THESIS

A STUDY OF SOME BACTERIA ISOLATED FROM RETTED FLAX FIBER

Antoinette Trevithick

1924

A STUDY OF SOME BACTERIA ISOLATED FROM RETTED FLAX FIBER

A STUDY OF SOME BACTERIA ISOLATED FROM RETTED FLAX FIBER

Thesis

Submitted to the Faculty of the Michigan Agricultural College in partial fulfillment of the requirements for the degree of Master of Science.

By

Antoinette Trevithick

1924.

THESIS

A STUDY OF SOME BACTERIA ISOLATED FROM RETTED FLAX FIBER.

Table of Contents.

I - INTRODUCTION

Cotton fiber, which is superior to flax fiber in purity, has frequently been the subject of investigations the purpose of which has been to determine the factors causing disintegration in cases of wet storage. Much more favorable are the conditions for microbial growth in scutched and hackled flax fiber. The reason for this lies partly in the chemical composition and partly in the different anatomical structure of the two fibers.

Bast fiber, which is found in flax, has no protective cuticle, and in the central lamellae there are mineral substances, carbohydrates, fatty and waxy substances.(1).

Due to the biological process of decomposition by which fiber is retted there is naturally a large variety of organisms present on the surface of the fiber. Such organisms as Bacillus mesentericus, B. subtilis, B. comesii, Clostridium amylobacter, Granlobacter pectinovorum, B. aerogenes and many molds and yeasts have been pointed out as the chief causal agents in flax retting. At least it is definitely considered that retting is a biological process.

Since a flax fiber is made up of individual cells held together by pectic substances which are exceedingly hygroscopic (15, 14), the purest fiber will always maintain its great water-attracting power. Even with moderate moistening of scutched fiber which contains such an abundance of food material, it seems reasonable to believe that many

organisms develop on the fiber. Under storage conditions, aerobic organisms probably develop.

This project was undertaken primarily with a view to determine the type of aerobic bacterial flora existing on the scutched flax fiber.

II - HISTORICAL REVIEW OF LITERATURE

Very little work has been done with the organisms present on retted flax fiber; more work has been done from the flax retting standpoint. One would naturally expect some of the organisms which are present in the retting solution to be found on the retted fiber.

In 1885 Winogradsky (6) found an aerobic spore-former which was designated as the specific organism causing retting.

In 1902 Naumann (18) stated that many microorganisms, such as B. mesentericus, B. subtilis, Streptothrix sp., Pseudomonas fluorescens, could ret flax. Beijerinck and VanDelden (1903) also found another organism, Granulobacter pectinovorum.

Rossi (1916)(19) described the specific organism, B. semesii.

Tanner (1922)(5) found that the flora from retting vats varied, but spore-forming bacteria of both aerobic and anaerobic types were common. The aerobic types were similar to the members of the subtilis-mesentericus group; they formed large spreading colonies on solid media and liquefied gelatin very rapidly. He found the anaerobe Clostridium amylobacter to be the most specific in retting. He describes

this organism as follows:

(1) Vegetative Cells, grown on common media, large rods with dense protoplasm.

(2) Spores, larger than vegetative cells, clostridium shape.

(3) Fermentation Reaction, large amounts of gas formed in lactose, glucose, saccharose and glycerol.

(4) Litmus milk, curd peptonized.

(5) Gelatin, quickly liquefied at 20° C.

(6) Plain broth, rendered cloudy.

(7) Nitrates, reduced with the formation of nitrites and ammonia.

Ruschmann (1) studied the organisms on the fiber of the hackled flax by staining them on the fiber. He used a process that had as its basis the ordinary Gram stain. He found that when the fiber was retted by the steeping method the predominating organisms were Gram positive, for example, B. mesentericus, B. Asterosporus, B. megatherium, B. subtilis, the cocci and streptococci. By his Gram coloring method he found cocci, a "tender" narrow bacillus, small oval bacillus, robust long bacillus, spores, yeast-like organisms. He could not find B. amylobacter on the fiber. He recognized the fact that artificial drying was able to kill a number of living germs. He though the majority of coccus types were due to air contamination. He also worked with living organisms on the fiber and found that the aerobic organisms occurring most

frequently on the hackled flaxes, water-retted and artificially dried, were B. mesentericus and B. megatherium. The first would include B. mesentericus fuscus, B. mesentericus vulgatus, related or varieties. These, with B. megatherium were the only aerobic spore-bearers found. He found that the anaerobic retting stimulator (Clostridium amylobacter) adhered to the fiber in a living form in spite of artificial drying, but he did not find it on the fiber when he applied the modified Gram stain. The vegetative forms of organisms were more numerous on the fiber of a naturally dried, dew-retted flax. While the aerobic organisms predominate on this, the anaerobic and "potentially" anaerobic organisms were more numerous on the fiber of the artificially dried water-retted flax. Most of the organisms were capable of decomposing pectic substances. He also stated that cellulose consumers are usually found.

Makrinov (11) referred to a pectin-fermenting organism isolated from soil. He named this organism Pectinabacter amylophilum. This is a motile, aerobic rod, measuring 4 to 6 microns in length by 5 microns in diameter. During sporulation the microbe takes on a fusiform aspect and in the enlarged end of the rod an ellipsoidal spore is formed. The organism is Gram positive. In bouillon a slight turbidity is found and at the surface, large lamellae are present. Milk is coagulated On boiled potato, viscid grey-white colonies are formed with a strong production of gas. The optimum temperature is 30°to 35°C. Fermentation is most energetic on fresh potato, less energetic

on cooked potato where the starch is partly hydrolized, and feeble on beets, carrots and turnips, which have sugar instead of starch. He decided that this organism was an active agent in pectin fermentation (in flax retting) under aerobic conditions.

According to Gustaf Sellergren (5), Antonio Bozzocchi (2), Thatcher (14) and others, the fiber bundles are composed of cellulose and the cells are held together with a substance called pectin.

There has been other work on flax retting and pectin fermentation, but only of a general nature.

