum* solution (8 ml of 20% commercial solution for 1000 ml of distilled water). After vigorous shaking, it was homogenized for one and two hours at room temperature (25°C), centrifuged for 15 minutes at 3000 rev/min. A fluid from over a sediment was decanted and the sediment was inoculated with 0.3 ml for 15 samples of Löwenstein-Jensen medium, 5 samples of which were incubated at 4°C, 25°C and 37°C for 10 weeks. Readings of colony growth were done weekly. The colonies were controlled in microscope smears stained by the Ziehl-Neelsen method and strains were identified into species. A blind test with chlorohexidine solution was adopted as control of research technique.

Technique to indicate oxygen bacteria and relative aerobes

The studies were based on inoculation of standard soil samples (ca 0.1 g) on the media: liquid and Sabourauda agar, thioglycolan liquid, liquid used in growth of microorganisms from blood *(Haemomedium)*, enriched with addition of 5% sheep blood and McConkey agar.

Cultures were incubated at 37°C. The cultures in the Sabourauda and thioglycolan media were run at 4°C, 25°C and 37°C. Observations of culture growing were done on 1st, 3rd, 5th, 14th and 21st incubation days. When cultures grew, microscopic preparations stained by the Gram method were done. After homogenization of the soil samples with chlorohexidine, microscopic smears stained with the Ziehl-Neelsen method were done to catch presence of acid-resistant microorganisms and other bacteria.

Results

They are presented in four tables. Table 1 presents studies of microscopic smears (by the chlorhexidine method) from sediments of homogenized soil

Table 1
Detectability of particular microorganisms in 53 microscopic soil samples from Spitsbergen dyed by the Ziehl-Neelsen method

Occurrence of microorganisms	Data	
	number	%
coccaceae (diplococci)	8	6.4
coccaceae (single)	23	18.4
streptococci (susp. *Str. faecalis)*	1	0.8
bearing spores bacilli (long)	20	16.0
bearing spores bacilli (short)	12	9.6
bacilli (susp. *B subtilis)*	14	11.2
bacilli (diminutive)	1	0.8
bacilli (in a small number)	1	0.8
corynebacteria	13	10.4
spiral forms	4	3.3
yeasts	12	9.6
fungi	14	11.2
acid-resistant bacilli	2	1.6
Total	125	100.0

samples after their staining by the Ziehl-Neelsen method to catch acid-resistant microorganisms. In microscopic picture acid-resistant microorganisms were observed twice: bacilla characteristic for acid resistant microorganisms and fragments of fungi spawns. Amongst other microorganisms there were coccaceae (25.6% cases) and single coccaceae (18.4%), diplococci (6.4%) and streptococci (0.8%). Bacilli of different sizes and spores were discovered (48.8%), including long forms with a few spores (16.0%) — short forms (9.6%), those resembling *B. subtilis* (11.2%) and corynobacteria (10.4%). There were also spiral forms (3.2%) yeasts (9.6%) and fungi (11.2%).

Quantitative analysis of particular bacterial forms indicated that a simple microorganism only was found in 18.9%, two in 43.3%, three in 28.3% and four or more in 7.5%.

Analysis of microorganism growth (Table 2) from soil samples on individual diagnostic media indicated considerable differences dependent on properties of the media used as well as on thermal conditions and time of microorganism growth.

Three cases of microorganism growth (5.7%) were obtained on the Löwenstein-Jensen egg medium at 4°C. At 25°C a growth was noted in 24 cases

(45.3%) but at 37°C in two cases only. Only same of the obtained primocultures resulted in secondary growth in subcultures.

A growth was much lower on a Sabourauda liquid medium (50.9% at 4°C, 71.7% at 25°C and 94.7% at 37°C) than on an agar medium (3.8% at 4°C, 5.7% at 25°C and 11.3% at 37°C). Similar values were obtained for a thioglycolane medium (47.2% at 4°C, 66.0% at 25°C and 98.1% at 37°C). Growth in medium for microorganisms from blood *(Haemomedium)* at 37°C gave positive results in 98.1% cases and in the McConkey medium — in 17.0%. Totally 212 cultures were run at 4°C but 318 at 37°C (Table 2).

Table 2

Analysis of microorganism growth from soil samples from Western Spitsbergen dependent on incubation temperature

Medium	Growth of microorganisms at temperature					
	4°C		25°C		37°C	
	number	%	number	%	number	%
Lowenstein-Jensen	3	5.7	24	45.3	2	3.8
Sabourauda liquid	2	3.8	3	5.7	6	11.3
Sabourauda agar	27	50.9	38	71.7	50	94.3
Thioglicolane	25	47.2	35	66.0	52	98.1
Haemomedium	—	—	—	—	52	98.1
McConkey	—	—	—	—	9	17.0
positive results (total)	57	26.9	100	47.2	171	53.8
number of founded cultures	212				318	

When analyzing growing of microorganismes in media after some observation days an increase of positive resultes with time was found. The data obtained are the lowest for the Sabouraud liquid medium from 1.9% after a single observation day to 11.3% on the 21st day. Growth on the McConkey and Löwenstein-Jensen media was noted from the 7th observation day. An increase with observation time was also found in this case (from 1.9% on 7th day to 45.3% on 21th day for the Löwenstein-Jensen medium).

An analysis of occurrence of various bacterial forms or species in soil samples (Table 3) showed that most isolated microorganisms included oxygen bearing spores bacilli, slightly dependent on a medium used in the studies (52.3% – 59.5%). Then a significant role of staphylococci should be ascribed (10.1% – 18.6% for the Sabourauda medium, 13.9% for the thioglicolane medium and 15.4% for *Haemomedium*). Numbers of negative cultures (except the McConkey medium) were rather small (1.9% compared with 79.2% for the McConkey medium).

