THESIS.

on

.....BACTERIA IN MILK.....

Submitted to the faculty of the

OREGON AGRICULTURAL COLLEGE.

for the degree of

BACHELOR OF SCIENCE

in

DOMESTIC SCIENCE AND ART.

by

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APPROVED:

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INTRODUCTION.

Bacteriology is one of the sciences, only recently developed. The objects with which it deals are so small that they cannot be seen by the naked eye; and the results of their activity are so common, as for instance decay, souring of milk and disease, that they were looked upon as natural and no cause was ever dreamed of.

The first microscope was invented by Hans and Zacharius Janson in 1590; but bacteria were not discovered until some time later and then by the use of a simple convex lens. The discovery was made by a man, now called the "father of microscopy", Antony Van Leuwenhock of Delft, Holland, who was an apprentice to a cloth merchant and had for this purpose learned lens grinding. Van Leuwenhock's work was interrupted by the advent of the bubonic plague through Europe; but the application of his discovery to disease was made only by his followers. In 1762 Plenciz carried the work along the common lines of decomposition again but the progress of the work was again side-tracked by that famous discussion which lasted a hundred years from 1750 to 1850, when the theory of "spontaneous generation" of life was advanced. Disproving this theory, Spallanzani showed that by prolonged boiling and exclusion of air, a meat infusion could be kept from putrefying.

In the 18th century the science made a decided advance. The microscope was improved, the cause of blue milk

and the connection between bacteria and disease were discovered. The name, bacteria, was not applied to the organisms until 1817 when Hoffman, a botanist, showed that bacteria were a separate group of plants from the yeasts and animalcules and applied the Greek name Ba´K T por, meaning, "little stick" to them. The first classification of bacteria was made about 1786. Pasteur proved that the souring of milk was due to bacteria by the ordinary processes of reproduction and growth. He also proved that specific organisms caused specific diseases and he did the greatest work in interesting the world in this important Science.

Bonnet, in 1762, originated the theory that bacteria might have eggs in order to account for irregularities in sterilizing. Schröder and Dusch first used cotton wool for filtering air in bacteriological experiments.

Although, about 1860, Pasteur showed a close connection between bacteria and the souring of milk, it was not until some twenty years ago that they were found to have a very close relation to dairying, by Müller of Copenhagen, who divided them into two classes "monas" and "vibrio". Further classification was made by Ehrenberg, in 1833, who with the aid of a better microscope divided them into four classes, "bacterium", "vibrio", "sprillum" and "spirochaeta".

Bacteriology is such a broad subject that a life-time spent in its perusal would fail of an entire knowledge of the subject, so the authors have chosen a field which is

certainly one that ought to interest every individual because it pertains to milk, one of the most important foods of the world. The advancement in decreasing the death-rate of infants in the large cities by scientific methods of handling milk is worthy of attention; and it is the purpose of this thesis to help in the work of disseminating the knowledge of these methods throughout the country and especially the dairy districts of Oregon. The samples of milk tested were taken from the mixed milk of dairies and the milk of private cows in and about Corvallis, Oregon. Those presented here will probably be found most common to all milk.

BODY OF THESIS.

Bacteria are unicellular plants of microscopic dimensions multiplying by fission and commonly found to be devoid of chlorophyl. Some idea of the minuteness of bacteria may be gained from the fact that there may easily be more than one billion in a single drop of milk. The average diameter of a transverse section is .001 m. m. or 1 micro millimetre. The forms of bacteria have been compared to lead-pencils, billiard-balls and cork-screws. Bacteria consist of an internal part or protoplasm surrounded by a cell wall or membrane. Many bacteria are motionless but some move freely about by means of thread like flagella and are thus enabled to come in contact with their food. Bacteria reproduce by means of fission but some have the power to produce spores. The spores however are not a means of reproduction but a means of resisting adverse conditions. Many spores require a temperature of 140° dry heat for fifteen minutes to destroy them. A temperature of from 10° C. to 40° C. is most conducive to their growth and for most bacteria a temperature of 100° C. is destructive. Although at the moment of secretion, milk from a healthy udder is said to be absolutely free from bacteria, it is a very difficult problem to prove it. About thirty years ago experiments were made with some success to prove that milk drawn with special precautions into sterilized vessels was practically free from bacteria. However, in recent years, even with all our improvements

in aseptic precautions, it has been impossible to draw any quantity of milk which was free from bacteria.

While the milk is in the glands, it is free from bacteria. This has been proven by making a bacteriological examination of the udder directly after the animal has been slain.

One of the sources of contamination is from the diseased udders. It has been found that the tubercle bacillus sometimes locates itself in the mammary glands and causes infection. Many times, however, the lesion may be so slight that there is no external indication. The milk secreted under these conditions is laden with bacteria. The question is often raised as to whether, from a cow suffering from a bacterial disease, the bacteria do not find their way into the milk. Conn says, "Bacteria press through the gland only when it is itself the seat of the inflammatory process."

Yet a more common source of contamination of the milk while yet in the udder is the entrance of bacteria from the outside through the teats. When this occurs, as naturally would be supposed, the milk drawn first contains the greatest number of organisms, while the last will be almost sterile. Thus the practice of not using the first few jets of milk is justified. The most peculiar occurrence connected with the bacteria present in the milk-ducts is the constancy of their numbers, though the time of milking may differ widely thus, although milk is not drawn

for several days, no bacterial change, as souring, will occur in the udder. This indicates that there is some check to their growth, most probably, the germicidal properties of the udder fluid. Another circumstance worthy of notice is that although the lactic acid bacteria are normally found in comparatively large numbers shortly after milking, they are very seldom found in the ducts. This shows that they are an exterior contamination. This brings us to the numerous causes of contaminations which take place after the milk is drawn; the cow, the air of the stable, the milker and the milking utensils themselves. Here, again the cow is an important factor. Even under the best conditions, dust will be present on the cow's body and in moving about, loose hairs and dust may fall into the milk. The likelihood of contamination is much augmented if the cow is not kept in the best condition. If hay is distributed during or just before milking, the air may be filled with myriads of dust particles and with it the bacteria which have previously grown upon the hay. The milker, although it is unnecessary, is often a source of contamination. His hands and clothing may carry bacteria and many times of a pathogenic type. It is usually by this means and by the water in which the milk vessels are washed that such a disease as typhoid fever is spread. Sedgwick and Batchelder found on an average, "that milk obtained in a clean stable, from a well kept cow, by milking in the usual way into a sterilized bottle, con-

tained 530 bacteria per cubic centimetre". "When, however, the milk man used the ordinary milk pail of flaring form, seated himself with more or less disturbance of bedding, and vigorously shook the udder over the pail during the usual process of milking, the numbers were very much higher, on an average 30,500 per cubic centimetre, immediately after milking." Sternberg says, "the average of fifteen samples taken from the tables of persons living in the suburbs of Boston was 69,143 per cubic centimetre. The average of fifty-seven samples of Boston milk, obtained directly from the milk wagons and plated at once was 2,355,500 per cubic centimetre. The average of sixteen samples from groceries in the city of Boston was 4,577,000 per cubic centimetre."

Some cities, since milk-inspection has been adopted, condemn milk containing over 200,000 bacteria per cubic centimetre.

Great stress must be laid on the sources of contamination and everything should be done to reduce the possibilities of contamination. Still even a more important point to be emphasized is the care of the milk after milking. The lack of good management in this respect is well illustrated by the enormous increase of bacteria of the common varieties in sample IX having a count of 516,000 per cubic centimetre which was milked and planted in relatively the same time as sample VI having a count of 19,000 per cubic centimetre. The former having been left standing

exposed in the kitchen of the house while the latter had been carefully handled and kept cool.

The first step taken to study the bacteria in milk, after it has been brought to the laboratory, it to make dilutions of the milk with sterilized water.

A one tenth dilution is made by taking one cubic centimetre of the milk and adding to it nine cubic centimetres of sterile water. Then for one one-thousandth dilution, one cubic centimetre of the one tenth dilution is added to ninety-nine cubic centimetres of water. For a one one-hundreth dilution, one cubic centimetre of the milk is added to ninety-nine cubic centimetres of sterile water.

This diluting is done because the milk is so opaque that it would be impossible to see the colonies in the plate cultures. The milk is then plated out. This consists in adding one cubic centimetre of one of the known milk dilutions to about ten cubic centimetres of agar agar, that has been cooled to body temperature, and after vigorously shaking pouring it into a petrie dish. The agar agar is a nutrient medium used for growing bacteria. These plates are then allowed to stand until the agar solidifies when they are placed in an incubator and left for about twenty-four hours at a temperature of ninety-eight degrees Fahrenheit.

While in the incubator the organisms multiply rapidly and at the end of the twenty-four hours it is possible to count the colonies.

A colony is the result of multiplication of one organism. Thus the count of the number of colonies represents the original number of organisms in the milk. A count of two thousand colonies on a one one-hundredth dilution plate would have to be multiplied by one hundred and it would then represent the number of organisms in one cubic centimetre of the milk.

Then by the aid of a sterilized platinum needle the colonies are segregated and transmitted to the various media employed.

The kinds of nutrient media used are agar slants, because they can be incubated; gelatine, to show whether the organism liquefies or not; potato, to show whether the organism develops spores; litmus milk, to show whether the organism produces an acid or alkaline reaction; plain milk, to show whether the organism coagulates and redissolves the casein.

Then by the aid of a microscope the morphology of the organism is examined in a hanging drop and on a smear preparation.

The motility is shown in a hanging drop. This is made by taking with a platinum loop one loopful of liquefied gelatine or some liquid culture, on a cover glass. The cover glass with its hanging drop is inverted over the cavity of a hollow slide and is then examined to ascertain the motility of the organisms and the character of their movements.

To make a smear preparation a straight platinum needle is used, and the organisms are taken from any of the media on which they are growing. As thin a film as possible is smeared on the cover glass and allowed to dry spontaneously. After it is dry it is passed three times through a flame, of a Bunsen burner, to fix the film on the cover slip and it is then covered with suitable staining solution which is allowed to remain until the organisms are sufficiently stained. Then it is thoroughly washed to remove superfluous stain and again dried. It is then mounted with Canada Balsam on a microscope slide.

The slide is then placed on the stage of the microscope, a drop of cedar oil being placed on the cover glass. The objective is immersed in the oil and it is ready for examination.

The smear preparation is for the purpose of determining the morphology of the organism.

Figure A.

FIG. A. Photograph of 1/10 dilution plate. Showing superficial, deep, chromogenic, non-chromogenic, and spreading colonies.

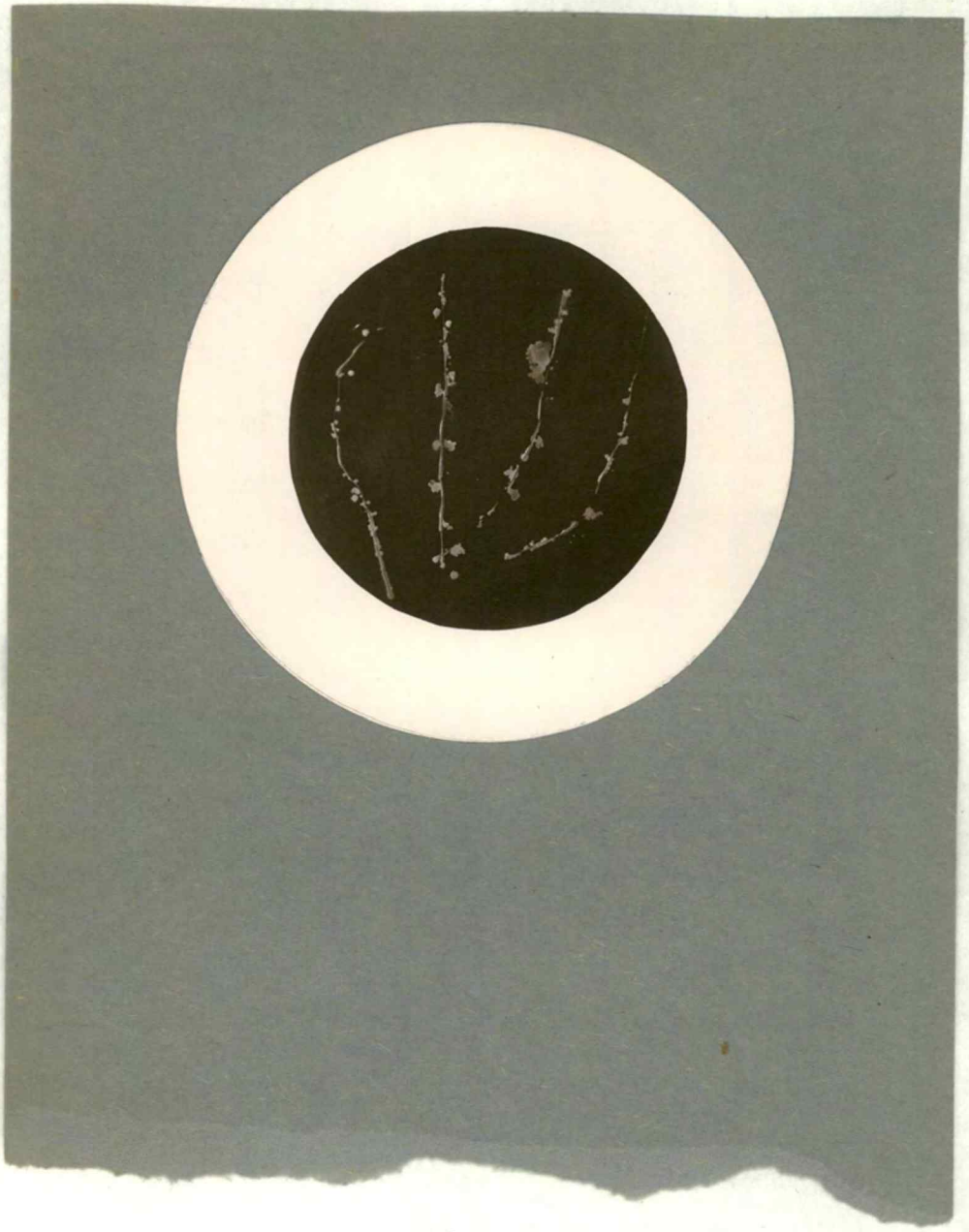


Figure B.

FIG. B. Photograph of agar-agar plate upon which four hairs were placed while the agar-agar was melted. The photograph shows the growth of the organisms which were upon the hairs at the time of planting. Upon one hair are 150 organisms. This illustrates the amount of contamination introduced by one hair falling into milk.

Figure C.

FIG. C. Painting of the plate photographed in Fig. B. to show different coloring of colonies.

Figure D.

FIG. D. Photograph of very unsanitary methods of treating milk and bad conditions surrounding the animals from which the milk was obtained.

Figure E.

FIG. E. Photograph showing well entirely too close to the stable. Seepage of water from the stable to the well is almost certain at this distance and the milk vessels washed in this water are sure to be contaminated.

Figure F.

FIG. F. Photograph of College barn where every thing is kept as sanitary as possible.

Figure G

FIG. G. Photograph of sanitary cow-lot with clean, well kept cows.

SAMPLE I.

Owing to a placing of the milk in hot agar-agar, the normal number of colonies did not develop and the sample was discarded.

