# BACTERIAL SOFT ROT OF LETTUCE.

Thesis presented for the Degree of

Master of Science.

Michigan Agricultural College.

by

Lionel E. Tisdale.

1921

## ACKNOWLEDGMENTS.

The writer is indebted to Doctors E. A. Bessey and G. H. Coons for many valuable suggestions throughout the course of experiments, and for thorough criticism and correction of the manuscript.

Table of Contents.

# BACTERIAL SOFT ROT OF LETTUCE.

## INTRODUCTION.

The growing of head lettuce (Lactuca sativa L.) on a commercial basis has proved very profitable, due to the extremely high demands on the market for this type of vegetable. Large areas in Michigan are well suited for growing this crop, especially for the early and late summer market. Since the trade demands a fresh crisp product, and two of the large market centers, Chicago and Detroit, are so accessible, there is great incentive for the development of the industry, with prospects of the state becoming one of the leading producers of this crop.

One of the limiting factors in this development is the matter of plant diseases. Lettuce in this regard is no exception in the list of intensive crops, and is subject in to several very serious parasitic diseases. Various root rots are common and loss from them has been very discouraging to growers. But the most serious obstacle is found in the so-called soft or black rot which, although common in the field under certain conditions reaches its height under transportation conditions. In recent years some attention has been directed to this type of disease, and several organisims have been described as a result of this work.

It is the purpose of this paper to show the results of experiments and investigations carried out during 1919, 1920 and 1921, which it is believed point to some of the underlying facts dealing with the cause of this disease and thus permit

the making of some recommendations as to possible methods of prevention of loss. The investigations also lead to the discovery of six new pathogenes, and these are herein described.

## ECONOMIC IMPORTANCE OF SOFT ROTS OF LETTUCE.

### Field Losses.

Since the market demands a fresh, sound product free from blemish and disease, it is very evident that soft-rot is a major disease of this crop. There have been several reports of epidemics of this disease in the fields in recent years. It was reported as completely destroying a 200 acre field in Louisiana during the winter of 1914-1915 (1) By actual count a field 3½ acres in South Carolina (2) was found to be 98 per cent diseased, and another field of 17 acres suffered at least 60 per cent loss. Other cases have been reported from Virginia and along the Rio Grande Valley in Texas. It is a generally known fact that when a field is once infected it is useless to try to put the product on the market, unless special precautions are taken to select those heads which are absolutely free from disease, and this would mean a loss of considerable time and money.

### Market Losses.

So common is the disease that at this Station during the course of these investigations it was difficult to procure head lettuce suitable for experimental work. Crate after crate secured on the market were rejected because of rot. Inspections

of stock at commission houses at various times commonly showed the stock on hand was suffering from rot, the outer leaves of the head being badly rotted and the inner leaves frequently partially decayed. Before this produce goes to the consumer, these outer rotted leaves are removed, along with such of the inner leaves as are rotted, and often times this shrinkage from blemished leaves amounts to pratically one-fifth to one-fourth of the entire head. Hence from this point of view it is the consumer who suffers the damage by receiving a smaller and inferior product. Taking this into consideration it is difficult to make an estimate of the amount of damage that is really done, but the loss may be constantly placed at from 25 to 50 per cent of the crop. Figures from the Plant Disease Survey Bulletin, Volume III, 1919, show that car load shipments from different states, reveal from 1 to 100 per cent infection, often as high as 20 to 40 per cent of the shipment being worthless.

## PREVIOUSLY DESCRIBED BACTERIAL SOFT ROT OF LETTUCE.

Bacterial diseases of lettuce of the type considered in this paper have been known in this country for a number of years. Such troubles have been reported from Vermont, Massachusetts, Louisiana, Florida, South Carolina, Virginia, Kansas and along the Rio Grande river in Texas.

A bacterial stem-rot of lettuce was reported by L. R. Jones in 1893 (3). He found a large Bacillus in the diseased

stems but did not make isolations. He reproduced the disease by setting plants in soil which had been inoculated with fragment of diseased plants; and also by pouring over the roots of healthy plants water in which a diseased lettuce head had been crushed.