III METHOD OF INVESTIGATION AND RESULTS

1. Isolation of Organisms and Source.

During the summer of 1921 in connection with some experimental work on flax retting, a large number of organisms was isolated from water-retted flax fiber. This fiber had been scutched, that is, after having been through the breakers, the shives were removed from the fiber by means of rotating beaters. Chilean, Dutch, Irish, Courtrai, Michigan and Japanese retted fibers were used. From these fibers aerobic dilution plates were made in the following way. A small piece of fiber was taken from the center of the fiber bundle and placed in a flask of normal salt solution, allowed to stand for a short time, and then shaken well. From these flasks ordinary dilution plates were made, using nutrient agar, pH 6.8. These plates were incubated 48 hours at 25° C. and 30° C. Representative types of colonies were picked from these plates, transferred to agar slants, grown 24 hours at 30° C. From the 24 hour growth gram stains were made. If the cultures were not pure they were replated until pure, as shown by microscopical examination. By this method 91 cultures were isolated.

2. Cultural Studies on Standard Media.

After purifying, the cultures were grown on the various kinds of media used in identification. For this work the descriptive charts recommended by the Society of American Bacteriologists

were used. For methods of media-making the directions given by the Society of American Bacteriologists and Giltner's Manual were followed. For methods of making nutrient agar (using 3 gms. beef extract per liter), gelatin, glycerin potato, nutrient broth (using 3 gms beef extract per liter), dextrose, lactose, saccharose and glycerin broth and starch agar the reader is referred to these two manuals. All of the media were adjusted to pH 6.8, and brom cresol purple was used as indicator in the sugar broths. In all cases the cultures were set up four different times in duplicate, and in all characteristics as given they checked. For the last two determinations a new set of media was made. Uninoculated controls were run with each experiment and all tubes were incubated at 37°C. with the exception of gelatin plates and tubes which were incubated at 20°C.

Results.

After purification the cultures were given stock numbers as follows:

Stock No.	Source of Isolation	Stock No.	Source of Isolation
2	Chilean fiber	22	Chilean fiber
4	" "	23	" "
5	" "	26	" "
6	" "	31	Dutch fiber
10	" "	33	Iriah "
12	" "	34	" "
13	" "	35	" "
17	" "	37	" "
19	" "	38	" "

Stock No.	Source of Isolation	Stock No.	Source of Isolation
40	Irish fiber	76	Japanese fiber
41	" "	78	" "
42	" "	79	" "
46	Courtrai fiber	82	" "
68	Michigan "	83	" "
69	" "	85	" "
72	" "	90	Michigan fiber
74	Japanese fiber	91	Courtrai fiber

Those numbers which are missing in the numerical sequence of the previous table belong to organisms which are duplicates of some of those described.

The organisms in the following descriptive charts have been grouped as follows:

(a). Group I.- Those rod-shaped organisms which have eccentric spores which are not enlarged on sporulation.

(b). Group II. Those rod-shaped organisms which have eccentric spores and which are club-shaped on sporulation.

(c). Group III. Those rod-shaped organisms which have central spores and which are spindle-shaped on sporulation.

(d). Group IV. Small rod-shaped organisms which have central spores and which are not swollen on sporulation.

(e). Group V. Rod-shaped organisms without spores.

(f). Group VI. Coccus forms.

ORGANISMS STUDIED

Group I.

Organism No. 5.

I. Morphology

1. Vegetative cells:

(a) Form, medium rods, round ends.

(b) Limits of size, 3.5 x 1 micron - 5.5 x 1.5 microns.

2. Endospores:

(a) Position, slightly eccentric

(b) Form, elliptical

(c) Rod not swollen on sporulation

3. Motility, true

4. Staining, Gram positive

II. Cultural Characteristics:

1. Agar stroke

(a) Growth, abundant

(b) Form of growth, echinulate

(c) Elevation of grwoth, flat

(d) Luster, dull

(e) Topography, contoured

(f) Optical characteristics, opaque

2. Gelatin stab.

(a) Growth best at top

(b) Line of puncture, filiform

(c) Liquefaction, saccate to stratiform

3. Potato media

(a) Growth abundant

(b) Form of growth, spreading

(c) Luster, dull

(e) Topography, contoured, wrinkled

(f) Chromogenesis, greyed

(g) Potato, darkened

4. Nutrient broth

(a) Surface growth, none

(b) Clouding, moderate

(c) Sediment, viscid

5. Agar colonies

(a) Growth, rapid

(b) Form, circular; sub-surface, irregular

(c) Surface, rough

(d) Elevation, flat

(e) Edge, undulate

6. Gelatin colonies

(a) Growth, rapid

(b) Elevation, flat

(c) Edge, lobate

(d) Liquefaction, complete in 2 days

III. Physiological Features

1. Fermentation tubes, brom cresol purple plus

(a) Dextrose (Gas, none
(Reaction, acid

(b) Lactose (Gas, none
(Reaction, neutral

(c) Saccharose (Gas, none
(Reaction, neutral

(d) Glycerin (Gas, none
(Reaction, acid

2. Litmus Milk, peptonization in 2 days

3. Ammonia test, positive in 10 days (nitrate peptone)

4. Indol, positive

5. Diastatic action, positive in 4 days

6. Temperature relations

(a) Grows at room temperature

(b) Grows best at 30° C.

(c) Grows at 45° C.

Organism No. 41.

This organism compares well with No. 5 except as follows:

I. Morphology

1. Vegetative cells

(a) Same

(b) Size, 2 x 1 micron - 3 x 1 micron.

II. Cultural Characterisites

1. Agar stroke

(a) Form of Growth, spreading

(b) Luster, glistening to dull

III. Physiological Characteristics

1. Fermentation, same except

Glycerin (Gas, none
(Reaction, neutral

Organism No. 69.

Same as No. 41 except

II. Cultural Characteristics

1. Agar colonies

(a) Form of growth, surface-irregular.

III. Physiological Characteristics

1. Fermentation

Saccharose (Gas, none
(Reaction, acid

Organism No. 10.

I. Morphology

1. Vegetative cells

(a) Form, medium rods, round ends

(b) Size, 8.5 x 1 micron - 3 x 1.5 microns

2. Endospores

(a) Position, about central

(b) Most rods not swollen

3. Motility, true

4. Staining, Gram stain positive

II. Cultural Characteristics

1. Agar stroke

(a) Growth, moderate

(b) Form growth, filiform

(c) Elevation, flat

(d) Luster, glistening

(e) Optical characteristics, opaque

(f) Chromogenesis, cream

2. Gelatin stab

(a) Growth, uniform

(b) Form, filiform

(c) Liquefaction, saccate

3. Potato

(a) Moderate

(b) Spreading

(c) Color, tan

(d) Smooth

(e) Shiny

4. Nutrient broth

(a) Surface, ring

(b) Clouding, slight

(c) Sediment, none

5. Agar colonies

(a) Growth, rapid

(b) Form, surface, circular; subsurface, irregular

(c) Surface, smooth

(d) Elevation, raised

(e) Edge, entire

6. Gelatin colonies

(a) Growth, rapid

(b) Form, punctiform

(c) Elevation, flat

(d) Edge, entire

(e) Slight liquefaction.