SAMPLE II. Nov. 10, 1909.

Milked 5:00 A. M.

Planted 3:30 P. M.

Average count of plates 534,000

per cubic centimetre.

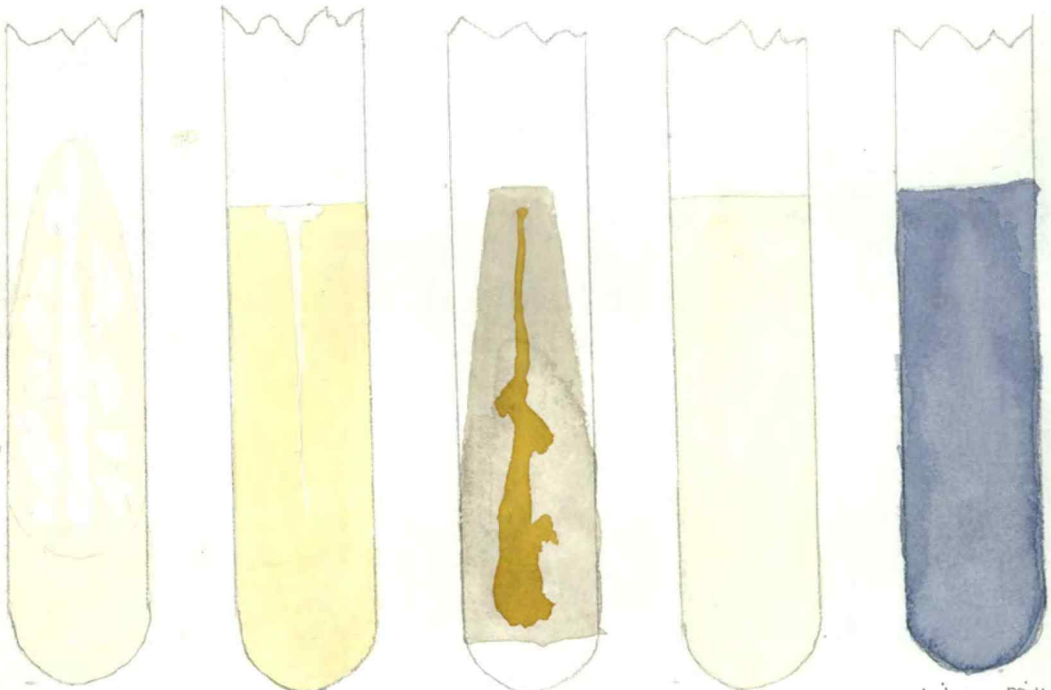
Sample II.

Colony I. Micrococcus Fervidosus.

Figure I

Agar Plate.

Microscope Field.



Agar Slant. Gelatine Potato Milk Litmus Milk.

L. E. Jackson.

Colony I, Figure I. Micrococua Fervidosus.

Micrococci--non-motile-- non-liquefying.

Agar-agar plate. Superficial, raised, smooth, round, brown colony with darker brown center by transmitted light. Milky white with heavier white center by reflected light.

Agar-agar slant. Smooth,white, thin growth along the line of inoculation with irregular borders somewhat thickened.

Gelatine. Non-liquefying--arobic, facultative--anarobic.

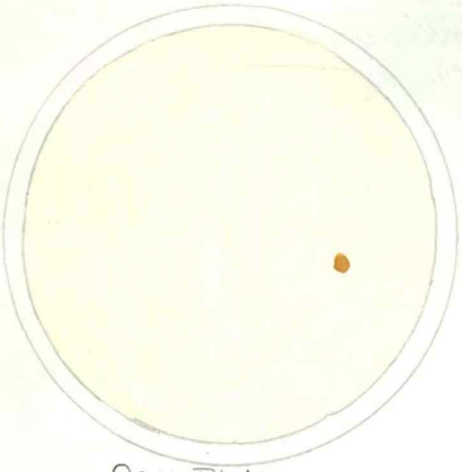
Potato. Dry, yellow, warty growth. Turns the potato dark grey or violet.

Milk. Apparently no reaction.

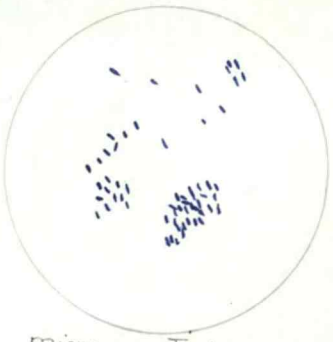
Sample II.

Colony II Bacillus Ochraceus.

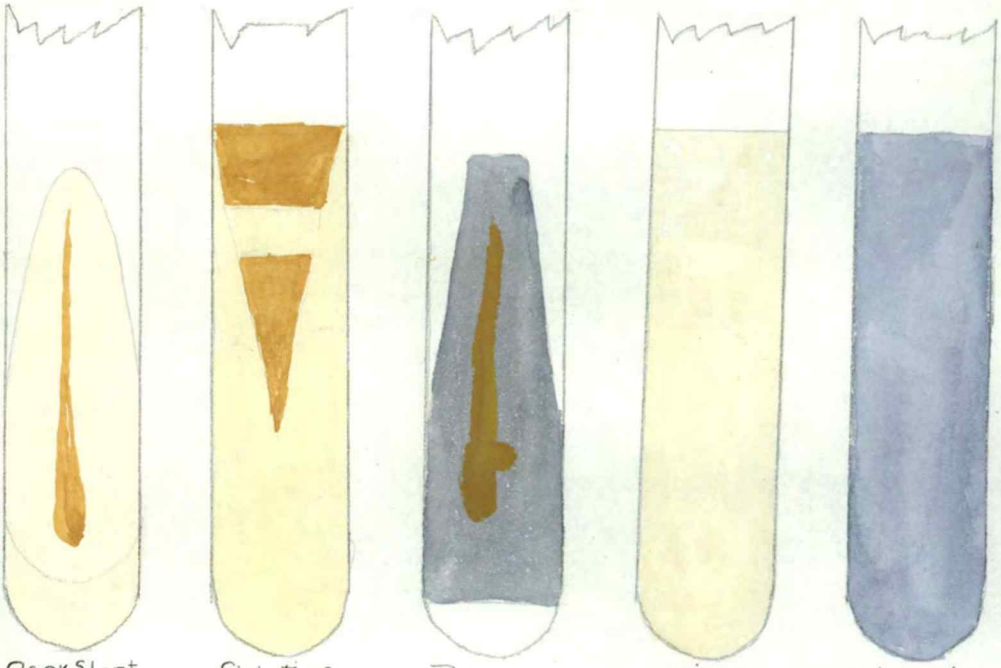
Figure II



Agar Plate



Microscope Field.



Agar Slant Gelatine Potato Milk Litmus Milk.

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Colony II, Figure II. Bacillus Ochraceus.

thick rod--slightly motile--liquefying.

Agar-agar plate. Round, shiny, yellow, raised colony with darker center by reflected light and cloudy yellow by transmitted light.

Agar slant. Shiny, yellow, thick, spreading growth along the line of inoculation. Raised.

Gelatine stab culture. Liquefaction occurs at first in funnel shape. Forms thick, brown microderma on top with streaks of clear and cloudy gelatine.

Potato. Thick, granular, orange growth. Does not discolor the potato.

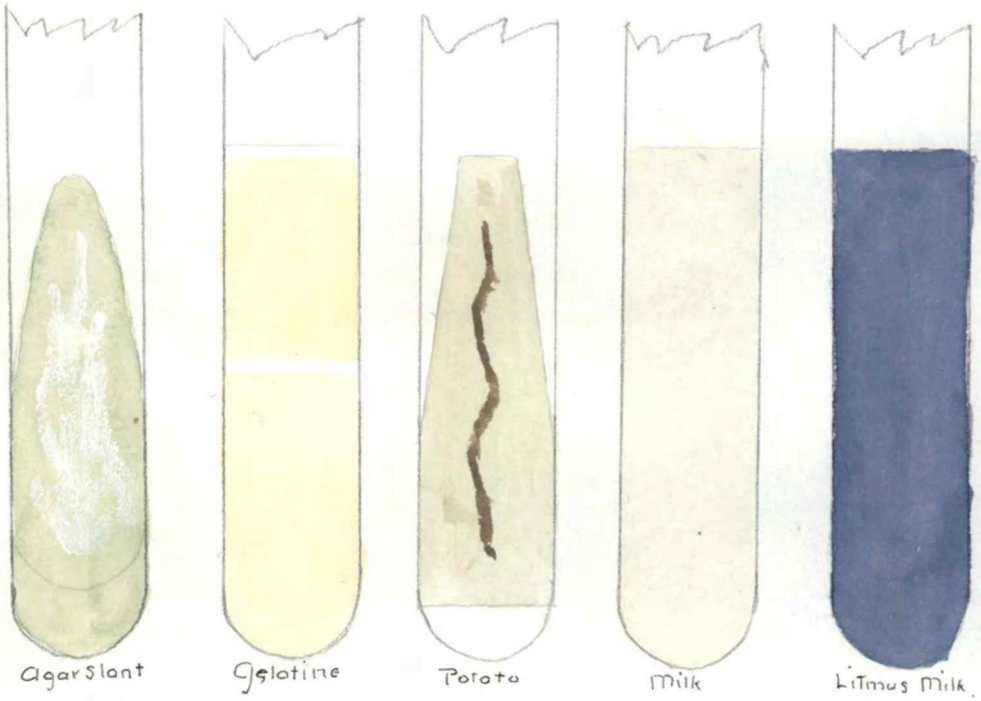
Milk. Coagulates and afterwards redissolves.

Litmus milk. Produces an acid.

Sample II.
Colony IV. Bacillus Fluorescens Liquefaciens.
Figure III

Agar Plate

Microscope Field.



Agar Slant Gelatine Potato Milk Litmus Milk.

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Colony III was the same as Colony I.

Colony IV, Figure III. Bacillus Fluorescens Liquefaciens. Rod shapes--actively motile.

Agar-agar plate. Thin, spreading colony with scalloped edges. Green florescence with reflected light. Almost transparent in transmitted light.

Agar-agar slant. Spreads over the entire surface of the slant and the agar-agar gains a green florescence.

Gelatine. Liquefies in cup shape, rapidly spreading to the edges. Liquefies about one-half inch in five days. Floor of gelatine has brownish precipitate and white microderma floats on top with a cloudy layer next it.

Potato. Turns potato light lavender. Warty yellowish growth.

Milk. Coagulates the milk and redissolves.

Litmus milk. No acid formed.

SAMPLE III. Dec. 5, 1909.

Milked 3:00 P. M. Dec. 5.

Planted 2:00 P. M. Dec. 6.

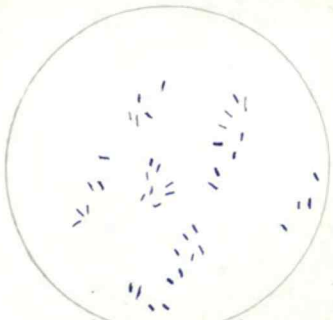
Average count of plates, 18,000 per cubic centimetre.

Sample III

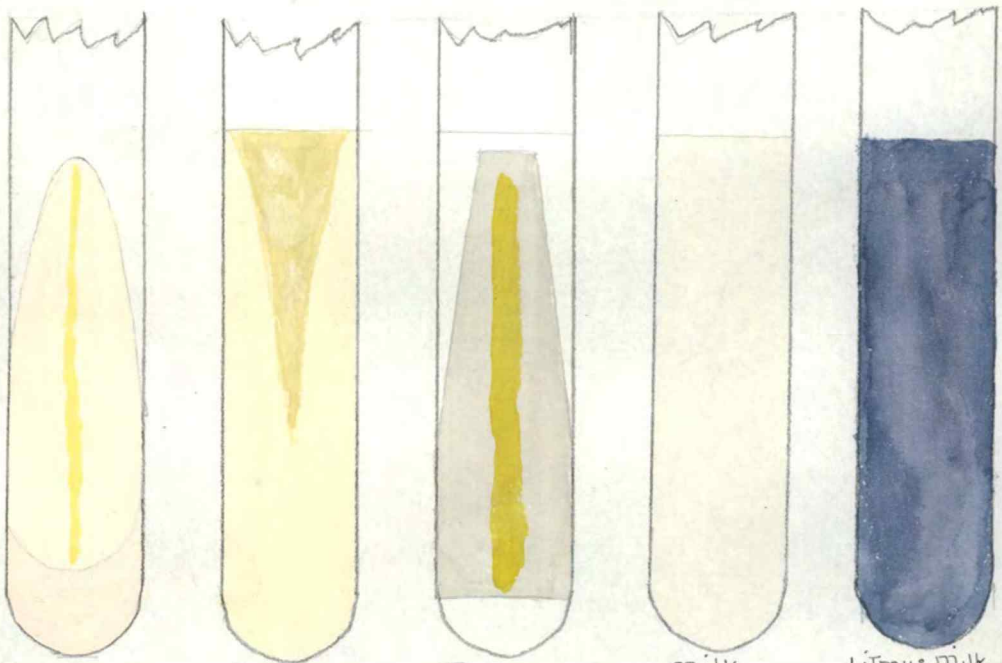
Colony A. Bacillus Figurans.

Figure IV

Agar Plate



Microscope Field.



Agar Slant Gelatine Potato Milk Litmus Milk

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Colony A, Figure IV. Bacillus Figurans.

medium long rods--rounded ends--non-motile--liquefying.

Agar-agar plate. Round, shiny, smooth, edged colony. Superficial, raised and cream colored by reflected light. Brown by transmitted light.

Agar slant. Raised,canary yellow growth just along the impfrish.

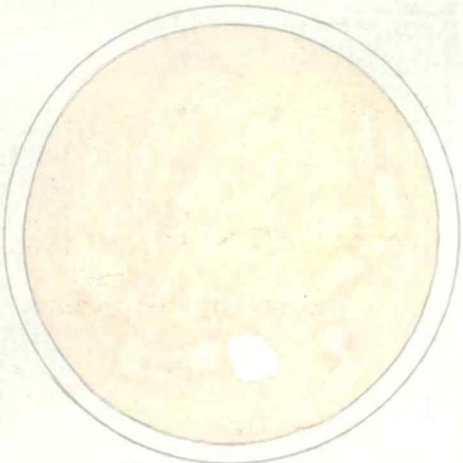
Gelatine stick culture. Liquefies in funnel shape with heavy microderma on top. Leaves the gelatine somewhat cloudy.

Potato. Heavy canary growth which later turns darker yellow. Potato later turns a dirty grey or violet.

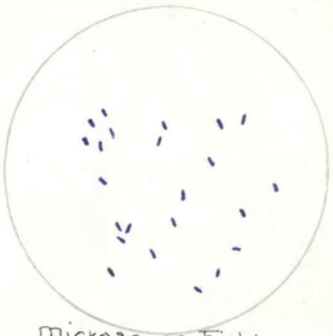
Litmus milk. Non-acid forming.

Milk. Does not coagulate the casein.

