THE BACTERIAL FLORA OF THE WATERSHEDS

OF

SEYMOUR AND CAPILANO CREEKS.

by

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1923.

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A Thesis submitted for the Degree of

MASTER OF ARTS

in the Department

of

BACTERIOLOGY.

THE UNIVERSITY OF BRITISH COLUMBIA

MAY, 1923.

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Two years ago McLean (1) investigated the Vancouver water supply collecting samples daily from a city faucet and a few from the watershed of Seymour Creek, at and above the intakes. The following were the main types of bacteria isolated;

1. Sarcinae and Rhodococci
2. Spore formers
3. Aerogenes - rapid lactose-fermenters
4. Slow lactose-fermenters
5. Fluorescent Bacteria.

The first two types were found in most of the samples but they are of little if any sanitary significance. The aerogenes type was isolated from samples of surface water or surface soil, the slow lactose-fermenters from deep soil and the fluorescent bacteria from practically all the samples.

The object of the present work is to determine:

1. The bacterial flora of the watershed of both Capilano and Seymour Creeks above the intakes.
2. The relative proportion of the different types found at any one point under varying climatic conditions.

3. The source of the aerogenes type previously isolated.

I. METHODS.

1. FIELD INVESTIGATIONS.

The watersheds at Seymour Creek have been previously described by McLean (1). Samples were collected from the watershed at and above the intake on two occasions, May 31, 1922, March 2, 1923 and from Capilano, July,5, 1922.

May 31, 1922. From May 24th to this date the weather had been warm and fine. On this particular day the temperature at the upper intake was 100°F. in the shade. Previous to May 24th the weather had been cold and wet. As a result of the early spring freshet the water in the Creek at this time was high. Floods had been severe resulting in numerous washouts both above and below the intakes.

March 2, 1923. During the winter months there had been the usual rain and rather heavy snowfalls but the day was bright and warm. Several inches of snow remained on the flats, above the intakes; the ground was frozen except where unprotected from the sun.

July 5, 1922. Very warm weather and no rain since May 21st.

METHODS OF COLLECTING SAMPLES.

The outfit for collecting samples consisted of:

1. Large, sterile test tubes 6" by 7/8".

2. Sterile spoons and tongue-depressors.

3. Alcohol lamp.

4. Scissors and forceps.

Samples of water, plants, surface soil and deep soil were taken in sterile tubes. Surface soil samples were obtained with sterile spoons. For deep soil samples the earth or clay was dug out with a handpick and the edge of the hole was scraped away with a sterile tongue-depressor. The plants were cut off with scissors flamed in an alcohol lamp, or pulled off with sterile tongue-depressors or forceps.

After collection the samples were transported to the laboratory as rapidly as possible and cultures sown the same day.

2. LABORATORY INVESTIGATIONS.

(1) Media.

All media used was made according to Standard Methods(2) with the exception of Eosin Methylene Blue Agar, Purple Lactose Agar, Endo's Agar, Starch Agar, Lead Acetate Agar which were the dehydrated Difco Media. Levine(3) recommends Koser's synthetic medium with Xanthine substituted for Uric acid. Xanthine was unobtainable so this medium was not used. The peptone in all the media except phosphate broth was Difco Bacto Peptone but in the latter, Difco Proteose Peptone was used. Liebig's meat extract was used throughout.

The sugar broths contained 1% sugar and Brom thymol blue Indicator.

In the first part of the work intermittent sterilization of all the sugar media was employed. Mudge(4) has shown that the time of exposure to heat was even more important than the degree of temperature. For the latter part all sugar media except phosphate broth were sterilized for twenty minutes at fifteen pounds pressure, care being taken in each case not to put media into autoclave until steam had begun to flow. After sterilization pressure was reduced as rapidly as possible. There was no reason to suspect any change in the constitution of the sugars. It was observed, however, that the Xylose broth appeared to increase slightly in hydrogen ion concentration.

(2) Procedure.

The procedure used on each of the three occasions varied slightly.

Seymour Creek, 1922. The samples were enriched with lactose broth and 1 cc. of enrichment fluid was sown immediately into a lactose broth fermentation tube. After 24 hours incubation at 37°C. cultures were recorded and plates made on eosin methylene blue agar, purple lactose agar and Endo, using a lactose broth fermentation tube as first dilution.

Capilano, 1922. Each sample was enriched as

before and incubated at 37°C. After 24 hours, plates were poured in the same manner as described above.