III. Physiological Characteristics

1. Fermentation tubes, brom cresol purple plus

(a) Dextrose (Gas, none; Reaction, acid

(b) Lactose (Gas, none; Reaction, acid

(c) Saccharose (Gas, none; Reaction, acid

(d) Glycerin (Gas, none; Reaction, neutral

2. Litmus milk

(a) Slightly acid first day

(b) Smooth curd second day

3. Ammonia test, positive (nitrate peptone)

4. Indol test, positive

5. Diastatic action, positive

6. Temperature relations

(a) Grows at room temperature

(b) Grows best at 30° C.

(c) Grows at 45° C.

Organism No. 34.

I. Morphology

1. Vegetative cells

(a) Form, medium rods, round end, filaments

(b) Limits of size, 4 x 1 micron - 3 x 1 micron

2. Endospores

(a) Location, slightly eccentric

(b) Size, fills most of cell

(c) Not swollen

3. Motility, none

4. Staining, Gram positive

II. Cultural Characteristics

1. Agar stroke

(a) Growth, abundant

(b) Form, echinulate

(c) Elevation, flat

(d) Luster, glistening

(e) Topography, smooth

(f) Chromogenesis, cream

2. Gelatin stab

(a) Growth, uniform

(b) Line of puncture, filiform

(c) Liquefaction, stratiform

3. Potato

(a) Growth, abundant

(b) Color, faded red-brown

(c) Luster, dull

(d) Growth, like scum on potato

4. Nutrient broth

(a) Surface growth, ring

(b) Clouding, clear

(c) Sediment, flaky

5. Agar colonies

(a) Growth, rapid

(b) Form, surface, circular; subsurface, irreg., small

(c) Elevation, raised

(d) Edge, undulate

6. Gelatin colonies

(a) Growth, rapid

(b) Form, irregular

(c) Edge, undulate

(d) Liquefaction, complete in 2 days

III. Physiological Characteristics

1. Fermentation tubes, brom cresol purple plus

(a) Dextrose { Gas, none; Reaction, acid

(b) Lactose { Gas, none; Reaction, neutral

(c) Saccharose { Gas, none; Reaction, neutral

(d) Glycerin { Gas, none; Reaction, neutral

2. Litmus milk

(a) Peptonized in 2 days

3. Ammonia test, positive (nitrate pepton)

4. Diastatic action, positive in 4 days

5. Temperature relations

(a) Grows at room temperature

(b) Grows best at 30° C.

(c) Grows at 45° C.

6. Indol, negative

Organism No. 68

I. Morphology

1. Vegetative cells

(a) Form, medium rods, ends round

(b) Limits of size, 2 x 1 micron - 3 x 1 micron

2. Endospores

(a) Position, eccentric

(b) Form, elongated or elliptical

(c) Rods not swollen

3. Motility, true

4. Staining, Gram stain positive

II. Cultural Characteristics

1. Agar stroke

(a) Growth, moderate

(b) Form, filiform

(c) Elevation, flat

(d) Luster, glistening

(e) Topography, smooth

2. Gelatin stab

(a) Growth, uniform

(b) Liquefaction, saccate to stratiform

3. Potato

(a) Color, white

(b) Shiny to dull

(c) Potato brown

(d) Topography, smooth

(e) Raised

(f) Potato, not changed

4. Nutrient broth, slight growth

5. Agar colonies

(a) Growth, slow

(b) Form, circular

(c) Surface, smooth

(d) Elevation, flat

(e) Edge, entire

(f) Shiny, white

6. Gelatin colonies

(a) Small

(b) Complete liquefaction

III. Physiological Characteristics

1. Fermentation tubes, brom cresol purple plus

(a) Dextrose (Gas, none
(Reaction, acid

(b) Lactose (Gas, none
(Reaction, acid

(c) Saccharose (Gas, none
(Reaction, acid

(d) Glycerin (Gas, none
(Reaction, neutral

2. Litmus milk, reduction in 10 days

3. Ammonia test, negative (Nitrate peptone)

4. Diastatic reaction, negative

5. Temperature relations

(a) Grows at room temperature

(b) Grows best at 30° C.

GROUP II

Organism No. 23.

I Morphology

1. Vegetative cell

(a) Form, medium rods, round ends

(b) Limits of size, 2.5 x.5 microns - 3 x .5 micro

2. Endospores

(a) Position, eccentric

(b) Rods, slightly club-shaped.

3. Motility, true

4. Staining, Gram stain positive

II. Cultural Characteristics

1. Agar stroke

(a) Growth, scanty

(b) Form of growth, echinulate

(c) Elevation of growth, flat

(d) Topography, contoured to rugose

(e) Optical characteristic, opaque

(f) Chromogenesis, white

2. Gelatin stab

(a) Growth, uniform

(b) Liquefaction, crateriform to stratiform

3. Potato

(a) Growth, moderate

(b) Form of growth, chalky or lichen-like

(c) Luster, dull

(d) Topography, wrinkled

(e) Potato, grey

(f) Color of growth, grey to tan

4. Nutrient broth

(a) Surface growth, pellicle

(b) Clouding, slight

(c) Sediment, viscid

5. Agar colonies

(a) Form of growth, irregular

(b) Surface, smooth

(c) Elevation, flat

(d) Edge, undulate

6. Gelatin colonies

(a) Growth, slow

(b) Form, circular

(c) Elevation, flat

(d) Edge, entire

(e) Liquefaction, complete in 4 days

III. Physiological Characteristics

1. Fermentation tubes, brom cresol purple plus

(a) Dextrose (Gas, none / Reaction, acid

(b) Lactose (Gas, none / Reaction, neutral

(c) Saccharose (Gas, none / Reaction, acid

(d) Glycerin (Gas, none / Reaction, neutral

2. Litmus milk, peptonized

3. Ammonia tests, positive in 10 days (nitrate peptone)

4. Indol, positive

5. Diastatic action, positive in 4 days

6. Temperature relations

(a) Grows at room temperature

(b) Grows best at 30° C.