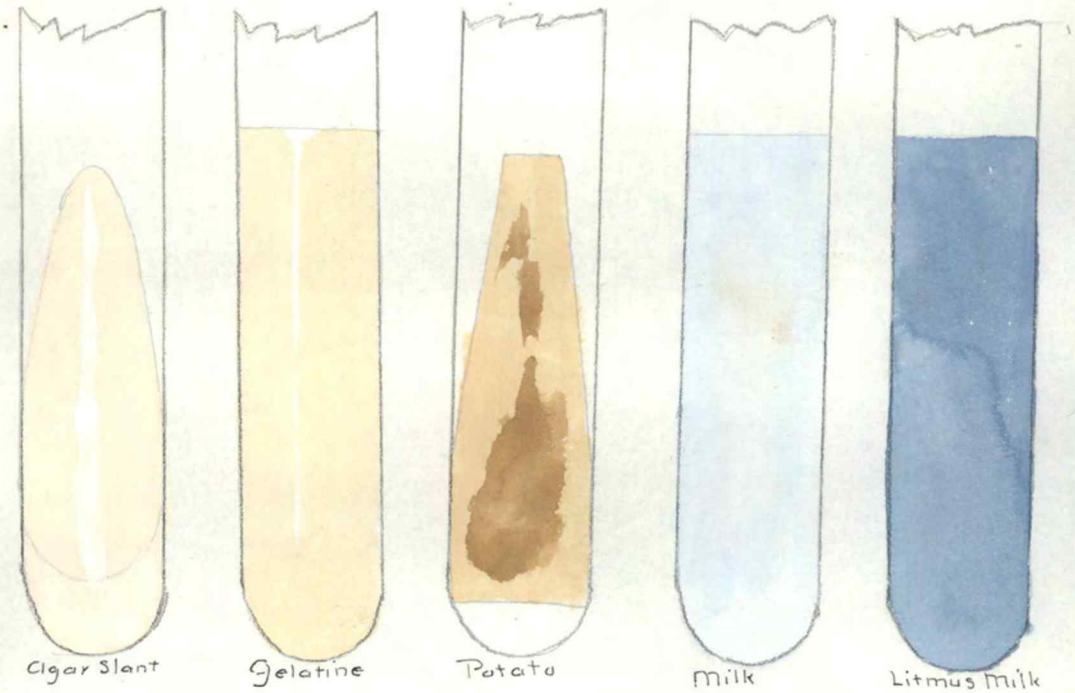
Sample III.
Colony B. Bacillus Nebilus.
Figure V.



Agar Plate



Microscope Field.



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Colony B, Figure V. Bacillus Nebilus.

Short rods with rounded ends--non-motile--liquefying.

Agar-agar plate. Round, white colony with radiating lines toward the outside. Cream by transmitted light.

Agar slant. Raised, heavy, white growth along the line of inoculation.

Potato. Light brown smear. Turns potato nearly the same color.

Gelatine. Liquefies and makes gelatine cloudy.

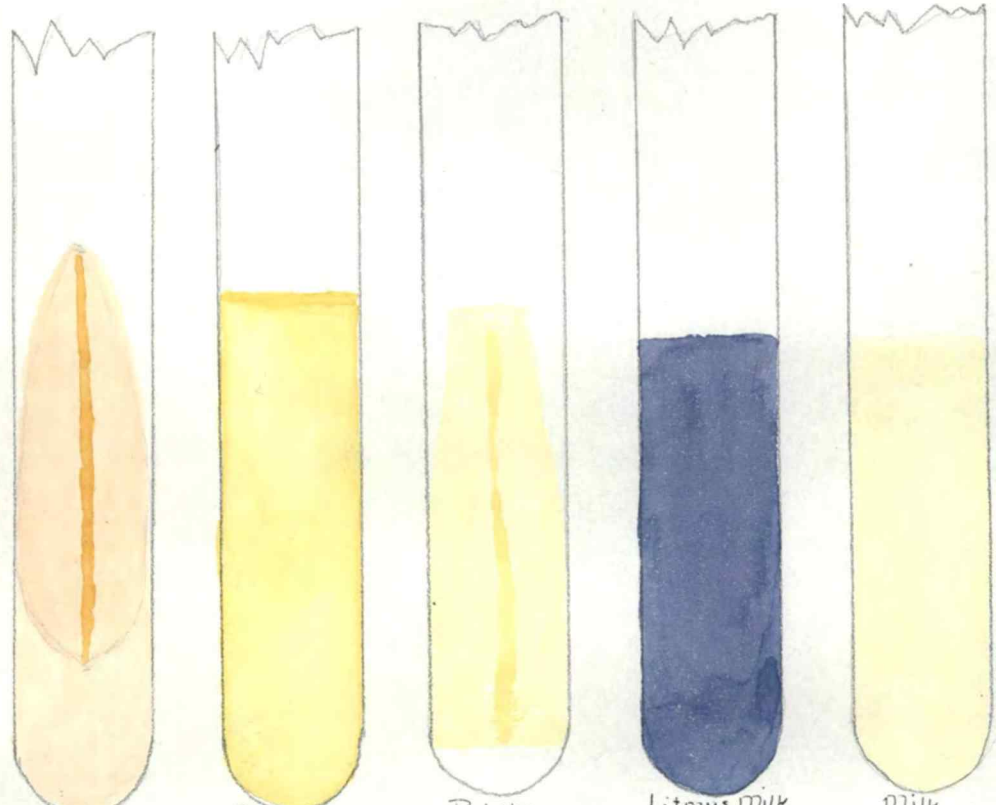
Milk. Does not coagulate the casein.

Litmus milk. Non-acid forming.

Sample III.
Colony C. Micrococcus Flavus Liquefaciens.
Figure VI.

Agar Plate

Microscope Field.



Agar Slant | Gelatine | Potato | Litmus Milk | Milk

L.E. Jackson.

Colony C, Figure VI. Micrococcus Flavus Liquefaciens.

Micrococcus, non-motile--liquefying.

Agar-agar plate. Oval, yellow, shiny, raised colony. Cream by transmitted light.

Agar slant. Smooth, raised, dark yellow or orange growth along line of inoculation.

Gelatine stick culture. Liquefies with heavy microderma on top.

Potato. Slight, thin, canary yellow growth. Does not discolor the potato.

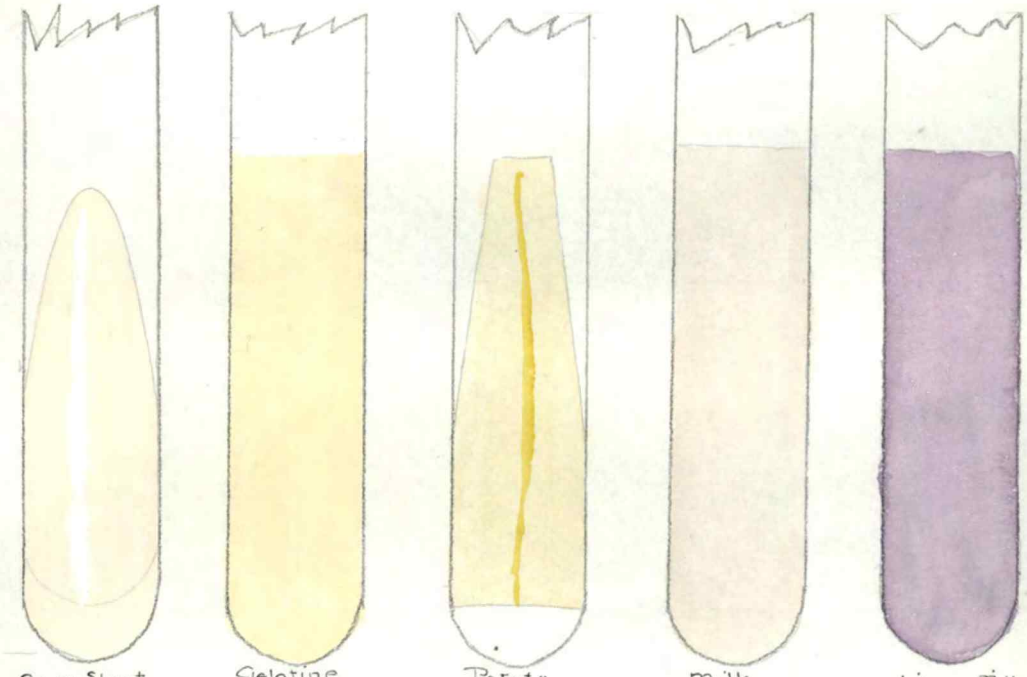
Milk. Does not coagulate the casein.

Litmus milk. Non-acid forming.

Sample III.
Colony D. Bacillus Superficialis.
Figure VII.

Agar Plate

Microscope Field.



Agar Slant Gelatine Potato Milk Litmus Milk

L.E. Jackson.

Colony D, Figure VII. Bacillus Superficialis.

Rod--slow motion like it had a terminal flagella. Involution forms found on potato. Liquefies.

Agar-agar plate. White, raised colony with thickened raised serrated edges. White with thick grey edge by transmitted light.

Agar slant. White growth just along the line of inoculation.

Gelatine stick culture. Liquefies with heavy white microderma on the top and suspended heavy floculi.

Potato. Very slight yellow growth just along the line of inoculation. Does not discolor the potato.

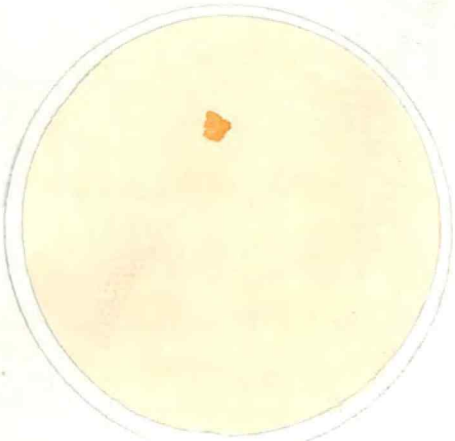
Milk. Does not coagulate the casein.

Litmus milk. Acid forming.

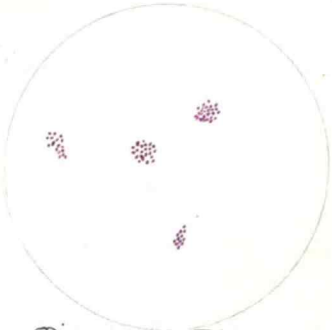
Sample III.

Colony E. Micrococcus Aurantiacus.

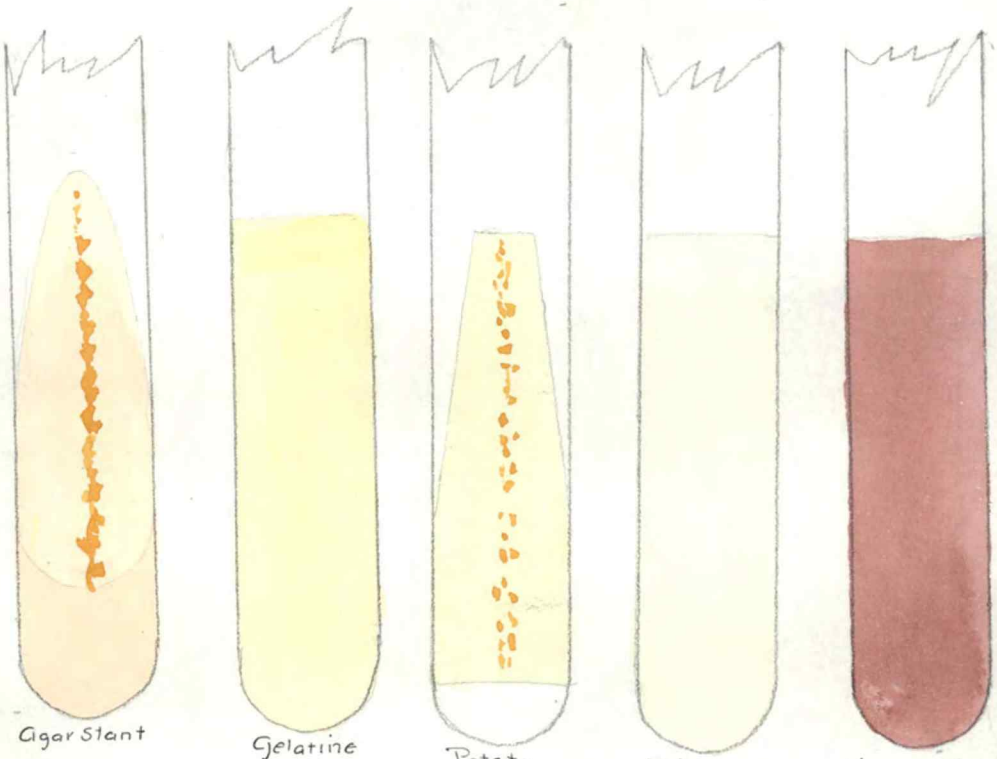
Figure. VIII.



Agar Plate



Microscope Field.



Agar Stant

Gelatine

Potato

Milk

Litmus Milk.

L.E. Jackson.

Colony E, Figure VIII. Micrococcus Aurantiacus.

Micrococcus of staphlo type--non-motile--non-liquefying. Grows best in incubator.

Agar-agar plate. Round, raised, orange colonies in bunches.

Agar-agar slant. Grows just along the impfrish in little hard knobs.

Gelatine stab culture. Non-liquefying.

Potato. Grows well along the impfrish in little knobs. Does not discolor.

Milk. Does not coagulate.

Litmus milk. Forms acid.

SAMPLE IV. Jan. 12, 1910.

Milked Jan. 11 at 5:00 P. M.

Planted Jan 12 at 3:00 P. M.

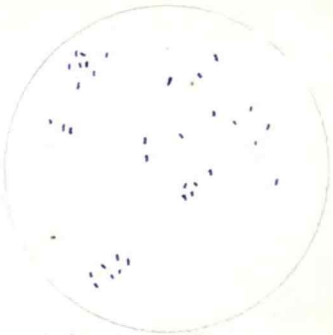
Average count 1650 per cubic centimetre.

Sample IV.

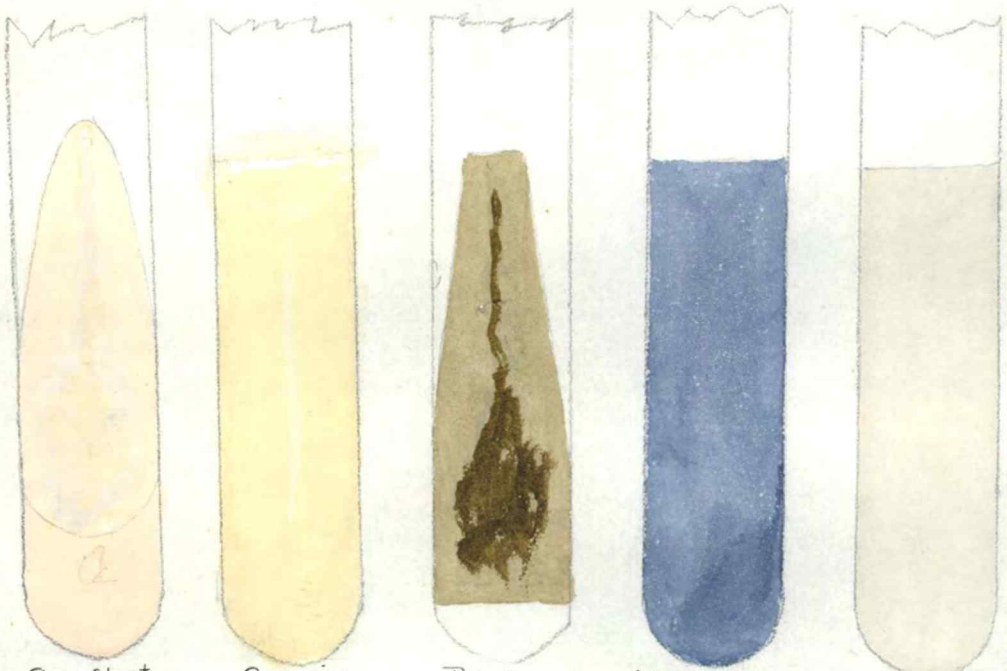
Colony I. Bacillus Muscoides.