Pietro Voglino (4) reported a bacterial disease of lettuce in Italy in 1904, and named the causal organism Bacillus lactucae. This disease was reported as causing serious trouble in the gardens around Turin, and had occurred annually for ten years before definite study was made. Maximum damage was done where the plants were heavily fertilized. Voglino describes the organism as a long rod form, producing on the lettuce gelatine, ivory white colonies which rounded up and after 15 days assume a rose-colored tint.

In 1907, G. E. Stone (5) gave a brief account of a bacterial disease, which he found occurring in the greenhouse on leaves of lettuce that had been rapidly forced. Stone believed this disease was the same as that investigated in his laboratory by Percival C. Brooks six years earlier. Brooks isolated an organism, and reproduced the disease by inoculations; but due to the belief that the disease was caused by the succulent growth of the plants, and was of very minor economic importance, he did not make any studies of the organism.

F. L. Stevens (6) in 1908 reported a bacterial disease of lettuce. He described the disease as causing a pale, green-yellow coloring of the leaf which finally becomes brown. The tissue of the diseased leaves when dry disintegrates, leaving

a striking net work of veins. Miscropic examinations of diseased tissue revealed numerous long rod forms of bacteria. He isolated the bacterium, but was not successful in reproducing the disease. He did not describe the organism.

In 1908 H. S. Fawcett (7) reported a bacterial disease of lettuce as producing dark brown irregular areas on the margin and other parts of the leaf. Occassionally these spots occurred only on one side of the leaf, while the opposite side remained green. Other characteristics of the disease were browning of the midrib, often times starting on the leaves that had already headed up and progressing from leaf to leaf until finally the entire head was blackened. He grew the organism in pure culture and reproduced the disease by inoculations. The organism stained well with carbol fuschin, Aqueous genetian violet, but with difficulty with Methylene blue. On standard agar the colonies had pearl white foci with irregular margins.

O. F. Burger, in 1912-1913 (8) described a bacterial disease of lettuce which caused browning of the margin of the leaves or spotting along the midrib; the entire leaf often being spotted. On the head the disease was observed to begin at the center, causing a blackening and softening of the head. The organism was large, motile, occurring in chains with endospore. Burger believed that the organism was a species of Pseudomonas. Inoculations of the organism upon healthy growing lettuce plants gave positive results.

C. W. Carpenter (10) in 1916, reported a bacterial disease of lettuce, from Texas along the Rio Grande Valley. He described the grass symptoms of the disease as (1) reddening

of the older leaves and blanching of the younger central ones; (2) restricted development of newly forming leaves, accompanied by small, dark-colored blister spots along the border; (3) development of numerous, lateral adventitious shoots, and (4) dry and dead small rotts. He proved the absence of parasitic insects and fungi in connection with the disease, but did not describe the organism.

In summing up the earlier investigations of the bacterial disease of lettuce, it appears that the descriptions of the pathogenes are very incomplete, and it is often impossible to make a comparison with those under consideration in this paper.

Miss Nellie A. Brown (1) in 1915 was the first to describe fully a bacterium causing a disease of lettuce. The diseased specimens were sent in to the U. S. Department of Agriculture from Louisiana. The disease was described as making its appearance first on the outer leaves which become spotted and darkened throughout. Positive results were produced by inoculating the organism which she isolated onto healthy plants. The bacterium grew well on steamed potato, producing a dark blue-green color, and due to this pecularity the name Bacterium Viridilividum was suggested for the organism.

In 1918 Miss Brown described two other new species of bacteria which were the cause of disease outbreaks in South Carolina, Virginia and Kansas.

The diseased plants from South Carolina were described as being of a lighter green color than healthy ones. In later stages the head may show rot through the center, on the top, or a general wilting of the head may occur without visible

signs of spots; and again only the outer leaves may be affected. The stems of the diseased plants were brittle, and in early stages a cross section through these stems showed a blue-green color and later it was brown. The name Bacterium Vitians was suggested for the organism. The same organism was isolated from diseased lettuce sent from Virginia.