(c) Grows at 45° C.

Organism No. 3

I. Morphology

1. Vegetative cells

(a) Form, medium rods, round ends

(b) Size, 3.3 x 1 micron - 2.2 x .05 micron

2. Endospore

(a) Position, eccentric

(b) Form elliptical

(c) Club-shaped rods

3. Motility, true

4. Staining, Gram stain positive

II. Cultural Characteristics.

1. Agar stroke

(a) Growth, moderate

(b) Form, filiform to spreading

(c) Elevation, flat

(d) Luster, glistening

(e) Topography, smooth

(f) Chromogenesis, cream

2. Gelatin stab

(a) Growth, uniform

(b) Line of puncture, filiform

(c) Liquefaction, saccate

3. Potato, slight

4. Nutrient broth

(a) Surface growth, none

(b) Clouding, slight

(c) Sediment, viscid

5. Agar colonies

5.Agar colonies

(a) Growth, moderate

(b) Form, circular

(c) Surface, smooth

(d) Edge, entire

6. Gelatin colonies

(a) Small

(b) Slight liquefaction

III. Physical Characteristics

1. Fermentation tube, brom cresol purple plus

(a) Dextrose (Gas, none / Reaction, acid

(b) Lactose (Gas, none / Reaction, acid

(c) Saccharose (Gas, none / Reaction, acid

(d) Glycerin (Gas, none / Reaction, acid

2. Litmus milk

(a) Coagulation,in 7 days

(b) Reduction in 7 days

3. Indol, positive

4. Ammonia test, positive (nitrate peptone)

5. Temperature relations

(a) Grows at room temperature

(b) Grows best at 30° C.

Organism No. 40

I. Morphology

1. Vegetative cell

(a) Form, Slender rods

(b) Size, 2 x .5 microns

2. Endospore, no spore

3. Motility, slight

4. Staining, Gram negative

II. Cultural Characteristics

1. Agar stroke

(a) Growth, scanty

(b) Form of growth, filiform

(c) Elevation, flat

(d) Luster, glistening

(e) Chromogenesis, light yellow

2. Gelatin stab

(a) Growth, uniform

(b) Line of puncture, filiform

(c) Liquefaction, none

3. Potato

(a) Growth, moderate, thin

(b) Color, lemon-yellow

(c) Topography, smooth

(d) Glistening

4. Nutrient broth, slight growth

5. Agar colonies, small

6. Gelatin colonies

(a) Small

(b) Liquefaction, slight if any in 1 month

III. Physiological Characteristics

1. Fermentation tubes, brom cresol purple plus

(a) Dextrose (Gas, none
(Reaction, acid

(b) Lactose (Gas, none
(Reaction, acid

(c) Saccharose (Gas, none
(Reaction, acid

(d) Glycerin (Gas, none
(Reaction, acid

2. Litmus milk, reduction in 7 days

3. H_2S test, positive in 4 days

4. Ammonia test, positive in 4 days

5. Temperature relations

(a) Grows at room temperature

(b) Grows best at 30° C.

GROUP VI

Organism No. 82

I. Morphology

1. Vegetative cells

Form, cocci, small

2. Motility, none

3. Staining, Gram negative

II. Cultural Characteristics

1. Agar stroke

(a) Growth, moderate

(b) Form of growth, filiform

(c) Elevation, flat

(d) Luster, glistening

(e) Topography, smooth

(f) Color, white

2. Gelatin stab

(a) Growth, best at top

(b) Form of growth, filiform

(c) Liquefaction, none

3. Potato, slight if any growth

4. Agar colonies

(a) Growth, moderate

(b) Form, circular

(c) Surface, smooth

(d) Elevation, flat

(e) Topography, smooth

(f) Optical Characteristics, opaque

(g) Chromogenesis, white

5. Gelatin stab

(a) Growth, best at top

(b) Line of puncture, filiform

(c) Liquefaction, none

6. Potato, slight if any growth

7. Agar colonies

(a) Growth, moderate

(b) Form, circular

(c) Surface, smooth

(d) Edge, entire

8. Gelatin colonies

(a) Growth, moderate

(b) Small

(c) Partial liquefaction in 7 days, complete in 1 month

III. Physiological Characteristics

1. Fermentation tubes, brom cresol purple plus

(a) Dextrose (Gas, none / Reaction, acid

(b) Lactose (Gas, none / Reaction, acid

(c) Saccharose (Gas, none / Reaction, acid

(d) Glycerin (Gas, none / Reaction, acid

2. Litmus milk

(a) Acid in 7 days

(b) Coagulation in 7 days

3. Ammonia test, positive (nitrate peptone)

4. Temperature relations

(a) Grows at room temperature

(b) Grows best at 30° C.

Organism No. 90

I. Morphology

1. Vegetative cell

Form, cocci, small

2. Motility, slight if any

3. Staining, Gram negative

II. Cultural Characteristics

1. Agar stroke

(a) Growth, moderate

(b) Form, filiform

(c) Elevation, flat

(d) Luster, glistening

(e) Chromogenesis, cream to white.

2. Gelatin

(a) Growth, uniform

(b) Line of puncture, filiform

(c) Liquefaction, none

3. Potato

(a) Growth, moderate

(b) Topography, smooth

(c) Color, cream white

(d) Luster, glistening

(e) Potato, not changed

4. Agar colonies

(a) Moderate growth

(b) Medium size

5. Gelatin colonies

(a) Small colonies

(b) No liquefaction in 1 month

III. Physiological Characteristics

1. Fermentation tubes, brom cresol purple plus

(a) Dextrose { Gas, none; Reaction, acid

(b) Lactose { Gas, none; Reaction, acid

(c) Saccharose { Gas, none; Reaction, acid

2. Ammonia test, positive (nitrate peptone)

3. Temperature relations

(a) Grows at room temperature

(b) Grows best at 30° C.