Figure IX.

Agar Plate



Microscope Field.



Agar Slant Gelatine Potato Litmus Milk Milk.

L.E. Jackson.

Colony I, Figure IX. Bacillus Muscoides.

Short rods with rounded ends--actively motile. Spores are formed at the ends of the rods. Non-liquefying.

Agar-agar plate. White, spreading colony, grows in fancy shapes like ferns.

Agar slant. White, spreading colony with fern like projections.

Potato. Dirty brown with tiny raised creases in all directions, spreading and turns the potato a dirty white.

Gelatine stab culture. Non-liquefying.

Milk. Does not coagulate the casein.

Litmus milk. Non-acid forming.

SAMPLE V. Jan. 26, 1910.

Milked 6:00 A. M.

Planted 3:00 P. M.

Average count 54,000 per cubic centimetre.

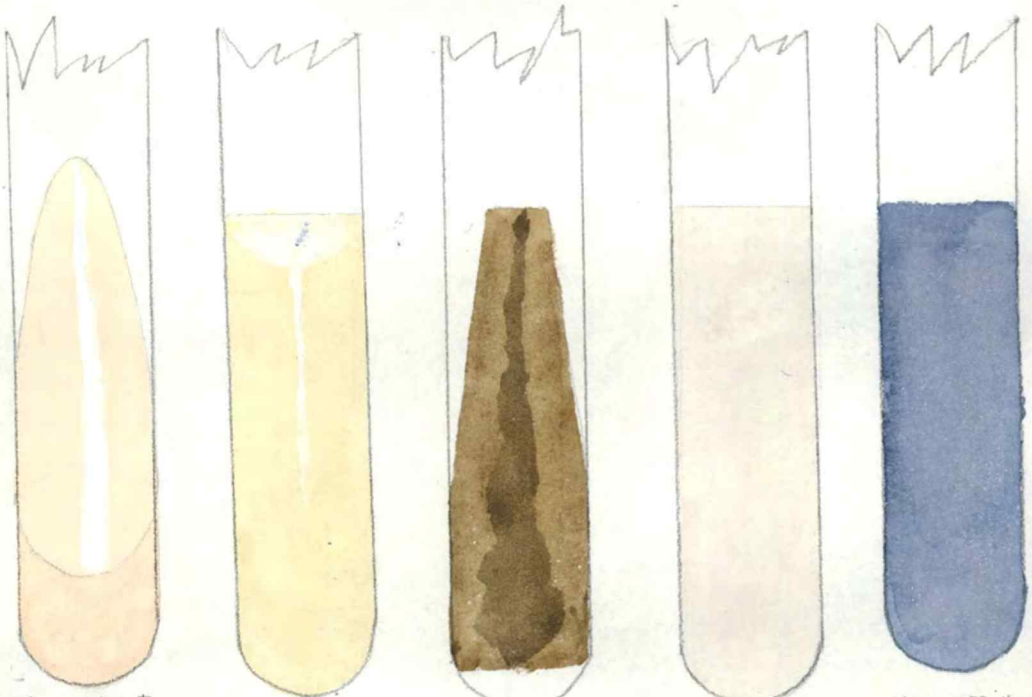
Sample V.

Colony A. Bacillus Aerolactes.

Figure X.

Agar Plate

Microscope Field.



Agar Slant — Gelatine — Potato — Milk — Litmus Milk

L.E. Jackson.

Colony A, Figure X. Bacillus Aerolactis. (Conn)

Rods small with rounded ends--non-motile--prolific spore producer--liquefying.

Agar-agar plate. White, spreading, wrinkled colony.

Agar slant. White, rather streaked growth along the impfrish.

Gelatine stab culture. Liquefies and forms a floculi.

Potato. Dirty yellowish brown. Discolors the potato.

Milk. Coagulates the casein.

Litmus milk. Non-acid forming.

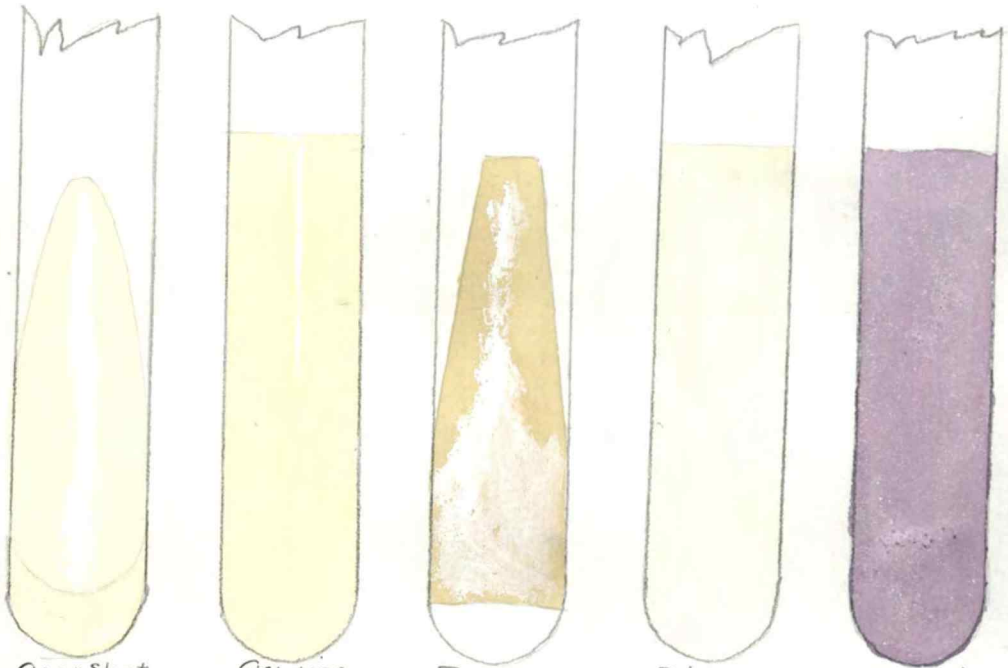
Sample V.

Colony C. Bacillus Acidi Lactici

Figure XI.

Agar Plate

Microscope Field.



Agar Slant Gelatine Potato Milk Litmus Milk.

L.E. Jackson.

Colony B same as C.

Colony C, Figure XI. Bacillus Acidi Lactici.

Large rod with rounded ends. Brownian motion. Forms spores in the center of the rod. Non-liquefying.

Agar-agar plate. White, almost transparent, deep growing colony.

Agar slant. Transparent white colony growing only along the impfrish.

Gelatine stab culture. Non-liquefying.

Potato. Spreading, white growth--raised, wrinkled and tenacious and turns the potato yellowish violet.

Milk. Coagulates and redissolves the casein.

Litmus milk. Acid forming.

SAMPLE VI. Jan. 3, 1910.

Milked 6:00 P. M. Jan. 3.

Planted 3:00 P. M. Jan.4.

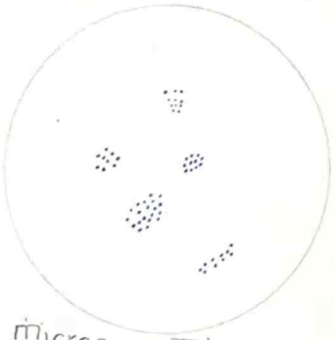
Average count 19,000 per cubic centimetre.

Sample VI.

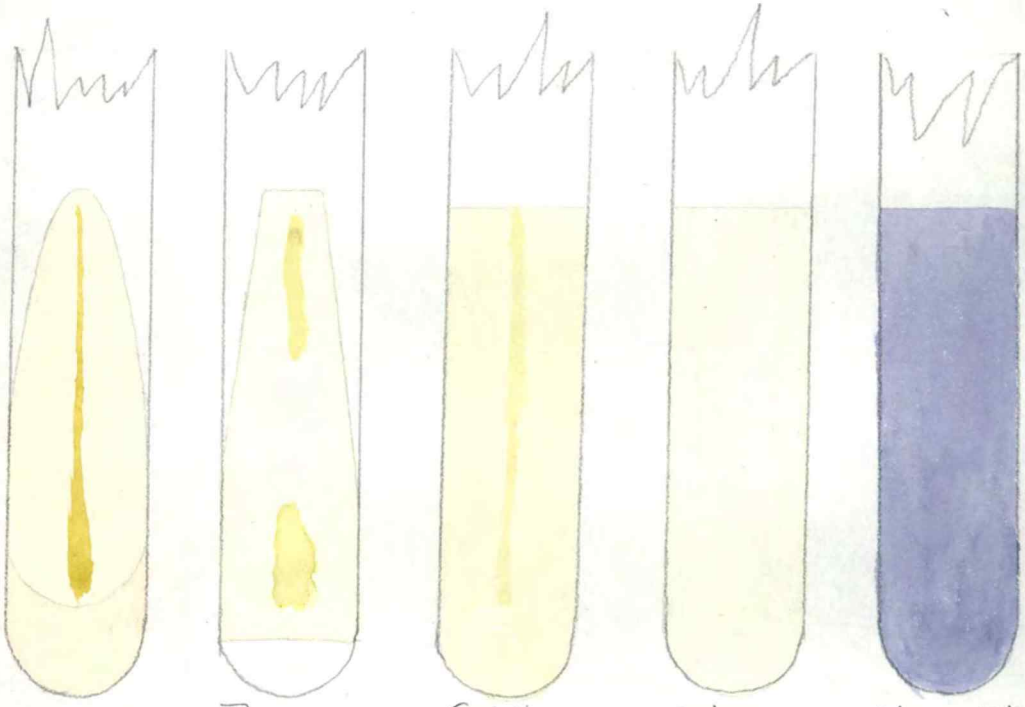
Colony III. Micrococcus Aurantiacus.

Figure XII.

Agar Plate



Microscope Field



Agar Slant | Potato | Gelatine | Milk | Litmus Milk.

L.E. Jackson.

Colony W, Figure XII. Micrococcus Aurantiacus.

Very small micrococcus--facultative anaerobic, non-motile--non liquefying.

Agar-agar plate. Round, greenish yellow colony.

Agar slant. Very pretty greenish yellow growth along the line of inoculation.

Gelatine stab culture. Non-liquefying. Good growth along impfrish.

Potato. Shiny yellow growth just along the line of inoculation. Does not discolor the potato.

Milk. Coagulates and redissolves the casein.

Litmus milk. Acid forming.

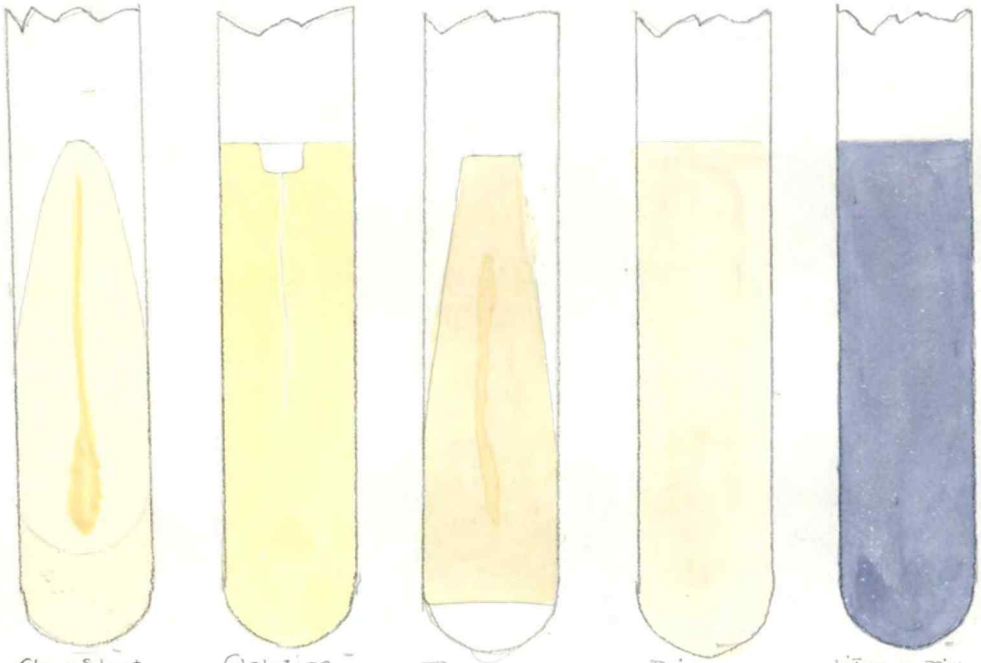
Sample VI.

Colony V Micrococcus Candicans

Figure XIII

Agar Plate

Microscope Field.



Agar Slant Gelatine Potato Milk Litmus Milk.

L.E. Jackson.

Colony V, Figure XIII. Micrococcus Candicans.

Micrococci, facultative anaerobic, non-motile--liquefying.

Agar-agar plate. Deep cream colored diamond shaped colony.

Agar-agar slant. Creamish yellow with heavy edges.

Gelatine stab culture. Liquefies in cup shape and dries out as fast as it liquefies.

Potato. Very slight growth. Does not discolor.

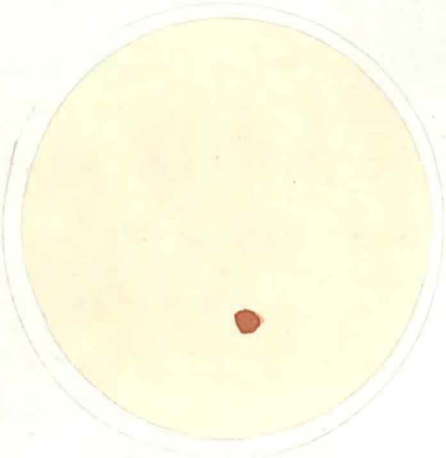
Milk. Does not coagulate the casein.

Litmus milk. Non-acid forming.

Sample VI.