The symptoms of the Kansas lettuce disease were described as the wilting of the tips of the leaves, first appearing as very small areas, which very often coalesce and become quite large. The vascular system often showed browning, causing the appearance to be marred but no decay of the heads. Miss Brown suggests the name Bacterium marginale for this organism. She also isolated the same organism from diseased lettuce sent in from Virginia.

## SYMPTOMS OF THE DISEASE UNDER CONSIDERATION.

The disease under consideration in this paper usually follows tip-burn or some form of injury of the host plant. When plants are affected the decay is rapid, often causing a rotting of the entire head. The older leaves are ordinarily attacked first, but in some instances the disease has been known to start on the younger leaves. The most pronounced signs of the disease are the spots appearing on leaves that have tip-burn. These spots are very small when first noticed, but in moist warm weather they increase rapidly in size and number and coalesce, until the entire leaf may become rotted.

The spots are first light green in color, are translucent with a water-soaked appearance. Soon they turn dark brown and slimy. When this rotted tissue becomes dry there remains the network of veins which are partially decomposed into stringy masses. On reaching the vascular system, a rapid browning ossurs throughout it. Cross sections of diseased main stems show a dark greenish-brown color of the vascular system, which on exposure becomes almost black.

In the Hortigultural Gardens at the Michigan Agricultural College an out-break of disease occurred in the fall of 1920 in a small patch of lettuce after a frost, which was followed by several warm, moist, cloudy days. The disease was first noticed on the center of the heads, which rapidly caused the decay of the entire head.

In transportation the disease most commonly occurs on the outer leaves. These rotted leaves are soft, brown to almost black and slimy. When the rot once gets started it goes rapidly from the outer to the inner leaves; finally the entire head often becoming a slimy, rotted mass.

## SOURCE OF DISEASE AND METHODS OF ISOLATION.

In September 1919 specimens of diseased head lettuce were brought to the Michigan Agricultural Experiment Station from the greenhouse at Kalamazoo, Michigan. On making miscropic examinations of sections from diseased tissue, numerous bacterial

rods were found. The organism was isolated and numerous inoculations made by needle puncture onto healthy growing lettuce plants and also heads in moist chambers, with positive results. The organism was reisolated and proved to be the same. Two weeks later on visiting one of the commission houses at Lansing, it was observed that they had just received a shipment of California Iceberg lettuce, which was very badly diseased. Two organisms were isolated from different heads of this lettuce and inoculations gave the same positive results.

In October 1920 a bacterial disease of lettuce started in the Horticultural gardens at the Michigan Agricultural College. Isolations were made and from three different spots on the same head. Three different organisms were found, which produced the disease from inoculation.

In making isolations, two methods were ordinarily employed viz. (1) method as described by Miss Brown. Small pieces of the diseased leaf were treated with Mercuric Chloride 1-1000 for one minute, washed in sterile distilled water, then crushed in a tube of broth, and dilutions were plated out on standard Nutrient Agar. (2) In case where the heads had started rotting, the outer leaves were carefully removed, and with a sterile platinum needle, bits of diseased tissue that had not been exposed to the air were transferred to broth tubes and plated out as above. By both methods it is possible to get an almost pure culture. The latter is preferred on account of being more convenient, but in some cases where the disease only shows up on the outer lower leaves it is necessary to use the former method.

For convenience during these investigations, each organism was numbered, and is referred to, through this paper as follows: Kalamazoo organisms #1. Organisms isolated from diseased lettuce secured from commission house #2, and #3 and organisms isolated from diseased lettuce from the Horticultural Gardens at Michigan Agricultural College #4, #5, and #6.

## PATHOGENICITY.

Soon after the organisms were isolated numerous tests of their pathogenicity were made by inoculating head lettuce in moist chambers. In testing the different organims, in case of all experiments, reinoculations of the organisms were made, also microscopic examinations of the diseased tissue were made to prove the absence of any fungi or other invading parasites.