Organism No. 91

I. Morphology

1. Vegetative cells

(a) Form, small short rods

(b) Size, 2 x 1.5 microns

2. Endospores, probably central

3. Motility, non-motile

4. Stain, Gram negative

5. Agar stroke

(a) Growth, moderate

(b) Form of growth, filiform

(c) Elevation, flat

(d) Luster, glistening

(e) Topography, smooth

(f) Chromogenesis, white

6. Gelatin stab

(a) Line of luncture, filiform

(b) Liquefaction, crateriform to stratiform

7. Potato

(a) Growth, smooth

(b) Color, cream

(c) Luster, glistening

(d) Potato, not changed

8. Nutrient broth, slight growth

9. Agar colonies, small white

10. Gelatin colonies

(a) Very small

(b) Complete liquefaction in 1 month

III. Physiological Characteristics

1. Fermentation tubes, brom cresol purple plus

(a) Dextrose (Gas, none / Reaction, acid

(b) Lactose (Gas, none / Reaction, acid

(c) Saccharose (Gas, none / Reaction, acid

(d) Glycerin (Gas, none / Reaction, neutral

2. Litmus milk

(a) acid

(b) Coagulation

3. Ammonia test, positive (nitrate peptone)

4. Temperature relations

(a) Grows at room temperature

(b) Grows best at 30° C.

Organism No. 76

I. Morphology

1. Vegetative cells, cocci, medium

2. Motility, non-motile

3. Staining Gram stain positive

II. Cultural Characteristics

1. Agar stroke

(a) Growth, moderate

(b) Form, filiform

(c) Elevation, flat

(d) Topography, smooth

(e) Chromogenesis, cream

2. Gelatin stab

(a) Growth, uniform

(b) Form, filiform

(c) Liquefaction, saccate

3. Potato

(a) Growth, moderate

(b) Color, light yellow

(c) mealy

4. Nutrient broth

(a) moderate

(b) Slightly cloudy

5. Agar colonies

(a) Growth, moderate

(b) Form, circular

(c) Surface, smooth

(d) Elevation, flat

(e) Edge, entire

(f) Luster, dull

6. Gelatin colonies

(a) Small

(b) Partial liquefaction in 7 days

(c) Complete liquefaction in 1 month

III. Physiological Characteristics

1. Fermentation tubes, bron cresol purple plus

(a) Dextrose (Gas, none
(Reaction, abid

(b) Lactose (Gas, none
(Reaction, acid

(c) Saccharose (Gas, none
(Reaction, acid

(d) Glycerin (Gas, none
(Reaction, neutral

3. Litmus milk

(a) Acid in 4 days

(b) Coagulation in 7 days

3. Ammonia test, positive (nitrate peptone)

4. Temperature relations

(a) Grows at room temperature

(b) Grows best at 50° C.

Organism No. 74.

I. Morphology

1. Vegetative cells

(a) Form, cocci

(b) Size, 1 x 1.5 microns

2. Motility, non-motile

II. Cultural Characteristics

1. Agar stroke

(a) Growth, moderate

(b) Form, filiform

(c) Elevation, flat

(d) Luster, glistening

(e) Chromogenesis, white

2. Gelatin

(a) Growth best at top

(b) Form, filiform

(c) Liquefaction, saccate in 1 month

3. Potato

(a) Growth, moderate

(b) Topography, smooth

(c) Color, cream

(d) Potato, dark grey

(e) Dull to glistening

4. Agar colonies

(a) Growth, moderate

(b) Form, circular

(c) Surface, smooth

(d) Elevation, raised

(e) Edge, entire

5. Gelatin colonies

(a) Growth, slow

(b) Small

(c) Partial liquefaction in 1 month

III. Physiological Characteristics

1. Fermentation tubes, brom cresol purple plus

(a) Dextrose (Gas, none
(Reaction, acid

(b) Lactose (Gas, none
(Reaction, acid

(c) Saccharose (Gas, none
(Reaction, acid

(d) Glycerin (Gas, none
(Reaction, neutral

2. Ammonia test, positive (nitrate peptone)

3. Temperature relations

(a) Grows at room temperature

(b) Grows best at 30 ° C.

III. Physiological Characteristics

1. Fermentation tubes, brom cresol purple plus

(a) Dextrose (Gas, none
(Reaction, acid

(b) Lactose (Gas, none
(Reaction, acid

(c) Saccharose (Gas, none
(Reaction, acid

(d) Glycerin (Gas, none
(Reaction, acid

2. Litmus milk, reduction in 7 days

3. H_2S test, positive in 4 days

4. Ammonia test, positive in 4 days

5. Temperature relations

(a) Grows at room temperature

(b) Grows best at 30° C.

GROUP VI

Organism No. 82

I. Morphology

1. Vegetative cells

Form, cocci, small

2. Motility, none

3. Staining, Gram negative

II. Cultural Characteristics

1. Agar stroke

(a) Growth, moderate

(b) Form of growth, filiform

(c) Elevation, flat

(d) Luster, glistening

(e) Topography, smooth

(f) Color, white

2. Gelatin stab

(a) Growth, best at top

(b) Form of growth, filiform

(c) Liquefaction, none

3. Potato, slight if any growth

4. Agar colonies

(a) Growth, moderate

(b) Form, circular

(c) Surface, smooth

(d) Elevation, flat

(e) Topography, smooth

(f) Optical Characteristics, opaque

(g) Chromogenesis, white

5. Gelatin stab

(a) Growth, best at top

(b) Line of puncture, filiform

(c) Liquefaction, none

6. Potato, slight if any growth

7. Agar colonies

(a) Growth, moderate

(b) Form, circular

(c) Surface, smooth

(d) Edge, entire

8. Gelatin colonies

(a) Growth, moderate

(b) Small

(c) Partial liquefaction in 7 days, complete in 1 month

III. Physiological Characteristics

1. Fermentation tubes, brom cresol purple plus

(a) Dextrose (Gas, none
(Reaction, acid

(b) Lactose (Gas, none
(Reaction, acid

(c) Saccharose (Gas, none
(Reaction, acid

(d) Glycerin (Gas, none
(Reaction, acid

2. Litmus milk

(a) Acid in 7 days

(b) Coagulation in 7 days

3. Ammonia test, positive (nitrate peptone)

4. Temperature relations

(a) Grows at room temperature

(b) Grows best at 30° C.

Organism No. 90

I. Morphology

1. Vegetative cell

Form, cocci, small

2. Motility, slight if any

3. Staining, Gram negative

II. Cultural Characteristics

1. Agar stroke

(a) Growth, moderate

(b) Form, filiform

(c) Elevation, flat

(d) Luster, glistening

(e) Chromogenesis, cream to white.