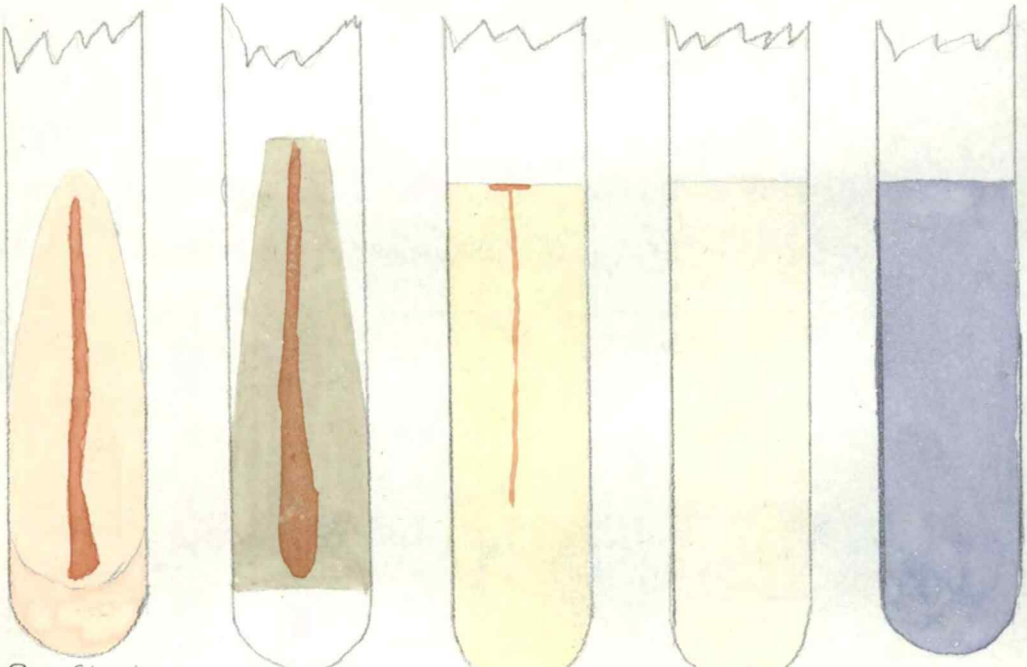
Colony Z Saccharomyces Prosacens.

Figure XIV.



Agar Plate

Microscope Field.



Agar Slant Potato Gelatine Milk Litmus Milk

L.E. Jackson.

Colony Z, Figure XIV. Saccharomyces Rosaceus.

Large round bodies about three microns long. Appear granular inside.

Agar-agar plate. Round, pink colony with dark pink center.

Agar-agar slant. Heavy, shiny, salmon-pink growth along the line of inoculation.

Gelatine stab culture. Non-liquefying. Button like growth at the top and slight growth along the line of inoculation.

Potato. Heavy pink growth in two days. Looks like wet paint. Not shiny.

Milk. Does not coagulate the casein.

Litmus milk. Non-acid forming.

This yeast makes a bread with a very delicate crust.

SAMPLE VII. March 11, 1910.

Milked 7:00 A. M. March 11.

Planted 2:30 P. M. March 11.

Average count 600 per cubic centimetre.

Mostly Bacillus Muscoides. No new colonies.

SAMPLE VIII. March 6, 1910.

Milked 6:00 P. M. March 5.

Planted 3:00 P. M. March 6.

Average count 45,000 per cubic centimetre.

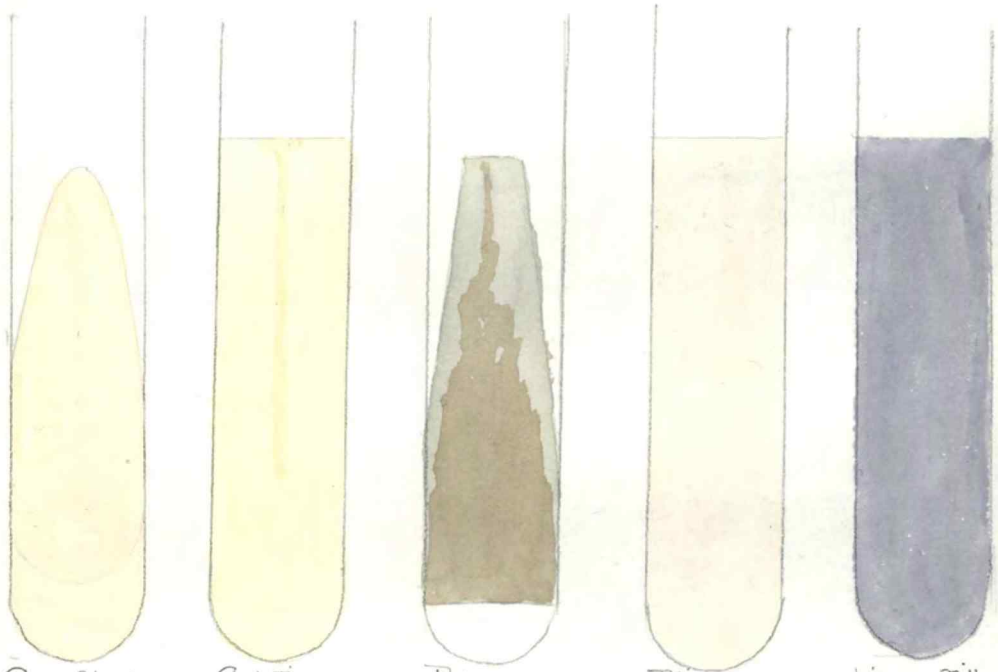
Sample VIII.

Colony I Bacillus Circulans

Figure XV.

Agar Plate

Microscope Field



Agar Slant Gelatine Potato Milk Litmus Milk.

L.E. Jackson.

Colony I, Figure XV. Bacillus Circulans.

Small rod about one and one-half microns long. Prolific spore producer. Actively motile. Liquefying.

Agar-agar plate. Raised, white, shiny superficial colony with many filaments. Brown by transmitted light.

Agar slant. Raised, flesh colored, slightly wrinkled growth just along the impfrish.

Gelatine. Liquefies very slowly in cup shape and dries out as fast as it liquefies.

Potato. Raised, wrinkled, spreading growth and discolors the potato.

Milk. Does not coagulate the casein.

Litmus milk. Non-acid forming.

SAMPLE IX. April 11, 1910.

Milked 5:00 P. M. April 9.

Planted 3:00 P. M. April 11.

Average count 516,000 per cubic centimetre. No new colonies.

SAMPLE X. April 11, 1910.

Milked 6:00 A. M. April 11.

Planted 3:00 P. M. April 11.

Average count 650 per cubic centimetre. The count was so low that a preservative was suspected.

SAMPLE XI. April 15, 1910.

Milked 6:00 A. M. April 15.

Planted 3:00 P. M. April 15.

Same milk as sample X. Tested for preservative but none was found. No colonies on the 1/100 or the 1/1000 dilution plate. 15,600 on 1/10 dilution plate. Irregularity not accounted for.

SAMPLE I. Nov. 3, 1909.

Milk used in Waldo Hall.

Count 1,380,000 colonies to

1 c. c.

SAMPLE II. Nov. 1909.

Milk used in Waldo Hall.

Count 480,000 colonies to 1 c.c.

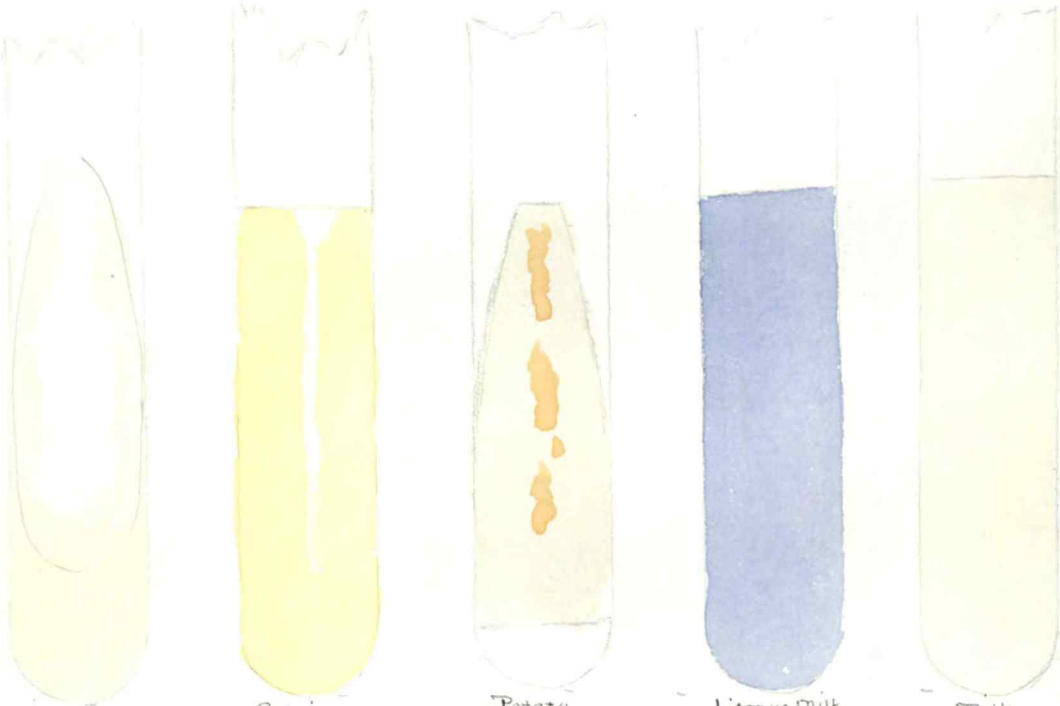
Sample II

Colony II. P II. Micrococcus Albus.

Figure I.

Agar Plate

Microscope Field.



Agar Slant — Gelatine — Potato — Litmus Milk — Milk

R. I. Hess.

Description of Figure I.

Colony II; Plate II. Micrococcus Albus.

By transmitted light on agar plate. Small, superficial, oval, raised, smooth, homogenous mass. Light brown.

By reflected light. Milky white.

Agar slant. Thin, white film showing many cross markings.

Potato. Dry, yellow, warty growth. Turns potato lavender. Growth only along line of inoculation.

Gelatine stab. Non-liquefying aerobic facultative anaerobic.

Litmus milk. No action.

Milk. No action.

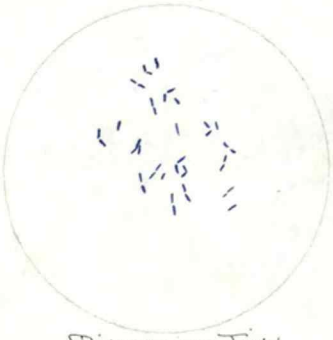
Form. Small micro-cocci.

Sample II.

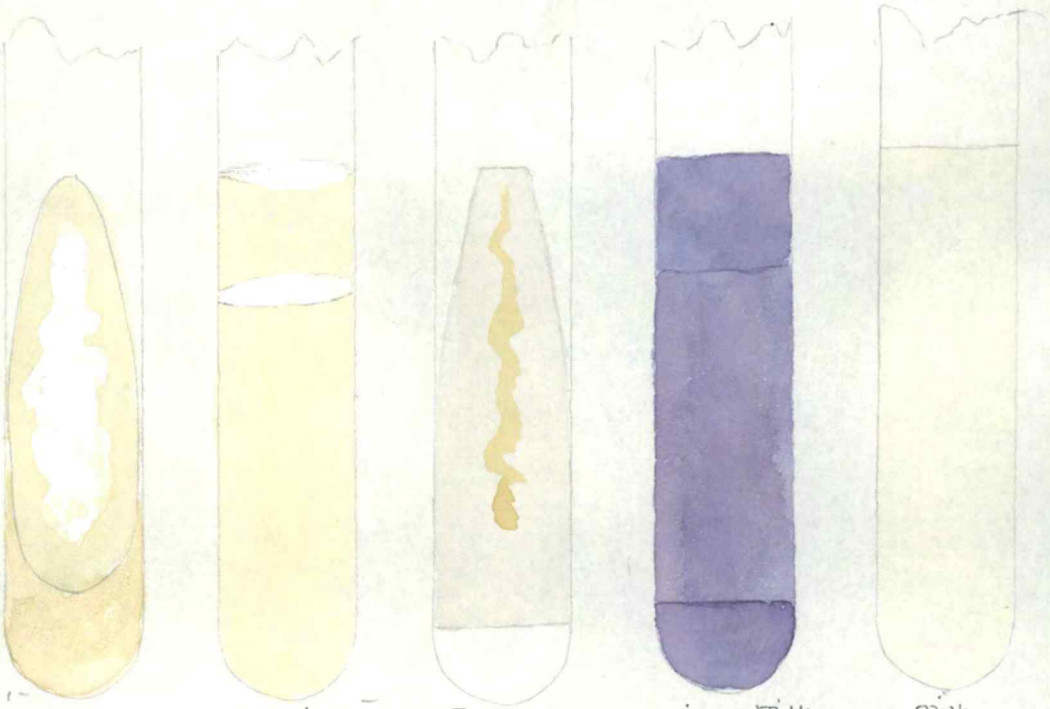
Colony III P II. Bacillus Fluorescens Liquefaciens

Figure II.

Agar Plate



Microscope Field.



Agar Slant | Gelatine | Potato | Litmus Milk | Milk

R.J. Hess.

Description of Figure II.

Colony III, Plate II. Bacillus Flurescens Liquefaciens.

By reflected light on agar plate. Greenish white, deep colony. Moss-like growth with many filaments.

Agar slant. White, spreading growth. Agar assumes green florescence.

Potato. Yellow, warty growth. Turns potato lavender.

Gelatine stab. Liquefies gelatine precipitate on floor of gelatine.

Litmus milk. Coagulates and redissolves casein. Acid reaction.

Milk. Coagulates and redissolves casein.

Form. Short, thick rods usually in pairs and slightly motile.

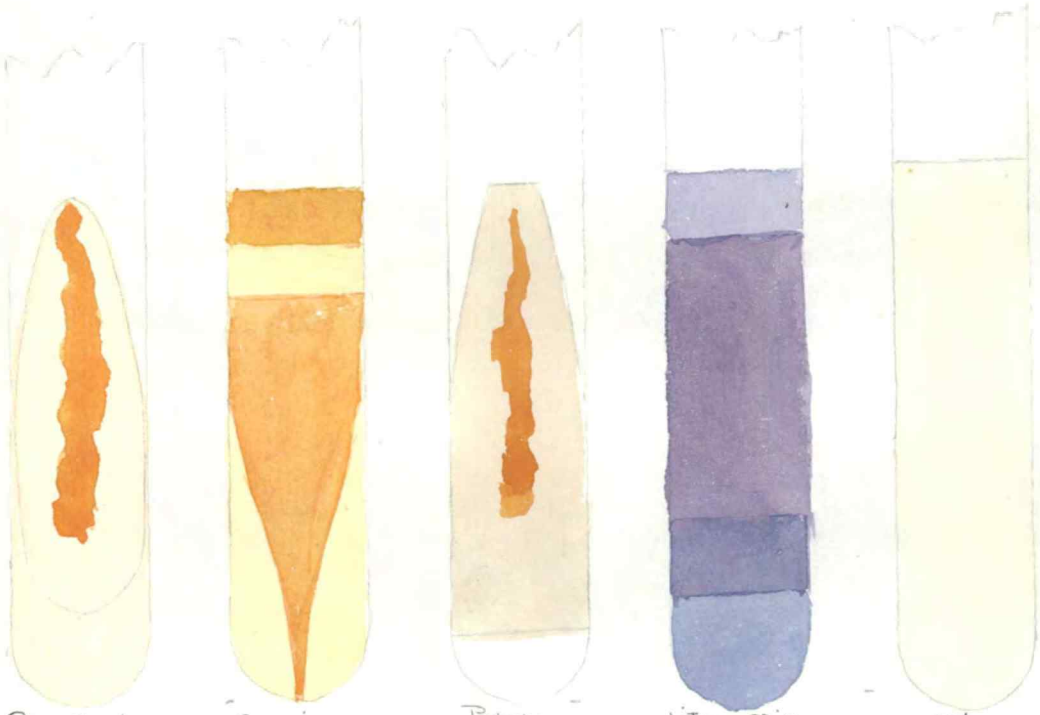
Sample II.