Seymour Creek, 1923. Samples were enriched with lactose broth and duplicate plates were made on eosin methylene blue agar and purple lactose agar using 1 cc. of enrichment fluid or in case of water samples 1 cc. of water. These were incubated at 22°C. giving the relative number of types from each location a factor not noted in the two previous investigations. After 24 hours incubation of original enriched samples, plates were made on eosin methylene blue agar using lactose broth fermentation tubes for the first dilution. These plates and fermentation tubes were incubated at 37°C.

(3) Picking of Colonies.

The colonies of different type were picked at 24 hours for 37°C. plates and at four to seven days for 22° plates and sown in lactose broth fermentation tubes. These were recorded after 24 hours incubation at 37°C. and each culture sown on slant agar. Morphology and relation to Gram's stain were determined after 24 hours at 37°C. Cultures were replated where the Gram stain indicated the possibility of contamination.

(4) Periods of Observation.

Sugars - The sugar media were incubated at 37°C. for three days and at room temperature for the remainder of the

week. Cultures were examined at the end of 24 hours, 48 hours, 72 hours and one week.

Milk - Fresh, separated milk containing sufficient litmus to give the required color was employed. Cultures were incubated at 37°C. and recorded after 24 hours, 48 hours, 72 hours, 96 hours, one week and two weeks.

Gelatin - Cultures were incubated at 22°C. and liquefaction recorded after three days, one week, two weeks, three weeks and one month.

Indol - Cultures sown in peptone water made with Difco Proteose Peptone after three days incubation were tested for Indol by Ehrlich's Method. The results were not entirely satisfactory and are recorded with reservations. Peptone water with and without dipotassium acid phosphate was tried without changing the results. One or two tubes with a peptone water to which a small amount of tryptophane was added (in addition to that said to be in the proteose peptone) gave much better results. There was not available sufficient tryptophane at this time to check all results by its use. Further investigation along these lines is anticipated and will be reported later. A good deal of difficulty has always existed in obtaining proper indol tests. Fellers(5) has described a method by distillation which apparently will give much more reliable results.

The Voges Proskauer and Methyl Red tests were carried

out according to standard methods(2). Cultures were incubated at 37°C. for five days. All results were confirmed by incubation at 22°C. without marked discrepancies, although a few additional positive methyl red tests were obtained at the latter temperature of incubation.

II. SAMPLING POINTS.

In obtaining the samples at Seymour Creek an attempt was made to duplicate as far as possible, those taken by McLean(1). A new trail had been cut, starting at the "outlook" and running over the clay bank instead of along the edge of the stream (see map) as that part of the old trail had been washed away.

The Government trail starts at the lower intake runs parallel with the other, from six to nine hundred feet back of the Creek and branches into the large slide. On the first investigation the Government trail was used to reach the big slide and the new trail to return. On the second, the lower trail only was used. No samples were obtained from the big slide as a recent smaller slide was found at the entrance to the former making it difficult of access. The sampling points are indicated on the annexed map, page 9a.

In the following list the Roman numerals represent the sampling points, the letters the types of samples and the Arabic numerals the investigations.

Seymour Creek Samples.