2. Gelatin

(a) Growth, uniform

(b) Line of puncture, filiform

(c) Liquefaction, none

3. Potato

(a) Growth, moderate

(b) Topography, smooth

(c) Color, cream white

(d) Luster, glistening

(e) Potato, not changed

4. Agar colonies

(a) Moderate growth

(b) Medium size

5. Gelatin colonies

(a) Small colonies

(b) No liquefaction in 1 month

III. Physiological Characteristics

1. Fermentation tubes, brom cresol purple plus

(a) Dextrose { Gas, none; Reaction, acid

(b) Lactose { Gas, none; Reaction, acid

(c) Saccharose { Gas, none; Reaction, acid

2. Ammonia test, positive (nitrate peptone)

3. Temperature relations

(a) Grows at room temperature

(b) Grows best at 30° C.

Organism No. 79

I. Morphology

1. Vegetative cells, large cocci

2. Motility, slight if any

3. Staining reaction, Gram stain negative

II. Cultural Characteristics

1. Agar stroke

(a) Growth, moderate

(b) Form, filiform

(c) Luster, glistening

(d) Topography, smooth

(e) Chromogenesis, cream

(f) Edge, echinulate

2. Gelatin

(a) Growth, best at top

(b) Form, filiform

(c) Liquefaction, none

Stratiform in 1 month

3. Potato

(a) Growth, moderate, thin

(b) Luster, dull

(c) Dry

(d) Topography, smooth

(e) Color, yellowish

4. Gelatin colonies

(a) Small

(b) Partial liquefaction in 7 days

(c) Almost complete in 1 month

III. Physiological Characteristics

1. Fermentation tubes, brom cresol purple plus

(a) Dextrose { Gas, none / Reaction, acid

(b) Lactose { Gas, none / Reaction, acid

(c) Saccharose { Gas, none / Reaction, acid

(d) Glycerin { Gas, none / Reaction, neutral

2. Litmus milk

(a) acid

(b) Coagulation

3. Ammonia test, positive (nitrate peptone)

4. Temperature relations

(a) Grows at room temperature

(b) Grows best at 30° C.

Organism No. 76

I. Morphology

1. Vegetative cells, cocci, medium

2. Motility, non-motile

3. Staining Gram stain positive

II. Cultural Characteristics

1. Agar Stroke

(a) Growth, moderate

(b) Form, filiform

(c) Elevation, flat

(d) Topography, smooth

(e) Chromogenesis, cream

2. Gelatin stab

(a) Growth, uniform

(b) Form, filiform

(c) Liquefaction, saccate

3. Potato

(a) Slightly spreading

(b) Creamy

(c) Normal potato

4. Nutrient broth

(a) Clouding, slight

(b) Sediment, viscid

5. Agar colonies

(a) Growth, rapid

(b) Form, circular

(c) Surface, smooth

(d) Elevation, raised

(e) Edge, entire

6. Gelatin colonies

(a) Growth, rapid

(b) Form, irregular

(c) Elevation, flat

(d) Edge, undulate

III. Physiological Characteristics

1. Fermentation

(a) Dextrose (Gas, none
(Reaction, acid

(b) Lactose (Gas, none
(Reaction, neutral

(c) Saccharose (Gas, none
(Reaction, acid

(d) Glycerin (Gas, none
(Reaction, acid

2. Litmus milk

(a) Coagulation, smooth in 9 days

(b) Reduction in 3 days

3. Indol, positive

4. Ammonia test, positive

5. Diastatic action, positive

6. Temperature relations

(a) Grows at room temperature

(b) Grows best at 30° C.

(c) Grows at 45° C.

Organism No. 21

This organism is similar to No. 17 except as follows:

I. Morphology

1. Motility, non-motile

II. Physiological Characteristics

1. Fermentation

(a) Dextrose (Gas, none
(Reaction, acid

(b) Lactose (Gas, none
(Reaction, acid

(c) Saccharose (Gas, none
(Reaction, acid

(d) Glycerin (Gas, none
(Reaction, neutral

Organism No. 85

This organism died before I had completed my study; therefore I could not check my results.

I. Morphology - as follows:

(a) Short thick rods

(b) Size 1 x 1.5 - 2 x 1 micron

(c) True motility

(d) Gram positive

3. Cellulose Decomposition.

All of the organisms were tested for cellulose decomposition in the following way:

Strips of filter paper were cut to fit into test tubes and these tubes were filled with Dunham's solution and sterilized. Two tubes were inoculated with each culture; one was left and called aerobic (conditions similar to those in retting); the other tube had sterile paraffin oil put on top of the liquid (anaerobic). Tubes with controls were placed at 30° C. for four months. From all cultures, both aerobic and anaerobic (when paper was tested with a platinum needle), the results were negative.

4. Cultural Studies on Synthetic Media.

Some of the typical organisms were tried on various kinds of synthetic media. In the first place the kinds of media listed in the Table of Contents were used in liquid form but it was impossible to tell anything about growth, due to sediment of chemicals and pectin, even when smears were made. These media were made as follows:

(a) Uschinsky's Asparagin medium.

1000 c.c. Uschinsky's Asparagin medium as given in Giltner's Manual was used as the basic substance, and 1.5 percent fermented agar was added. When this solution was

divided into four parts and made into the following:

1. Uschinsky's Asparagin medium plus agar pH 7.2.

2. Uschinsky's Asparagin medium plus agar pH 7.2 plus 1 percent pectin.

3. Uschinsky's Asparagin medium plus agar (natural pH).

4. Uschinsky's Asparagin medium plus agar (natural pH) plus 1 percent pectin.

This gives four different combinations from this one medium. The pectin used was a German product, probably "free pectin".

(b) Conn's solution as given in Giltner's Manual was made up as above, but due to the great amount of precipitate when NaOH was added, the medium was adjusted to pH 6.8.

(c) Winogradsky's medium for symbiotic nitrogen fixation as given in Giltner's Manual was made up into four combinations as described previously and half of it adjusted to pH 7.2.

(d) Conn's Asparaginate agar.

Agar	30 gms.
Sodium asparaginate	1 gm.
Monobasic ammonium phosphate ($NH_4H_2PO_4$)	1.5 gm.
$CaCl_2$	0.1 gm.
$MgSO_4$	0.2 gm.
KCl	0.1 gm.
$FeCl_3$	trace
H_2O	1000 c.c.

Half of this was adjusted to pH 6.8 and the other half to pH 7.2+; to this last was added 5 gms. of pectin. The agar was washed in distilled water and allowed st stand over night.