Colony IV P II. Bacillus Ochracens

Figure III.

Agar Plate

Microscope Field



Agar Slant — Gelatine — Potato — Litmus Milk — Milk

R. J. Hess.

Description of Figure III.

Colony IV, Plate II. Bacillus Ochracens.

By reflected light on agar plate. Small, round, smooth, yellowish white, raised, shiny colony having darker center.

By transmitted light. Deep brown center. Outer part lighter brown.

Agar slant. Smooth, shiny; yellowish growth. Growth only along line of inoculation.

Potato. Orange colored granular growth. Growth only along line of inoculation.

Gelatine stab. In gelatine stab cultures liquefaction occurs in funnel form. Forms a brown microderma on floor of gelatine and a brown deposit at point of funnel. Cloudy on floor of gelatine then clear streak then cloudy streak.

Litmus milk. Coagulates and redissolves casein. Acid reaction.

Milk. Coagulates and redissolves.

Form. Short slender bacillus. Motile.

SAMPLE III. From King's Dairy.
Milked 3:00 P. M. Dec. 5, 1909.
Plates made 2:00 P. M. Dec. 6,
Count 18,000. It was impossible
to pick up any colonies as there
was a white filmy colony over the
entire plate.

SAMPLE IV. From O. A. C. Farm.

Milked A. M. Dec. 15, 1909.

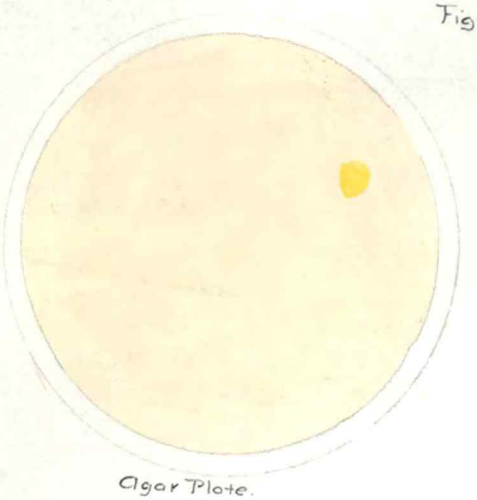
Plates made P. M. Dec. 15, 1909.

Count 13,200.

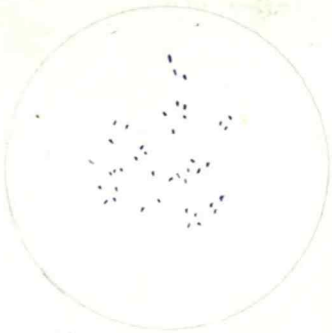
Sample IV.

Colony I P III. Bacillus Fluivus

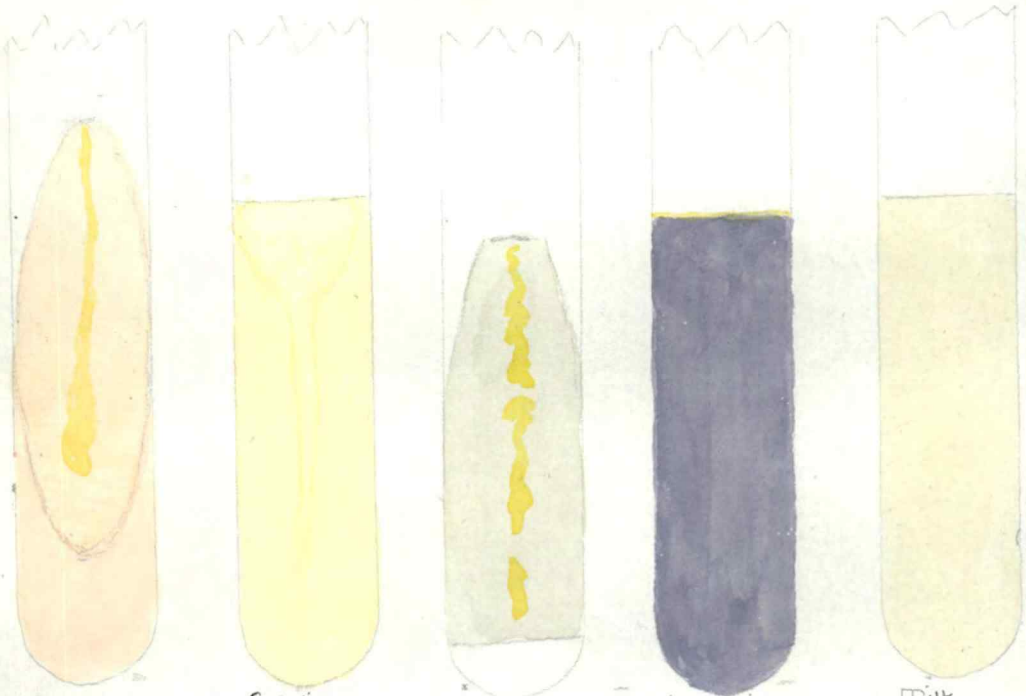
Figure IV.



Agar Plate.



Microscope Field.



Agar Slant Gelatine Potato Litmus Milk Milk.

R.J. Hess.

Description of Figure IV.

Colony I, Plate III. Bacillus Fluivis.

By reflected light on agar plate. Small, oval, shiny, yellow, raised, superficial colony.

By transmitted light. Light brown.

Agar slant. Raised, smooth, shiny, lemon yellow growth. Growth only along line of inoculation.

Potato. Lemon yellow, shiny colony. Growth only along line of inoculation. Wrinkled with deep fissures. Turns potato dirty white.

Gelatine stab. Liquefies gelatine. Aerobic facultative anaerobic. Liquefaction very slow. It occurs in cup shaped form.

Litmus milk. No action.

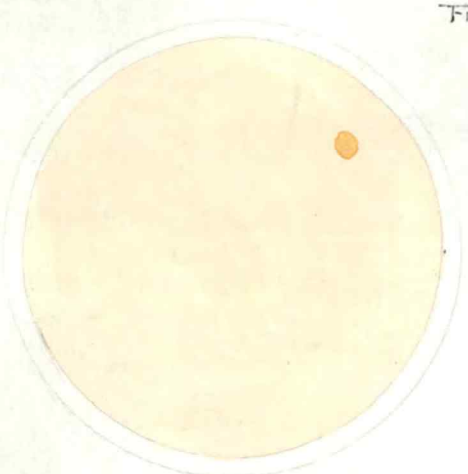
Milk. No action.

Form. Short rods. Transverse divisions. Non-motile.

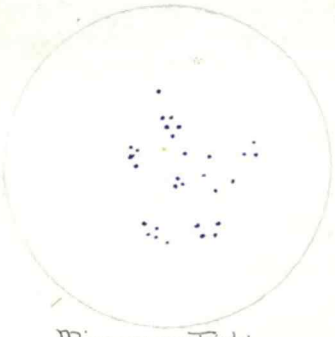
Sample IV.

Colony II P III Micrococcus Lactis Aurens. (Conn).

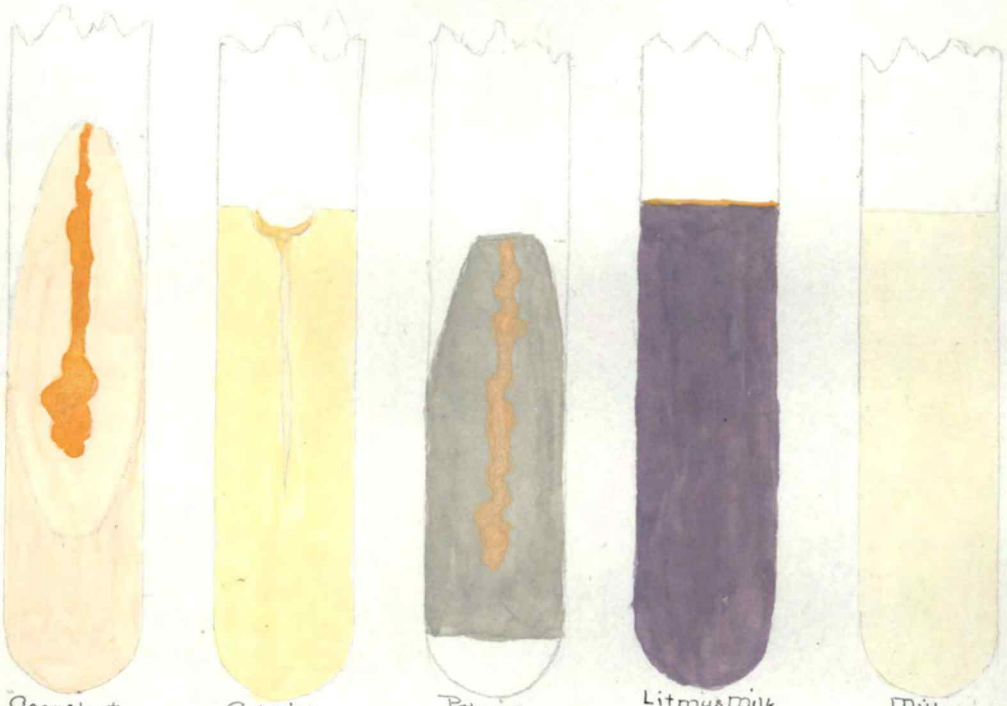
Figure V.



Agar Plate



Microscope Field.



Agar slant Gelatine Potato Litmus Milk Milk.

R.J. Hess.

Description of Figure V.

Colony II, Plate III. Micrococcus Lactis Aureus. (Conn)

By reflected light on agar plate. Small, raised, round, shiny, flesh colored, superficial colony.

By transmitted light. Light brown.

Agar slant. Smooth, raised, shiny, orange colored growth. Growth only along line of inoculation.

Potato. Shiny, orange colored growth. Turns potato dirty grey. Growth only along line of inoculation.

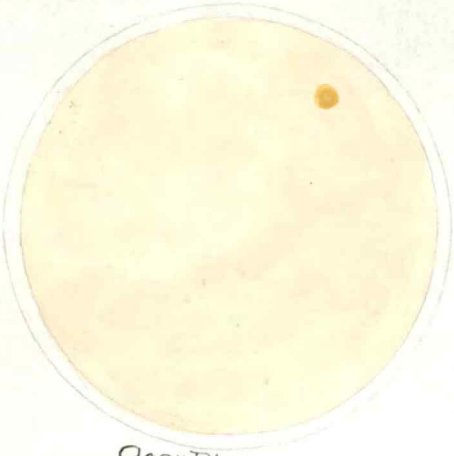
Gelatine stab. Does not liquefy gelatine. Yellow microderma on floor of gelatine.

Litmus milk. Orange microderma on floor of milk. Acid reaction. Action very slow. Coagulates milk.

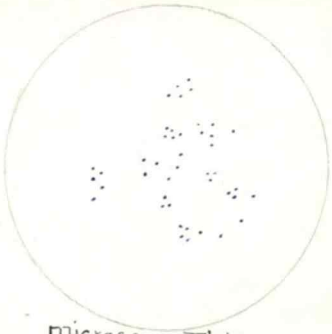
Milk. Slight yellow growth around tube at upper surface of milk. Coagulates milk.

Form. Medium small micro-cocci.

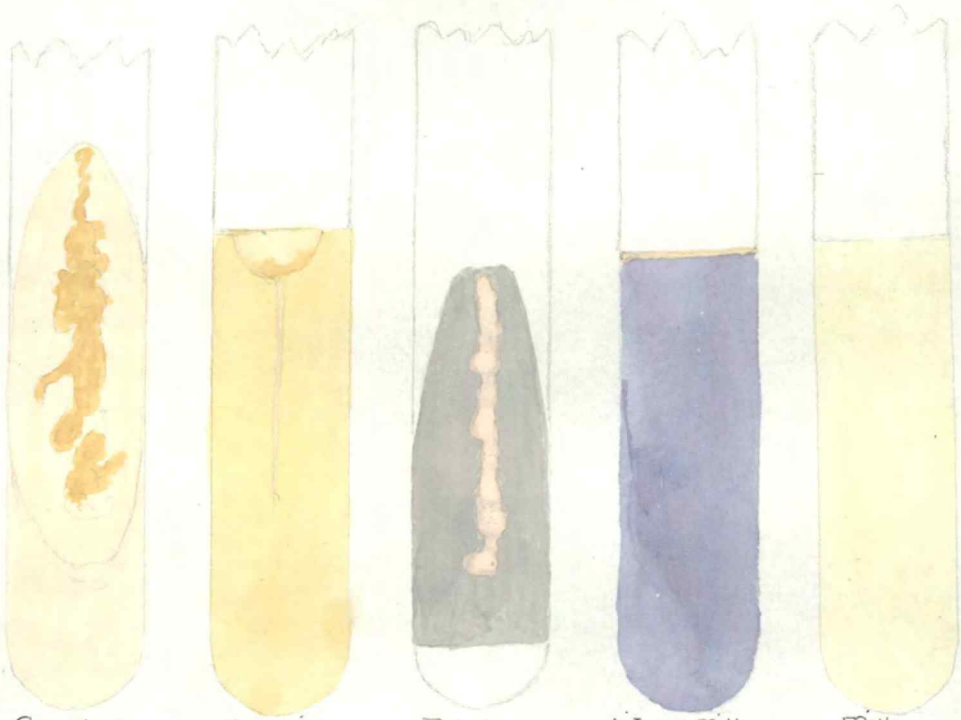
Sample IV.
Colony I PII Micrococcus Lactis Varians
Figure VI.



Agar Plate



Microscope Field



Agar Slant | Gelatine | Potato | Litmus Milk | Milk

R.J. Hess.

Description of Figure VI.

Colony I, Plate II. Micrococcus Lactis Varians.

By reflected light on agar plate. Small, raised, shiny colony, with serrated edges.

By transmitted light. Light brown.

Agar slant. Light ochrans yellow growth. Raised and wrinkled with deep fissures.

Potato. Dull flesh colored granular growth. Growth only along line of inoculation. Turns potato dirty grey.

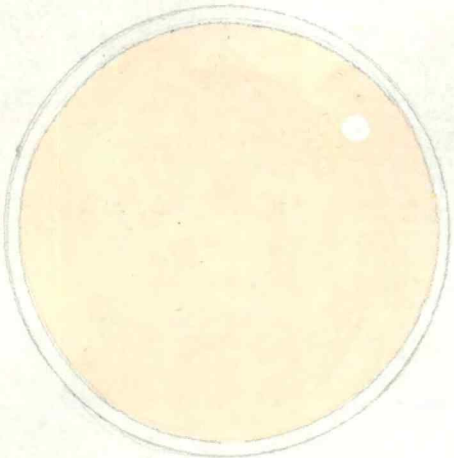
Gelatine stab. Liquefies gelatine in cup shaped form. Aerobic facultative anaerobic.

Litmus milk. Yellow microderma formed on floor of milk. Coagulates milk.