I. "Outlook" on Creek, above the upper Intake.

A1 - surface clay

B1 - clay 8" deep

C1 - moss

D2 - water from Creek

A2 - clay under snow

II. Half-way up trail above the "outlook".

A2 - loam

C2 - moss

III. Below trail on top of clay bank.

A1 - surface clay

B1 - clay 4" deep

D2 - water and sand

IV. Stream at stake #16 - between clay bank and slide.

D1 - water from stream

D2 - water from stream

A2 - sand

C2 - moss from tree near stream

V. New small slide

A2 - surface clay at base

B2 - clay 6" deep at base

AX2 - surface sand high up bank

BX2 - sand 6" deep high up bank

VI. Between stakes 17 and 18 on the flats

C2 - fern

R2 - ranunculacea

P2 - small pine

T2 - trillium

VII. Government trail - near the top where trail branches

A1 - surface soil

C1 - moss

VIII. West bank, in mouth of "big slide".

A1 - surface clay

B1 - clay 12" deep

D1 - water from fall

IX. Near top of west bank of "big slide"

A1 - surface clay

B1 - sand below gravel

C1 - weed

X. Small stream in floor of "big slide" about half way from mouth

D1

XI. Top of clay bank

C1

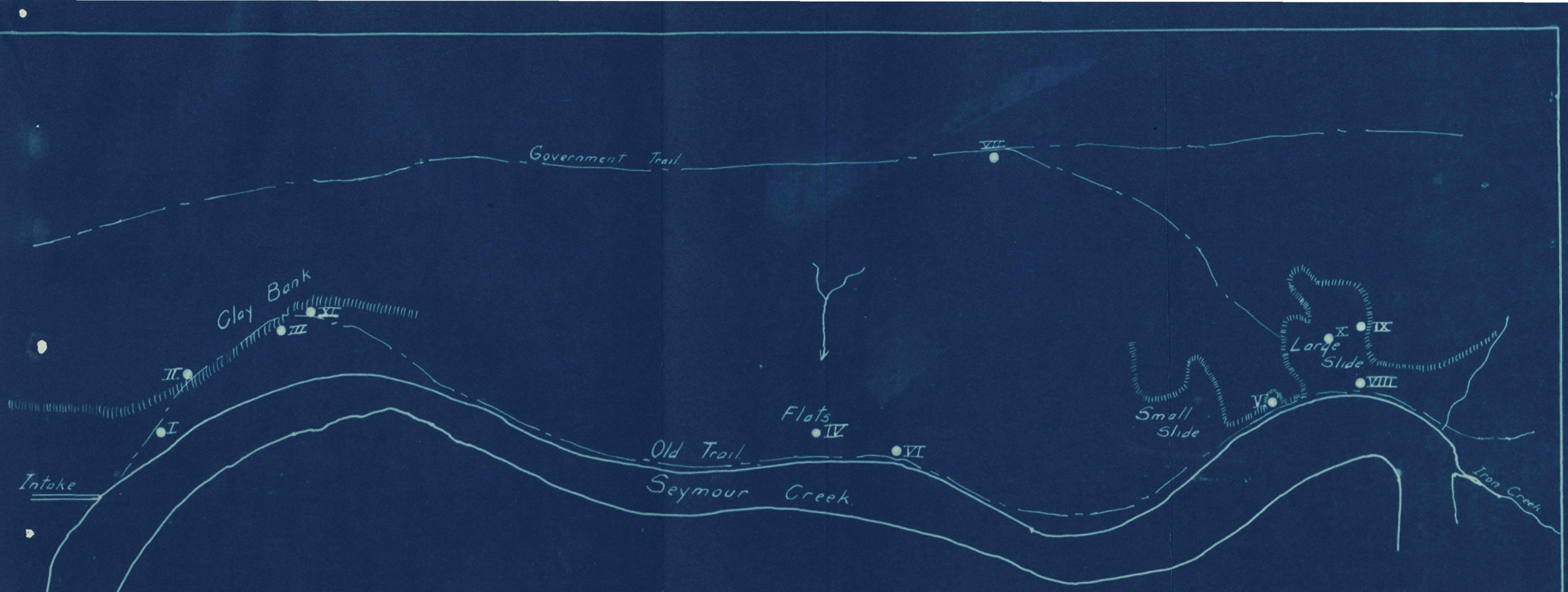
XII. Upper Intake

DT2 - water from right tank

DS2 - water below screen

D2 - water from flume

XIII. Lower Intake



Plan
of
Part of Seymour Creek.
Scale: 300' = 1".

DL2 - left tank

DR2 - right tank

XIV. Canyon view - part way to intake

AD2 - sand and water.

Capilano Samples.

The Creek was crossed at the intake and a logging train taken to the end of the track. The roadbed was then followed to the end of the construction in progress at the time, just beyond the cook house.

XV. Spring near cook house at end of track.

D - water

XVI. End of road

D - water from leak in pipe

CT - leaf of tree

B - soil 3" deep

C - moss

XVII. Capilano Creek below cook house

D - water

XVIII. Capilano Creek at bridge.

D - water

XIX. Five hundred feet north of Sister's Creek

A - brown sand near right of way

XX. Intake

D - water from tank near the screens.

III. OUTLINE OF CLASSIFICATION.

188 cultures were isolated.

Slant agar cultures were made of all organisms from the lactose broth fermentation tubes sown from colonies picked from plates. These were examined for morphology using Gram's stain. Each culture was sown on the following media:

1. Plain broth - for motility.
2. Sugar media - dextrose, maltose, mannit, xylose, arabinose, rhamnose, dulcite, lactose, salicin and sucrose.
3. Phosphate broth - for Methyl red and Voges Proskauer tests.
4. Peptone water - for indol test.
5. Starch agar - for diastase production (6).
6. Plain gelatin - for liquefaction.
7. Litmus milk.
8. Eosin Methylene Blue agar - for differential appearance of colonies.