(e) Conn's agar for actinomycetes

Agar	30 gms.
Glycerin	10 gms.
Sodium asparaginate	1.0 gm..
Monobasic ammonium (hydrogen) phosphate	1.5 gm.
$MgSO_4$	0.2 gm.
Calcium Chloride	0.1 gm.
Ferric Chloride	trace

Half of this was adjusted to pH 6.8, the other half to pH 7.2; to this last was added 5 gms. of pectin.

(f) Carrot-juice agar.

Carrots were cleaned and prepared, cut into small pieces and cooked in water as follows: 250 gms. of raw carrot plus 300 c.c. of distilled water. This was added to a half-liter of 3 percent fermented agar plus 5 gms. of peptone and 2 1/2 gms of salt, and adjusted to pH 7.0

Results.

It was found that in some cased when pectin was added the medium would not solidify. This was probably due to the acid formed when the pectin was sterilized. Therefore, I had the following solid media to work with:

Synthetic Media.

A - Nutrient agar.

I - Carrot agar.

II - Conn's agar for actinomycetes, pH 7.2, plus pectin.

III - Conn's agar for actinomycetes, pH 6.8.

IV - Conn's asparaginate agar, pH 7.2, plus pectin.

V - Conn's asparaginate agar, pH 7.2.

VI - John's Solution, pH 6.8, plus pectin.

VII - John's Solution, pH 6.8.

VIII - John's Solution, pH 5.8.

IX - Jschinsky's asparagin agar, pH 7.2.

X - Winogradsky's medium, pH 6.4.

XI - Winogradsky's medium, pH 7.2.

Not having time to work with all of the organisms, I tried to pick out six of the most typical rod-shaped organisms and I grew these on the above media for 24 hours at 30° C. The results are shown in Table 1.

TABLE I.

Growth of Six Organisms on Synthetic Media.

Types of Media	Growth of organisms No. 61			Growth of organism No. 58			Growth of organism No. 35			Growth of organism No. 6			Growth of organism No. 5			Growth of organism No. 2			Uninoculated controls		
	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3
Nutrient agar	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	-	-	-
Carrot agar	+	++	++	+	++	++	±	++	++	+	++	++	+++	+++	+++	+	++	+	-	-	-
II	++	++		++	++	++	±	++	++	++	++		+++	+++		++	++		-	-	
III	++	++	++	++	++	++	++	++	++	++	++	++	+++	+++	++	++	++	+	-	-	-
IV	+	+	±	++	++	++	+	++	++	++	++	++	++	++	++	+	++	++	-	-	-
V	++	++	++	++	++	+	+	++	++	++	++	++	++	++	++	+	++	++	-	-	-
VI	+	+	±	+	±	±	+	+	+	+	++	±	++	++	++	±	+	+	-	-	-
VII	++	+	++	++	+	+	++	+	±	+	++	++	++	++	++	++	++	++	-	-	-
VIII	+	++	++	++	++	++	++	++	+	±	++	++	+	+	++	+	+	+	-	-	
IX	++	++		++	++		++	++		++	++		+	+		+	+		-	-	-
X	+	+	++	+	+	++	++	+	+	++	++	++	+	+	++	+	+	±	-	-	-
XI	++	++	++	++	++	++	++	++	++	++	+	++	+	+	++	+	+	+	-	-	-

+++ = abundant growth

++ = moderate growth

\+ = slight growth

± = very slight growth

\- = no growth

IV DISCUSSION OF RESULTS

The scope of this project was limited, due to the inadequate knowledge available on the cultural characteristics of the organisms present on flax fiber. Had more been known about this subject, different media might have been used in isolating the organisms and in that way more groups might have been added to the collection.

The organisms studied have been divided into six groups as follows:

Group I included organisms Nos. 5, 41, 69, 10, 34 and 68. Organisms 5 and 10 were isolated from Chilean fiber, 34 and 41 from Irish fiber and 68 and 69 from Michigan fiber. This group contains organisms which are medium sized rods with rounded ends, measuring about 3 x 1 microns, having slightly eccentric spores which do not affect the shape of the rods. They are all Gram positive. All but 34 are very motile, and all of them liquefy gelatin. They grow very well on potato media, and vary as to action on milk and sugar broths. All of them attack starch.

In this work an attempt was made to name these organisms with the aid of Bergey's (16) and Chester's (32) Manuals. It was found, however, that the descriptions were too meager. It was also found that very little work has been done with aerobic spore-bearing organisms. Therefore it was impossible definitely to name any of the organisms isolated. Numbers 68, 5, 41 and 69

are similar to B. megatherium (Bergey) except that they are not so large. These characteristics throw this group into the mesentericus-megatherium group (16).

Group II includes Nos. 23, 2, 38, 33 and 13. Organisms 23, 2 and 13 were isolated from Chilean fiber, and 33 and 38 from Irish fiber. This group contains organisms which are medium sized rods with rounded ends, measuring about 2.5 x 5 microns, and having eccentric spores which cause the rods to appear club-shaped. They are Gram positive and in all cases there is true motility. Gelatin is liquefied; growth on potato is moderately abundant; the organisms vary as to action on milk and sugar broths; they all attack starch.

It is hard to decide just where such organisms as Nos. 2, 23, 38 and 13 belong because the shape of the sporulating rod is not definite. In these cases the organisms seem to be pleomorphic (21). Numbers 2, 23 and 38 vary from rods not swollen to slightly club-shaped rods, which would place these organisms either in Group II, or the megatherium-mesentericus group (Bergey) (16). Number 13 varies from spindle-shaped to club-shaped. If they are considered club-shaped, they fall into the group which, according to Bergey (16) included B. pseudotetanicus, B. terminalis and B. macerans. They would fall into Group VII (Laubach, Rice and Ford) (20), the round, terminal-spored group or into Group VIII (Laubach, Rice and Ford), the cylindrical, terminal-spored group, or according to the Chester classification they would fall into Class XIX, the tetanus type, which included B. sublanatus and B. putrificus.