There were two kinds of moist chambers ordinarily employed for carrying out the experiments; tin boxes 12 x 10 x 6 inches and glass dishes 10 inches in diameter. These chambers were always sterilized before use with 1 - 1000 mercuric chloride, and towel paper was placed inside and moistened with sterile water. The tin boxes proved to be much more convenient, where whole heads of lettuce were to be used due the size, where as the glass dishes were preferred in case of single leaf inoculations.

Sound heads of lettuce were selected, washed and then rinsed in sterile water. Single leaves and whole heads were placed in these moist chambers, and inoculated. The inoculations were made by placing a loopful of a 24 hour bouillon culture

diluted in sterile distilled water on the leaf and then rupturing the epidermis with a sterile platinum needle through the drop. In case of the whole heads, inoculations were made near the center. These inoculations were made in triplicate and kept at room temperature. The results of this experiment are shown in Table I. The results were identical for the six organisims used.

TABLE I.

Pathogenicity: Test of Head Lettuce in Moist Chambers.

| Method of inoculation | Results. | | | |
|---|---|---|---|---|
| | 2 days | 3 days | 5 days | 10-12 days |
| Drop of 24 hour Bouillon culture place on leaf and epidermis rupture with needle through drop | Light green yellow water soaked area 1 mm in diameter at p point of puncture. | Rotted area brown, translucent, voscular system browning | All outer leaves of head rotted spots on inner leaves. | Head a slimy motted mass through out dark brown bad odor. |
| Control | Sound | Sound | Sound | Sound |

As shown in the table in this experiment, in the inoculations began to show light green yellow areas for 1 mm immediately around the point of inoculation in two days. These areas had a water soaked appearance, turning brown on the third day. The vascular system became brown in three days, and from this

time on a rapid decay of the leaf followed, burning complete in five days. At this time the inner leaves were showing rotted areas. In 10-12 days the rot had progressed through the entire head, leaving a dark brown, slimy rotted mass. The checks remained sound.

A series of healthy lettuce plants that were just beginning to form heads was selected. These plants were growing in 6 inch pots. Inoculations with the organisms were made by placing a drop of bouillon culture diluted in sterile distilled water on one of the leaves in the center of the head, and rupturing epidermis with a sterile platinum needle. Large bell jars containing pieces of moistened filter paper were placed over the pots. Six plants were inoculated with each organism and the same number untreated were kept under similar conditions for control. The results of this experiment are shown in Table II

TABLE II

Pathogenicity: Test of Growing Plants Under Bell Jars.

| Organism | Method of inoculation | Results. | | | |
|---|---|---|---|---|---|
| | | 2 days | 3 days | 5 days | 12-14 days |
| 1 | 1 drop bouillon culture diluted in sterile distilled water. Epidermis punctured. | ——— | Light green yellow areas. water soaked appearance 1 mm in diameter. | Vascular system of entire inoculated leaf brown rotted area 1 mm in diameter irregular | Plants all dark brown fallen over, slimy. |
| 2 | Do. | 2 out of 6 plants show very small rotted areas. light green. | All plants show rotted areas at point of inoculation. Vascular system browned in 2 of plants | Vascular system. All brown spots on inner leaves brown. | Do. |
| 3 | Do | ——— | As in #1 | As in #1 | Do |
| 4 | Do | ——— | Do | Do | Do |
| 5 | Do | ——— | As in #2 | As in #2 | Do |
| 6 | Do | 3 out of 6 plants show very small light green yellow areas. | As in #2 | As in #2 | Do |
| Control | Untreated | Plants healthy | Plants healthy | Plants healthy | Plants healthy |

This experiment shows that infection took place from two to three days after inoculations were made. It indicates that organisms #2 and #6 are more virulent than No's 1,3,4 and 5,as infections were noted to take place one day earlier. The vascular system became brown in three days in these two cases, and in five days the vascular system was browned in all cases, the decayed areas of the leaf being typical as described in the preceding experiment. In five days all the outer leaves were browned and on examination the inner leaves were found to be spotted. The decaying progressed very rapidly, extending from leaf to leaf until at the end of twelve to fourteen days the plants were all dark brown, almost black, slimy masses and part of them had fallen down. The controls all remained healthy.