Table 3

Analysis of isolated microorganisms from soil samples from Western Spitsbergen based on microscopic studies of smears from particular cultures

Microorganism	Number of perceptible microorganisms in the media									
	Sabourauda				Thioglico-lane		*Haemome-dium*		McConkey	
	liquid		agar							
	number	%	number	%	number	%	number	%	number	%
staphylococci	9	10.1	16	18.6	11	13.9	12	15.4	—	—
streptococci	6	6.7	5	5.8	6	7.6	3	3.8	—	—
packed-forming	5	5.6	4	4.7	1	1.3	3	3.8		
bacilli	49	55.1	45	52.3	47	59.5	44	56.4	—	—
corynobacteria	7	7.9	5	5.8	5	6.3	5	6.5	3	12
gram negative rod bacteria	5	5.6	5	5.8	3	3.8	3	3.8	14	56.0
gram negative yeats	3	3.4	3	3.5	4	5.1	5	6.5	2	20.0
fungi	5	5.6	3	3.5	2	2.5	3	3.8	3	12.0
Total number of negative tests	—	—	1	1.9	1	1.9	1	1.9	42	79.2
Total number of isolated microorganisms	89	100.0	86	100.0	79	100.0	78	100.0	25	100.0

When analyzing amounts of microorganisms in particular soil samlpes, estimated from microscopic smears of individual cultures stained by the Gram method, no microorganisms were found in 1,9% of the studied samples only. In 26.5% only a single microorganisms was found, in 39.6% there were two, in 25.4% — three and in 7.5% — four and more.

No acid resistant microorganisms were detected in soil samples collected from Tomtodden and Barentsburg (Table 4). Only for soil samples from outwash of the Renard Glacier concurrent results by both methods were obtained. In other cases twofold increase of research results was obtained by use of culture method compared with a bacterioscopic one.

Table 4

Detectibility of Mycobacteria by the methods: microscopic and culture in soil samples from Western Spitsbergen

Sampling	Number of positive samples	Positive results obtained by methods:			
		microscopic		culture	
		number	%	number	%
Longyearbyen	20	5	25.0	12	60.0
Outwash of Renard Glacier	5	3	60.0	3	60.0
Viking (cottage of whalers)	13	6	46.2	9	69.2
Tomtodden	5	—	—	—	—
Barentsburg	10	—	—	—	—
Total	53	14	26.4	24	45.3

Amongst 24 primocultures of acid resistant strains (45.3%) in subcultures, in 19 cases a strain growth was only at 25°C and in one case at 37°C. None of examined acid-resistant strains showed sufficient data for complete identification according to the accepted criteria in this range (Janowiec 1975, Janowiec and Sobiech 1975, Janowiec *et al.* 1977, 1986, 1987).

Summing up results on occurrence of bacterial flora in soil samples, an attention should be paid to isolation of acid resistant microorganisms grown in a primoculture: in three cases at 4°C (5.7%), 24 cases at 25°C (45.3%) and two cases at 37°C (3.8%). Using identification tests for secondary identification at 25°C and 37°C (Janowiec 1975, Janowiec and Sobiech 1975, Janowiec *et al.* 1977, 1986, 1987) 20 strains were used but only in one case a growth was obtained at 37°C (enabling a complete identification). The strain was included into the group IV — according to Runyon difficult to be identified (susp. *Myc. friburgensis)*. Of 19 strains giving growth at 25°C which are the only that make a complete identification possible, the growth was obtained on the 8th observation day in six and on the 5th day in other cases. Studied strains were colourful and produced orange dye at different shades (from yellow to pink) in most cases. In microscopic smears dyed by the Ziehl-Neelsen method, particular strains showed great differentiation if their acid resistance is concerned. In most cases many non-acid resistant forms were found among acid resistant ones of different sizes from coccaceous to rodshaped forms. Smears were susceptible to decolourization by acid alcohol (which is a typical feature for acid-resistant saprophytes).

When analyzing occurrence of other microorganisms (basides mycobacteria) in soil samples as in previous studies (Janowiec *et al.* 1990, Jóźwik 1992) bearing spores oxygen bacilli and oxygen bacilli (55.7%), coccaceae (15.4%) and staphylococci were found as most numerous among those perceptible in cultures and microscopic smears.

Conclusions

1. In 53 soil samples from Western Spitsbergen an acid-resistant microorganisms, not fully identified (susp. *Myc. friburgensis* with characteristics of acid-resistant saprophytes was isolated.

2. In 54.7% of samples the acid-resistant strains which grow at 25°C only, were isolated.

3. In the studied samples bacilli (*ca* 55.7%) and coccaceae (*ca* 15.4%) were isolated.

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Streszczenie

W roku 1991 w ramach Wyprawy Geograficznej na Spitsbergen Uniwersytetu Marii Curie Skłodowskiej w Lublinie pobrano próbki gleb celem izolacji drobnoustrojów. Próbki pochodziły z miejsc, gdzie kiedyś żyli ludzie (miejsce pobytu łowców wielorybów) jak również z terenów, gdzie obecnie żyje człowiek (Barentsburg i Longyearbyen) (tab. 4).

Wśród 53 próbek glebowych izolowano drobnoustrój kwasooporny (susp. *Mycobacterium friburgensis*) o cechach saprofitu kwasoopornego (tab. 1–3). W 54.7% izolowano szczepy kwasooporne dające wzrost tylko w 25°C (tab. 2). Izolowano także laseczki (55.7%) i ziarenkowce (15.4%) *por.* tab. 1 i 3).