Group III includes Nos. 6, 4 and 35. Organisms 6 and 4 were isolated from Chilean fiber, and 35 from Irish fiber. The organisms of this group are medium sized rods with rounded ends, measuring about 3.5 x 1.5 microns, having centrally located spores. Some of the spore-bearing rods are spindle-shaped. They are all Gram positive and have true motility; they liquefy gelatin; there is little if any growth on potato; milk is peptonized by all: there is a variation in their sugar reactions: they give a positive diastatic test. Due to the pleomorphic characteristics of these organisms, as in Group II, their identity is doubtful. Due to the close resemblence to mycoides and subtilis they seem to belong to this group (16).

Group IV includes Nos. 91, 78 and 72. Organism 91 was isolated from Courtrai fiber, 72 from Michigan fiber and 78 from Japanese fiber. This group contains organisms which are small rods measuring about 2 x 1.5 microns. They have spores which are very small and centrally located. Numbers 91 and 78 are Gram negative and 72 is Gram positive. All of the organisms in this group except 72 are non-motile; gelatin is liquefied by 91 and 78 but not by 72; the organisms grow very well on potato; they vary as to reactions on litmus milk and sugar broths. These seem to belong to the group having B. subtilis as type species (16), which places 72 in the aerobic motile group and 91 and 78 in the aerobic non-motile group.

Group V consists of one organism, No. 40, which was isolated from Irish fiber. It is a slender rod with round ends, measuring about 2 x 5 microns. It is not a spore-producer, is only slightly motile and is Gram negative. It does not liquefy

gelatin and grows well on potato. It reduces litmus milk and produces acid in dextrose, lactose, saccharose and glycerin broths.

This organism belongs to the Family Bacteriaceae (16), Tribe Chromobactereae and Genus Flavobacterium, but due to lack of description it was impossible to trace this organism further.

Group VI includes Nos. 82, 90, 79, 76, 74, 19, 12, 26 and 17. Numbers 12, 19, 17 and 26 were isolated from Chilean fiber, 74, 76, 79 and 82 from Japanese fiber, and 90 from Michigan fiber. This group contains all of the coccus forms. Organisms 82, 76, 26 and 17 are Gram positive, while 90, 79 and 12 are Gram negative. Numbers 72, 90, 26, 17 and 21 do not liquefy gelatin, while 82, 79, 76, 74, 19 and 12 do. These organisms vary in action on litmus milk and sugar broths. They all grow well on potato except 19.

It was found that none of the organisms tested would decompose cellulose by the method used.

All of the organisms grow well on synthetic media as shown in Table I.

V SUMMARY

1. The predominating organisms found on flax fiber were Gram positive, spore-bearing rods. Some coccus forms were also present.

2. The subtilis, mycoides and mesentericus-like organisms predominated.

3. They attack the common carbohydrates and most of them attack starch.

4. Clostridium-shaped organisms were found which may be the same as Kuschmann's "potentially anaerobic" forms.

The writer wishes to acknowledge her indebtedness to Dr. Ward Giltner, Mr. R. L. Tweed, Mr. F. W. Fabian and Mr. G. L. A. Ruehle for the assistance and helpful suggestions received during the course of these investigations.

BIBLIOGRAPHY.

(1)* Ruschmann, G.
Entwertung der Schwungflachses durch Mikroorganism.
Translated by Edwin Leydel.
Faserforschung, Vol. III, No. 2, 1923.

(2) Bozzocchi, Antonio
Sulla Macerazione Industriale Dell Peante Lissili.
Translated by Violet Kelk.

(3) Tanner, Fred
Microbiology of Flax retting.
Bot. Gazette, October 1922, Vol. LXIV.

(4)* Muller, W., and F. Tobler
Retting Bacteria: Mode of Penetration.
Faserforschung, 1922, No. 2, 21-28. Translation.

(5)* Bellergren, Justaf
Native Fiber Plants. Translation.

(6)* Winogradsky, Sergius
Sur le rouissage du lin et son agent microbien.
Comptes Rendus de L'Acad. Des Sci. de France,
November 1895, p. 742. Translated by R. L. Davis.

(7)* Tadokoro, T.
Studies on Flax Retting.
Reprinted from the Jour. of the College of Agr.,
Tohokii Imperial University, Sapporo, Japan.
Vol. V, part II, March 1913.

(8) Clayson, Donald, Frederick Norris and Samuel Schryver.
The Pectic Substances of Plants.
Biochem. Jour. Vol. XV, part II, No. 5, p. 41.

(9) Schmidt, E. G., W. H. Peterson and F. B. Fred.
Destruction of Pentosans by Molds and Other
Microorganisms.
Soil Science Vol. XV, June 1923.

(10) Laubach, G. A., J. L. Rice and W. W. Fred.
Studies of Aerobic Spore-Bearing Non-Pathogenic
Bacteria.
Jour. of Bact., part I, May 1916, Vol. III.,
part II, Sept. 1916, Vol. I, No. 5

(11)* Makrinov, I. A.
Sur un Nouveau Microorganime la Fermentation de
l'Amidon et des Matieres Pectiques.
Translated by R. L. Davis.
Arch. des Sci. Biol., Vol. XVIII, 1915, p. 440.

* = not verified by the writer, simply complete translations read.

(12) Sucharipe, Rudolph
Protopectin and Some Other Constituents of Lemon Peel.
Jour. of American Chem. Soc., Vol. XLVI, Jan. 1924,
No. 1.

(13)
Notes on Hydrolysis of Pectin.
Biochem. Jour. 17, 83(1923), No. 1.

(14) Thatcher
Chemistry of Plant Life, p. 20-31, 67, 70.

(15) Willaman, J. J.
Pectin Relations of Schrotinia Cineres.
Bot. Gaz. Vol. LXX, p. 221.

(16) Bergey
Manual of Determinative Bacteriology.

(17)* Behrens, J.
Retting Bacteria Organisms.
Cent. f. Bakt. Par. Inf. II Abt., Bd. X, 1903,
No. 16/17, pp. 524-530.

(18) Rossi, G.
Industrial Retting of Textile Plants by
Microbiological Action.
International Review of Science and Practise of
Agriculture, Year VII, No. 8, August 1916.

(19) Giltner, Ward,
Laboratory Manual in General Microbiology.

(20) American Society of Bacteriologists
Manual of Methods for Pure Culture Study of Bacteria.

(21) Löhnis, F. and N. R. Smith
Studies upon the Life Cycles of the Bacteria.
Part II Life History of Azotobacter,
Reprint from Jour. of Agr. Res.

(22) Chester, Frederick D.
A Manual of Determinative Bacteriology.

* = not verified by the writer, simply complete translations read.