After noting in the preceding experiments that, when the heads of lettuce were once infected, the decay progressed from leaf to leaf until the entire head was decayed, a more careful experiment was planned. Heads were inoculated in moist chambers, care being taken to rupture the epidermis only. It was noted that spots began to appear under the inoculated areas in one to two days. This rot continued from leaf to leaf very rapidly through the entire head.

Carrots and potatoes were selected, washed, dried and heated with mecuric chloride 1-1000 for one minute and then washed again in sterile water. With a clean knife they were cut up in disks about 1 cm in thickness. The outer layers of onion bulbs were pealed away and disks cut in the same manner.

Single leaves of cabbage were also used, and lettuce was used as a check for the experiment. This material was placed in moist chambers and inoculated. The inoculations were made on the carrot, potato and onion by placing a loopful of diluted bouillon culture on the cut surfaces of the disks. The cabbage and lettuce leaves were inoculated by placing a drop of the diluted culture on the leaf and rupturing the epidermis through the drop. Moist chambers were kept at room temperature.

The results of this experiment are shown in table III

TABLE III

Pathogenicity: Test of carrots, onions, potatoes and cabbage.

+ sign indicates beginning of rot - indicate no effect.

| Organism | Method of inoculation | Carrot | | | Onion | | potato | | cabbage | | lettuce. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1d | 2d | 10d | 2-3 d | 10d | 3d | 10d | 2d | 10d | 2d | 10d |
| 1 | Drop of 24 hour bouillon on surface of disk lettuce and cabbage inoculated as in preceding exp. | + swelling | watery exudation | exudation and rotted tissue brown | + | very offensive odor | - | - | + | Blackening of 2mm in diameter | + | all rotted. |
| 2 | do | + | do | do | ? | do | - | - | + | do | ? | do |
| 3 | do | + | do | do | ? | do | - | - | + | do | + | do |
| 4 | do | + | do | do | + | do | - | - | + | do | + | do |
| 5 | do | + | do | do | ? | do | - | - | + | do | ? | do |
| 6 | do | + | do | do | + | do | + yellow growth | tissue gray rotted mass | + | do | ? | do |
| control | untreated | - | - | - | - | - | - | - | - | - | - | - |

The experiment shows that the organisms are all pathogenic to carrots, onions and cabbage, while organism No. 6 is the only one pathogenic to potatoes. In case of the carrots swelling occurred on the surface of the disk in one day. In two days a clear watery exudation appeared which turned dark brown in three days, together with the tissue beneath the exudation. In ten days the rot had extended through to the under side of the disk, the whole disk being almost completely rotted.

In three days at the point of inoculation on the onions appeared small water soaked areas, which turned black two to three days later. The disks were a slimy, rotted mass in twelve-fourteen days with a very offensive odor.

Only organism No. 6 produced infections on potato. In three days there appeared an orange-yellow slimy growth on the surface of the disk. In five days the tissue had become brown over the entire surface of the disk. In ten-twelve days there was a sunken area in the disks at the point of inoculation, the rotted tissue being a grey, watery mass.

In case of the cabbage there appeared blackened areas 1mm in diameter at the point of inoculation in two days. These spots never became very large. At the end of the experiment the spots were about four to six mm in diameter, and had a dry appearance. The controls in this experiment all remained sound.

During the course of these experiments it seemed advisable to test various methods of inoculating plants. In the following experiment, young plants were inoculated and bell jars

with pieces of moistened blotting paper in the tops, were placed over the pots. Inocluations were made as follows: (1) a loopful of a diluted bouillon culture was placed on the leaf and then the epidermis was ruptured through the drop; (2) a transfer was made with the diluted culture with a needle direct into the tissue of the leaf; and (3) in each of the above cases moistened absorbent cotton was placed over the points of inoculation. The results of this experiment is shown in Table IV The rotting is indicated by a plus sign (+) The number of them indicating the degree of the rot. A minus sign (-) indicates no rotting.