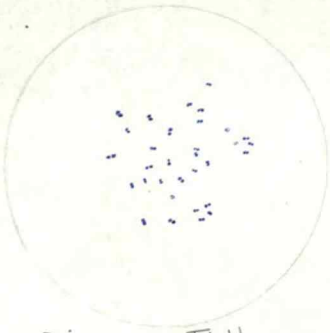
Milk. Coagulates milk. Yellow microderma formed on floor of milk.

Form. Very small micro-cocci.

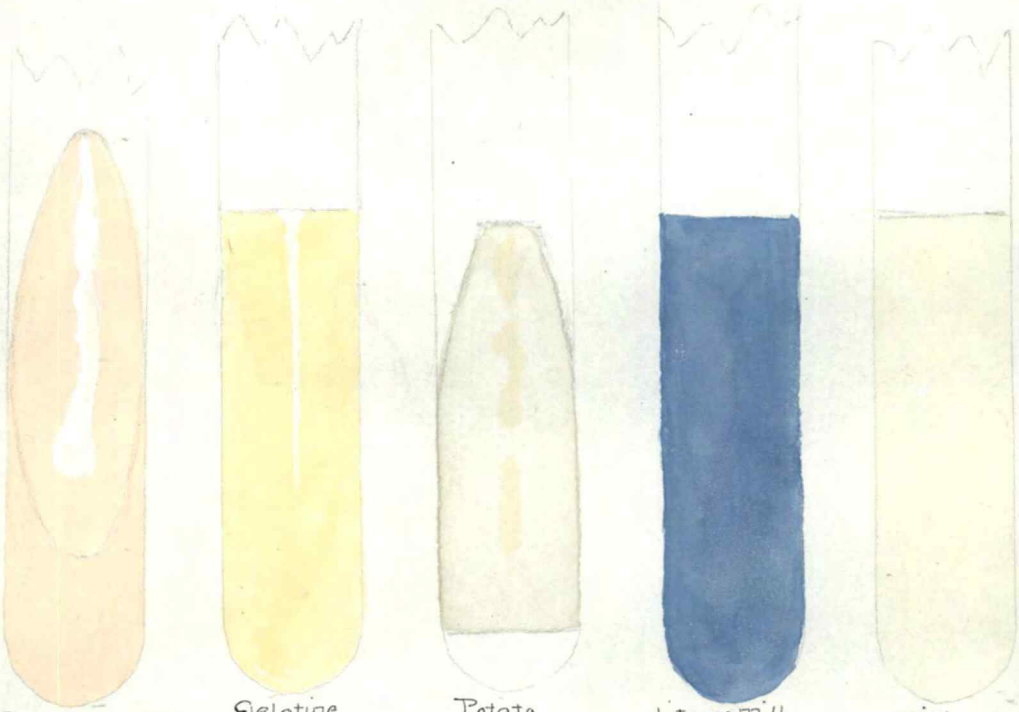
Sample IV
Colony II P II Micrococcus Ferviolosus.
Figure VII.



Agar Plate



Microscope Field.



Agar Slant. Gelatine Potato Litmus Milk Milk

R.J. Hess.

Description of Figure VII.

Colony II, Plate II. Micrococcus Fervidosus.

By reflected light on agar plate. Small, smooth, shiny, superficial, white colony.

By transmitted light. Light brown.

Agar slant. Smooth, shiny, white growth. Growth only along line of inoculation.

Potato. White, shiny growth. Turns potato dirty white. Growth very scant.

Gelatine. Does not liquefy gelatine. Growth very scant.

Litmus milk. No action.

Milk. No action.

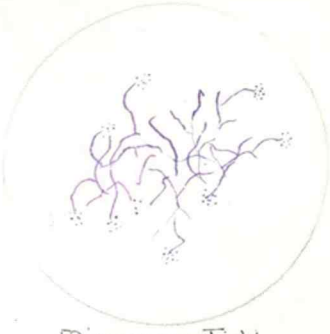
Form. Diplo-cocci--non-motile.

Sample IV.

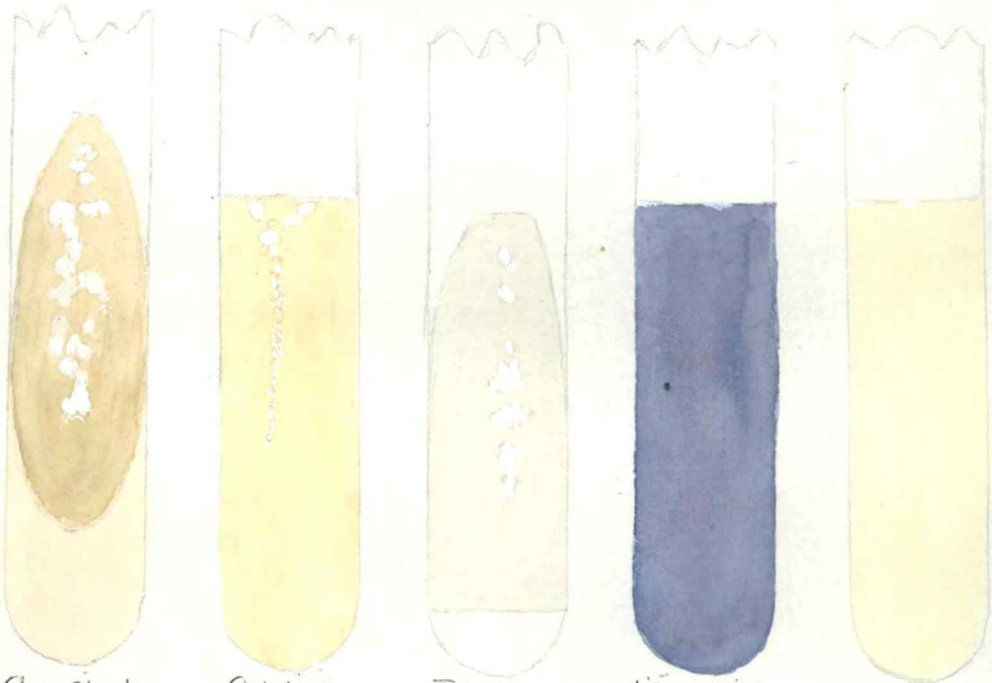
Colony III P II Cladothrix Dichotoma.

Figure VIII.

Agar Plate



Microscope Field.



Agar Slant | Gelatine | Potato | Litmus Milk | Milk

R.J. Hess.

Description of Figure VIII.

Colony III, Plate II. Cladothrix Dichatoma.

By reflected light on agar plate. Smooth, white, superficial, shiny colony. Opaque with dull white center.

By transmitted light. Light brown with white center.

Agar slant. Raised, white, warty growth. Gives off odor of mould or decaying wood.

Potato. Same growth as on agar slant.

Gelatine. Liquefies gelatine in cup shaped form. Aerobic facultative anaerobic.

Litmus milk. Casein is coagulated and redissolved. Acid reaction.

Milk. Light yellow growth around tube on surface of milk. Coagulates and redissolves milk.

Form. Long thread-like filaments having group of spores at the ends. The filaments are branching.

SAMPLE V. From Irvine's, Private cow.

Milked A. M. Jan 14, 1910.

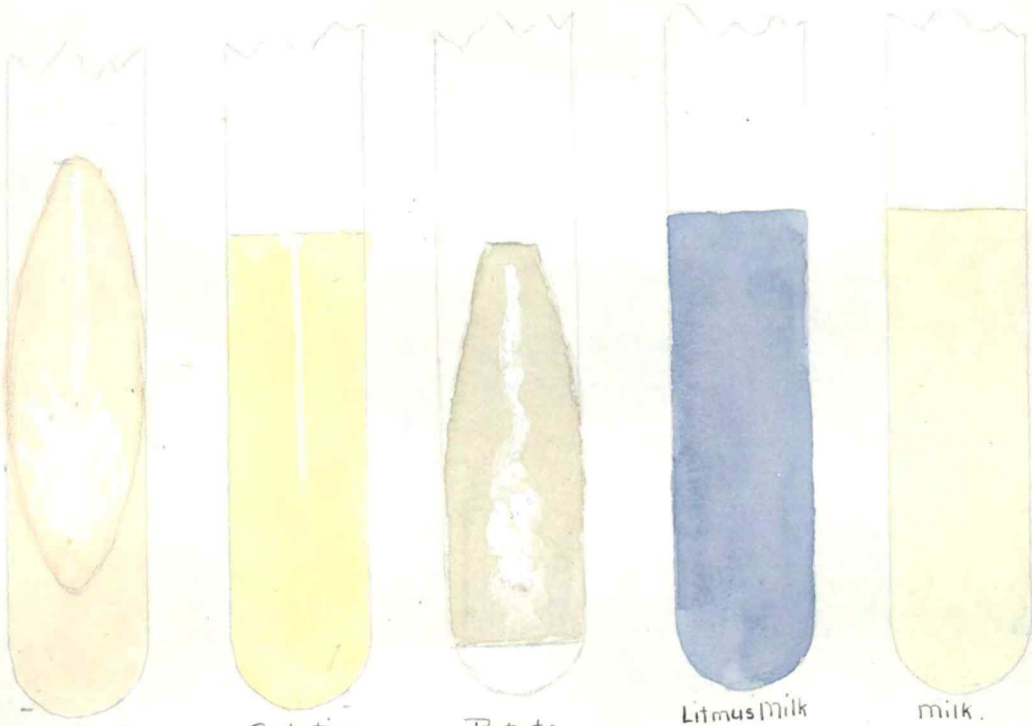
Plates made 2:30 P. M. Jan. 14.

Count 19,200.

Sample V.
Colony II P III Bacillus Ubiquitus.
Figure X

Agar Plate.

Microscope Field.



Agar Slant Gelatine Potato Litmus Milk Milk.

R.J. Hess.

Description of Figure X.

Colony II, Plate III. Bacillus Ubiquitous.

By reflected light on agar plate. Deep white, spreading, moss-like growth, with many filaments.

Agar slant. Clear, white, spreading growth, with many filaments.

Potato. Clear, yellowish white, wrinkled, spreading growth. Turns potato grey.

Gelatine. White, scanty growth. Does not liquefy gelatine.

Litmus milk. Coagulates and redissolves casein.

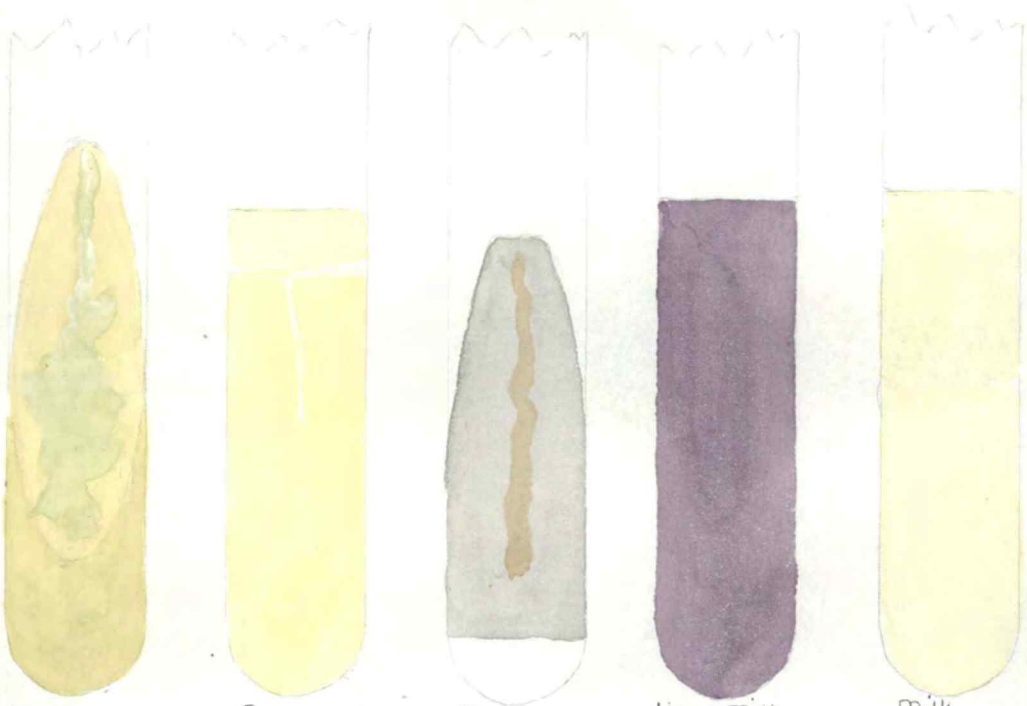
Milk. Coagulates and redissolves casein.

Form. Short thick rods. Non-motile.

Sample V.
Colony III P III Bacillus Pyocanus
Figure XI

Agar Plate

Microscope Field



Agar Slant Gelatine Potato Litmus Milk Milk.

R.J. Hess.

Description of Figure XI.

Colony III, Plate III. Bacillus Pyocanus.

By reflected light on agar plate. Greenish white, round, raised colony.

By transmitted light. Light brown, deeper brown center.

Agar slant. Raised, shiny, greenish white, spreading growth. Gives agar green florescence.

Potato. Brownish white, granular growth. Growth only along line of inoculation. Turns potato grey.

Gelatine. Liquefies gelatine. Action very slow. White deposit of floor of gelatine. Scanty growth along line of inoculation.

Litmus milk. Dissolves casein.

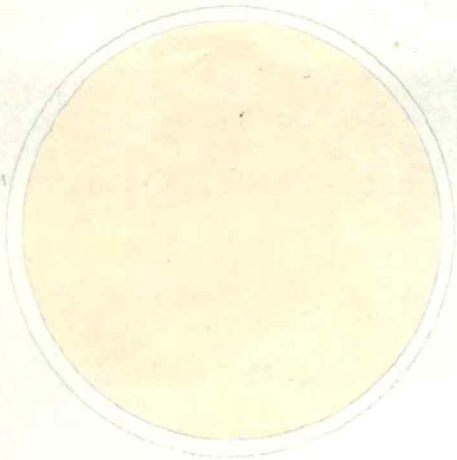
Milk. Dissolves casein.

Form. Long rods. Motile.

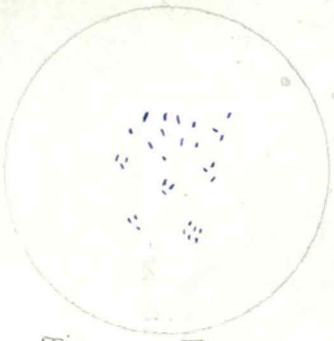
Sample V.