Observations were made as previously indicated and tabulated. An analysis of this tabulation indicates a certain definite grouping of the different cultures. The several features, best correlated are the following: lactose dulcite, Methyl red, Voges Proskauer, milk, gelatin, motility, morphology and eosin methylene blue agar. In certain forms pigmentation gives an easy differentiation. Starch agar was

used but appeared to be of value only in the pigmented forms. The primary division is made from the action of the organisms in lactose dividing them sharply into two classes, first, those fermenting lactose either to acid alone or to both acid and gas; and second, those showing an alkaline reaction. Quantitative gas production was not studied as it does not appear to add any points of differential value and is not included in the table. Slight variations in maltose, mannite, xylose, arabinose, rhamnose, salicin and sucrose do not assist in classification. They are recorded but not used to create different groups.

Types I to IX inclusive, embracing 167 cultures are tabulated in Table I which shows morphology, action on sugars, gelatin, milk and the number of cultures in each type. Types X to XII inclusive, embracing 21 cultures are not included in the Table for reasons indicated under each type.

IV. DESCRIPTION OF BACTERIA.

DIVISION I. BACTERIA FERMENTING LACTOSE.

Type I.

Twelve cultures of this type were obtained from the following sources:

May 31, 1922. Surface sand below trail on clay bank. Moss and surface clay at "outlook".

Capilano. Water from Creek below cook house and

Table I.

Type	No of cultures	Morphology	Motility	Dextrose	Maltose	Mannite	Xylose	Arabinose	Rhamnose	Dulcite	Lactose	Salicin	Sucrose	M.R.	V.P.	Milk	Gelatin
I	12	Short rods gram negative	0	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	0	#	ACR	0
II	27	Short rods gram negative	#	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	0	#	ACR	#
III	32	Short rods gram negative	#	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	#	0	A	0
IV	11	Short rods gram negative	0	AG	AG	AG	AG	AG	AG	ak	AG	AG	AG	#	0	ACR	0
V	12	Short rods gram negative	0	AB	AB	AB	AB	AB	AB	AB	Ar	AB	AB	#	0	0	0
VI	10	Short rods gram negative	#	AG	AG	AG	AG	AG	ak	ak	ak	AG	AG	0	#	ACD	#
VII	3	Short rods gram negative	#	AG	AG	AG	AG	AG	AG	ak	ak	ak	ak	0	#	Ar	0
VIII	52	Short rods gram negative	#	A	ak	ak	ak	ak	ak	ak	ak	ak	ak	0	G	CRD	#
IX	8	Short rods gram negative	#	ak	ak	ak	ak	ak	ak	ak	ak	ak	ak	0	0	0	0

A - Acid
G - Gas
B - Bubble
ak - Alkaline
G - Green

C - Coagulation
R - Reduction
D - Digestion
r - Reversion

- plus

from tank at intake. Surface sand, near road bed north of Sister's Creek. Moss from end of road.

Morphology

Short, non-motile, gram negative rods without spores or capsules.

Carbohydrates

Acid and gas are produced in the following sugars: dextrose, maltose, mannite, xylose, arabinose, rhamnose, dulcite, lactose, salicin and sucrose. One culture produced no gas in lactose.

Methyl Red and Voges Proskauer Reactions

All cultures give a negative methyl red and a positive Voges Proskauer reaction.

Litmus Milk

All cultures are acid after 24 hours, coagulated and reduced at the end of three days. After six days there is extrusion of whey and dissolving of curd.

Gelatin

No liquefaction.

Indol

None of the cultures produced indol.

Identification

This type is identified as Bact. aerogenes.

Type II.

Twenty-seven cultures of this group were isolated from the following sources:

March 2, 1923. Water from stream at stake 16. Water and sand from below trail on clay bank. Surface and deep soil from "new slide". Water from both tanks at lower intake, from the flume and behind screen at upper intake. Water from "outlook". Sample of sand and water from Canyon-view on way to intake.

Capilano. Brown sand 500 feet north of Sister's Creek.

Morphology

Short, gram negative rods. All strains are motile without spores or capsules.

Carbohydrates

Dextrose, maltose, mannite, xylose, arabinose, rhamnose, dulcite, lactose, salicin and sucrose are fermented to acid and gas.