TABLE IV

Effect of Different Methods of Inoculations.

Test of Growing Lettuce Plants.

| Organism | Methods of Inoculation and Results. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | (1) Drop of diluted culture placed on leaf and epidermis ruptured thru drop. | | | (2) Direct Transfer of culture by puncture of leaf tissue with needle | | | (3) Same as 1. with moistened absorbent cotton placed over point of inoculation | | |
| | 2d | 3 2 | 5 d | 2 d | 3 d | 5 d | 2 d | 3 d | 5 d |
| 1 | + | + + | + + + + | - | - | + | - | - | - |
| 2 | + | + + | + + + + | - | + | + + | - | - | - |
| 3 | + | + + | + + + + | - | - | + | - | - | - |
| 4 | + | + + | + + + + | - | - | + | - | - | - |
| 5 | + | + + | + + + + | - | - | + | - | - | - |
| 6 | + | + + | + + + + | - | + | + + | - | - | - + |

This experiment shows that the method of placing a drop of culture of the leaf and then rupturning the epidermis is very successful; while the adding of absorbent cotten after inoculations were made seems to retard or check the growth of the organism. Disease appeared in the former method in two days in every case; while only one inoculation was positive in the latter The case of slow growth where the direct transfer was made with a needle may be explained in the fewer number of organisms. There seems to be some difference due to the amount of inoculation used.

Similar experiments were performed with carrot disks. Carrots were prepared and disks were cut as already described. These disks were placed in moist chambers and inoculated as follows. (1) A loopful of diluted culture of the organism was placed on the cut surface of the disk; (2) same as one, with a small piece of moistened absorbent cotton placed over the point of inoculation. (3) same as one with a small filter paper cone placed over the disk and kept moistened with sterile water, Moist chambers were incubated at room temperature.

The results of this experiment are shown in Table V

TABLE V

Effect of Different Methods of Inoculation.

Test on Carrot.

+ signs indicate degree of rotting. - signs indicate no rotting

| Organism | Methods of Inoculation. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | (1) Loopful of organism placed on cut surface of disk | | | (2) Same as 1, with moistened absorbent cotten over point of inoculation. | | | (3) Same as 1 with a small moistened filter paper cone over disk | | |
| | Results | | | Results | | | Results | | |
| | 1 d | 2 d | 3 d | 1 d | 2 d | 5 d | 1 d | 2 d | 5 d |
| 1 | + - | + | + + + | - | - | - | + | + + | + + + + |
| 2 | + - | + | + + + | - | - | - + 1 in 6 | + | + + | + + + + |
| 3 | - | + | + + + | - | - | - | + | + + | + + + + |
| 4 | - | + | + + + | - | - | - + 1 in 6 | + | + + | + + + + |
| 5 | - | + | + + + | - | - | - | + | + + | + + + + |
| 6 | + - | + | + + + | - | - | - + 1 in 6 | + | + + | + + + + |

This experiment checks with the preceding. It seesm that the absorbent cotton almost completely checks the growth of the organisms. There were six inoculations made with each organism and only one of these inoculations with three of the organisms was positive, and the disease was very slow in making its appearance. By placing a moistened filter paper cone directly over the disk after inoculation, hastens infection. Watery exudation appeared in case of each organism in one day. This experiment shows that the organism produced the greatest amount of damage under moist conditions.

That there is considerable difference in the progress of the disease, where the host is infected at different points is shown in the following experiment. Healthy growing lettuce plants and head lettuce in moist chamber were inoculated at the different points on the leaves as follows: (1) in the parenchyma tissue of the leaves between the veins; (2) in the small veins; and (3) in the midrib and main stems. Bell jars with moistened pieces of blotting paper in the top were placed over the growing plants. The results of this experiment were practically the same with each organism as shown in Table VI

TABLE VI

Effect of Inoculating at Different Points:

Test of Lettuce Plants.