Colony IV P III. Bacillus Candicans

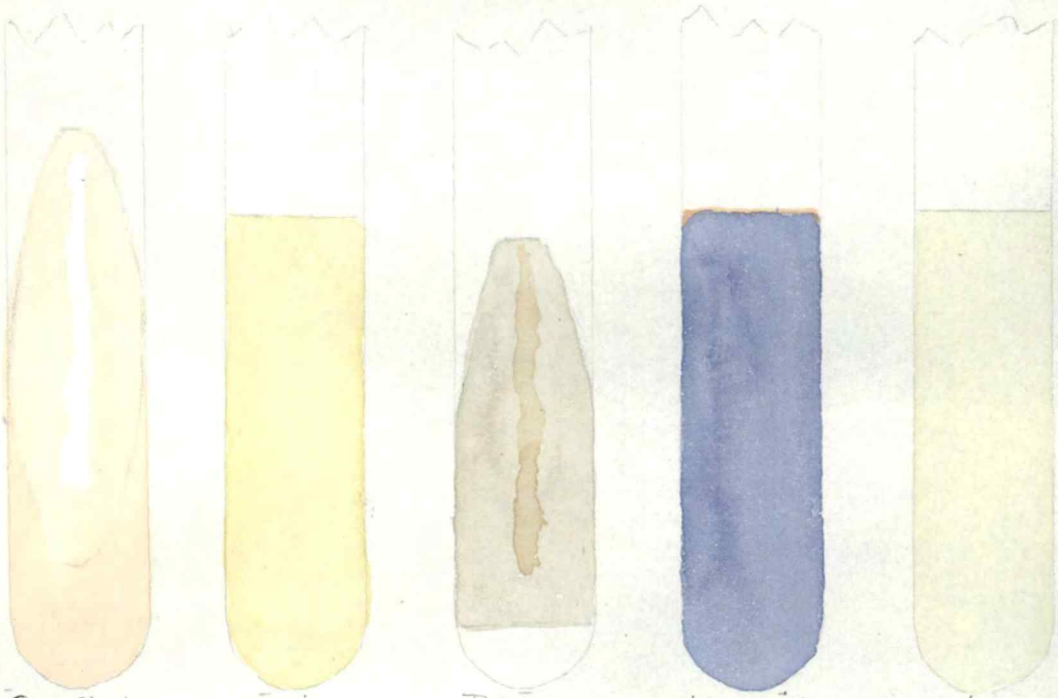
Figure XII



Agar Plate.



Microscope Field.



Agar Slant. Gelatine Potato Litmus Milk Milk

R.N. Hess.

Description of Figure XII.

Colony IV, Plate III. Bacillus Candiccus.

By reflected light on agar plate. Smooth, white, round, raised, shiny colony.

By transmitted light. Light brown.

Agar slant. White, raised, shiny growth. Growth only along line of inoculation.

Potato. Dirty brownish white, granular growth. Growth only along line of inoculation. Turns potato grey.

Gelatine. Does not liquefy gelatine. Growth scanty along line of inoculation.

Litmus milk. Coagulates milk. Light brown deposit around tube.

Milk. Coagulates milk.

Form. Short thick rods. Non-motile.

SAMPLE VI. Morgan's private cow.

Milked A. M. Feb. 7, 1910.

Plates made 2:30 P. M. Feb. 7.

The plates made were not good.

SAMPLE VII. From Smith's private cow.

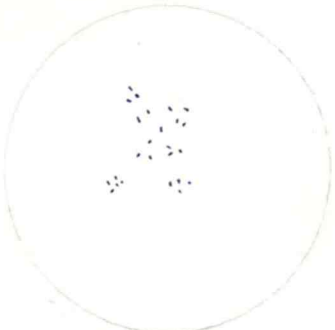
Milked P. M. March 6, 1910.

Plates made P. M. March 7, 1910.

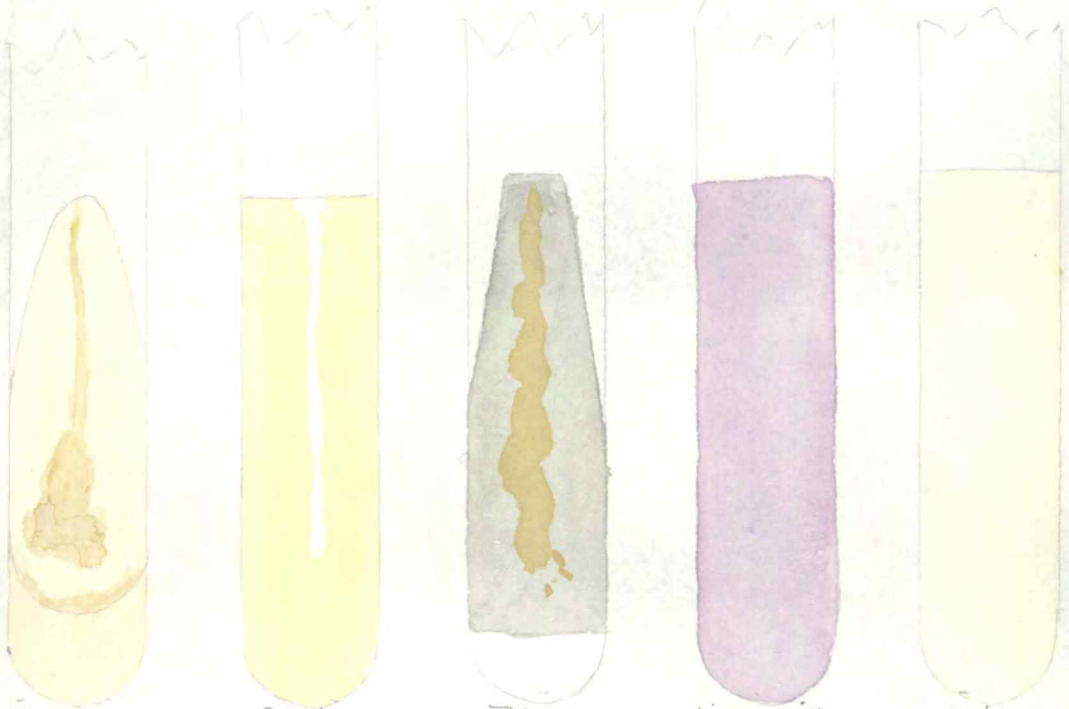
Count 45,000.

Sample VII.
Colony III PI Bacillus Limbatus Acidi Lactici
Figure XIII

Agar Plate



Microscope Field



Agar Slant Gelatine Potato Litmus Milk Milk

R.J. Hess.

Description of Figure XIII.

Colony III, Plate I. Bacillus Limbatus Acidi Lactici.

By reflected light on agar plate. Clear, white, round, deep colony.

By transmitted light. Light brown.

Agar slant. Light yellow, shiny, raised growth.

Potato. Brownish yellow, moist, shiny, slightly spreading growth. Turns potato grey.

Gelatine. Does not liquefy gelatine. Scant growth along line of inoculation.

Litmus milk. Acid reaction. Dissolves casein.

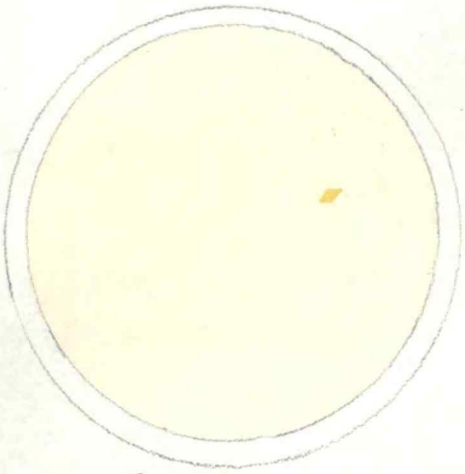
Milk. Dissolves casein.

Form. Short bacillus. Non-motile.

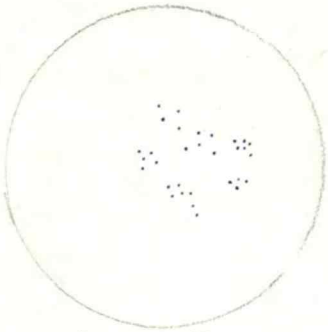
Sample VII.

Colony I P III. Micrococcus Aurantiacus.

Figure XIV.



Agar Plate



Microscope Field.

Agar Slant

Gelatine

Potato

Litmus Milk

Milk.

R.W. Hess.

Description of Figure XIV.

Colony I, Plate III. Micrococcus Auranticus.

By reflected light on agar plate. Raised, shiny, light yellow, diamond shaped colony.

By transmitted light. Light brown.

Agar slant. Orange yellow, with lighter yellow edges, smooth, shiny colony.

Potato. Moist, light brown, pink tint, shiny growth. Growth only along line of inoculation.

Gelatine. Does not liquefy gelatine. Scant growth along line of inoculation.

Litmus milk. Slightly acid.

Milk. No action.

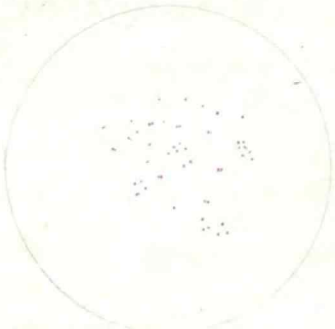
Form. Micro-cocci.

Sample VII.

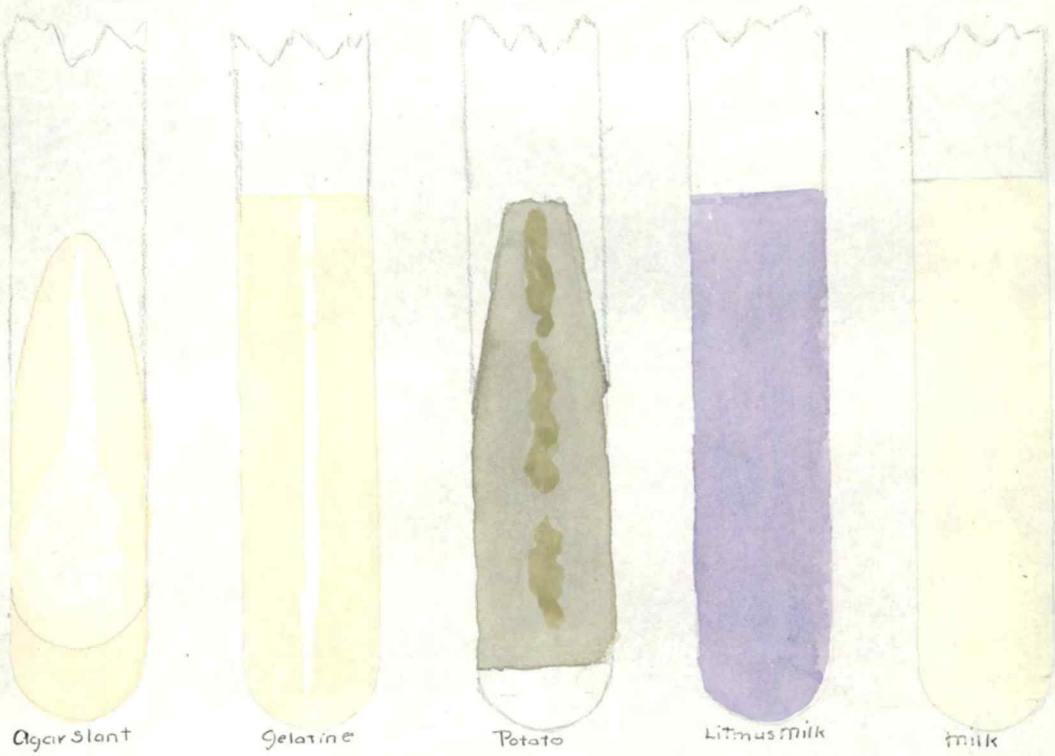
Colony II ~~P~~ III Micrococcus Concentricus.

Figure XV.

Agar Plate.



Microscope Field.



Agar Slant | Gelatine | Potato | Litmus Milk | Milk

R.J. Hess

Description of Figure XV.

Colony II, Plate III. Micrococcus Concentricus.

By reflected light on agar plate. Small, oval, raised, white, shiny colony.

By transmitted light. Light brown.

Agar slant. Thin film, white, spreading growth.

Potato. Dirty white, raised, granular growth. Growth only along line of inoculation. Turns potato grey.

Gelatine. Does not liquefy gelatine. Growth very scant along line of inoculation.

Litmus milk. No action.

Milk. No action.

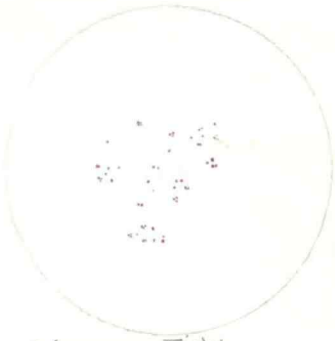
Form. Micrococci singly or in pairs.

Sample VII.

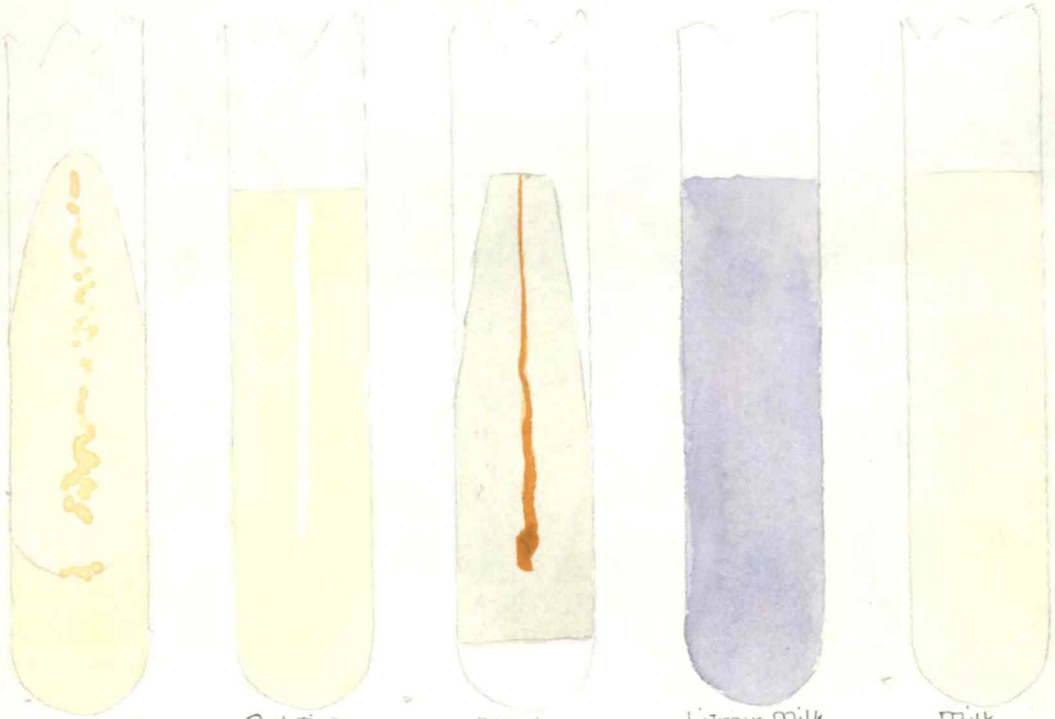
Colony III P III Micrococcus Citreus.

Figure XVI.

Agar Plate.



Microscope Field.



Agar Slant Gelatine Potato Litmus Milk Milk

R.J. Hess.

Description of Figure XVI.

Colony III, Plate III. Micrococcus Citrens.

By reflected light on agar plate. Small, oval, raised, shiny, yellow colony.

By transmitted light. Light brown.

Agar slant. Pinkish yellow, shiny growth. Growth very scant.

Potato. Orange colored growth, very slight along line of inoculation.

Gelatine. Does not liquefy gelatine. Slight white growth along line of inoculation.

Litmus milk. No action.

Milk. No action.

Form. Cocci varied in size. Singly, in pairs, in threes or in mosses.