Methyl Red and Voges Proskauer Reactions

All cultures give a negative methyl red reaction and all but four give a positive Voges Proskauer reaction, one of these remains unchanged and the other three give a green color, similar to that described under type VIII below, instead of the usual eosin-pink.

Litmus Milk

All cultures are acid after 24 hours and coagulat-

ed after three days. Reduction takes place on the fifth day.

Gelatin Stab

All cultures are completely liquefied in from three to five days.

Indol

Three strains of this type give the required cherry-red color when tested for indol by Ehrlich's method (7).

Identification

This type is identified as B. cloacae.

Type III.

Thirty-two organisms of this group were isolated from the following sources.

May 31, 1922. Samples of surface soil, deep soil and water taken from the west bank, mouth and stream in floor of "big slide". Water from stream at stake 16 samples of surface clay and moss at "outlook" and surface soil on government trail.

March 2, 1923. Samples of water and clay from "outlook". Water from tank at upper intake. Weed between stakes 17 and 18. Water and sand below trail on clay bank.

Capilano. Water samples from Creek below cook house and from leak in pipe at end of road.

Morphology

All strains are short rods, gram negative and actively motile, without spores or capsules. No fluorescence.

Carbohydrates

Dextrose, maltose, mannite, xylose, arabinose, dulcite, lactose, rhamnose, salicin and sucrose are fermented to acid and gas.

Methyl Red and Voges Proskauer Reactions

All cultures give a positive methyl red reaction and a negative Voges Proskauer reaction.

Litmus Milk

The majority of the cultures turned the milk slightly acid after 24 hours growth. Three of the cultures required five to seven days to produce acidity. The milk did not coagulate.

Gelatin Stab

With the exception of two cultures there was no liquefaction.

Identification

This type is identified as Bact.neapolitanus

Type IV.

Eleven cultures of this type were obtained from the following sources:

March 2, 1923. Samples of water and moss at stake 16. Surface sand half way up trail above "outlook" and from a weed between stakes 17 and 18.

Capilano. Samples of water from Creek at bridge and sand 500 feet north of Sister's Creek.

Morphology

Short, gram negative rods, non-motile, without spores or capsules.

Carbohydrates

Dextrose, maltose, mannite, xylose, arabinose, rhamnose, lactose, salicin and sucrose are fermented to acid and gas. Dulcite shows an alkaline reaction.

Methyl Red and Voges Proskauer Reactions

Six cultures give a negative methyl red reaction and a positive Voges Proskauer reaction. Five cultures give a negative Voges Proskauer and a negative methyl red, confirmed by repeated tests. A satisfactory explanation is not at present available but the matter is under investigation. They are included in this type since all their other reactions are similar.

Litmus Milk

Acid is formed after 24 hours in all eleven cultures. At the end of five days coagulation and reduction

are complete with one exception in which the milk is not coagulated but decolorized.

Gelatin Stab

No liquefaction.

Type V.

Twelve organisms of this type were obtained from the following samples:

March 2, 1923. Clay under snow at "outlook".

Capilano. Water from Creek below cook house and at bridge. Surface soil near road bed north of Sister's Creek. Deep soil from end of road.

Morphology

Short gram negative rods, non-motile, no spores or capsules.

Carbohydrates

Lactose becomes acid and reverts in 48 hours, remaining alkaline. Dextrose, maltose, mannite, xylose, arabinose, rhamnose, dulcite, salicin and sucrose are fermented to acid and a very small amount of gas. One culture did not ferment dulcite.

Methyl Red and Voges Proskauer Reactions

All cultures give a positive methyl red reaction and a negative Voges Proskauer reaction.

Litmus Milk

All cultures remain unchanged.

Gelatin

No liquefaction.

DIVISION II. BACTERIA NOT FERMENTING LACTOSE.

Type VI.

Ten cultures of this group were obtained from the following sources:

March 2, 1923. Moss from trail half way up clay bank. Water and sand from Canyon-view.

May 31, 1922. Moss at "outlook".

Capilano. Deep soil from end of road.

Morphology

Short gram negative rods without spores or capsules. Motility is variable.

Carbohydrates

Dextrose, maltose, mannite, xylose, arabinose, salicin and sucrose are fermented to acid and gas, a large quantity of gas being formed in each case. Rhamnose, dulcite and lactose give an alkaline reaction. One culture ferments rhamnose.

Methyl Red and Voges Proskauer Reactions

All give a negative methyl red and a positive