| Organism | point inoculated | Results. | | | |
|---|---|---|---|---|---|
| | | 2 days | 3-4 days | 5-8 days | 10-12 d. |
| 1-6 inc. | Parenchyma tissue between veins | diseased area 1 mm in diameter | disease reached veins, browning 1 cm | disease spread through Vascular system to other leaves. | plants dark brown and rotted. |
| 1-6 inc. | Small veins | browning of veins 1 cm from point of inoculation | Vascular system all brown in leaf | Entire plant dark brown | Rotted mass |
| 1-6 inc. | midrib | browning 1 cm from point of inoculation | Do.- also browning of veins in other leaves | Do | Do |
| Control | no treatment | Healthy | Healthy | Healthy | Healthy |

As shown in this experiment there is a marked difference in the progress of the disease, when inoculations are made at different points on the leaf, however, actual decay of the tissue at the point of inoculation begins about the same time, in two days. In case where the parenchyma tissue was inoculated, the progress of the disease was rather slow until

it reached the veins, then a rapid browning of the vascular system occurred, as in case of the direct inoculations of the veins and midribs. In two days in case of the direct inoculations into the vascular system, browning occurred 1 cm from the point of inoculation. A decay of the accompanying parenchyma tissue was observed to take place very rapidly. In two or three cases where the main stem was inoculated, the plants wilted in four to five days. Examinations showed that the vascular system was plugged. Cross sections of diseased main stems in the early stage, show the vascular system as being dark greenish brown in color, and turning black on exposure to the air.

A series of dilution flasks was prepared by adding 10 cc of bouillon culture of the organism to 90 cc of sterile distilled water, making 1-10 dilutions. From these flasks dilutions of 1-100, 1-1000, 1-10,000 and 1-1,000,000 were made in the ordinary way. Inoculations were made on healthy lettuce heads in moist chambers by placing a drop of each of these dilutions on the leaves and then rupturing the epidermis through the drop with a needle. Heads were also inoculated with an undiluted bouillon culture of the organism. The moist chambers were incubated at room temperature. Results of this experiment are tabulated as plus signs in case of positive results and minus signs in case of negative results, and are shown in Table VII

Table VII

Effects of Different Dilutions of Inoculum:

Test on Head Lettuce in Moist Chambers.

| Organisms | Dilutions | Results. | | | | |
|---|---|---|---|---|---|---|
| | | 1 day | 2 days | 3 days | 4 days | 5 days |
| 1-6 inc. | Bouillon | + | + + | + + + | + + + + | + + + + + |
| 1-6 inc. | 1-10 | + | + + | + + + | + + + + | + + + + + |
| 1-6 inc. | 1-100 | - | + | + + | + + + | + + + + |
| 1-6 inc. | 1-1000 | - | - | + | + + | + + + |
| 1-6 inc. | 1-10,000 | - | - | - | + | + + |
| 1-6 inc. | 1-1,000,000 | - | - | - | - | + |
| Control | No treatment. | - | - | - | - | - |

The results of this experiment were very striking, as shown the dilutions containing the larger amount of organisms, produced infection much earlier than the weaker dilutions. But after the disease once made its appearance, it progressed rapidly in all cases.

Relations of the Disease to Tip Burn.

Early in the investigation an experiment was performed by spraying a diluted culture of the organisms onto healthy uninjured plants. Bell jars with moistened pieces of filter paper in the top were placed over these plants. At the end of two weeks the plants were still healthy. This was repeated several times with the same negative results.

It was noted in the field that this disease nearly always accompanied tip-burn which is an extremely common disease of lettuce. In growing plants in the greenhouse for experimental work, invariably a large per cent were affected with this type of trouble. One instance was particularly noticeable. There had been about a week of cool, damp, cloudy weather, which was followed by several warm, bright sunny days. Shortly afterwards practically all of the plants that were nearing maturity began to show the typical dying along the margin of the leaves; the older leaves becoming affected first.

A series of plants was selected that showed the typical burning on the margins of the leaves, and also perfectly healthy plants were selected. These healthy plants and those having tip-burn were inoculated with a 1-100 dilution of the organism, by spraying with an atomizer. Bell jars with moistened pieces of filter paper were placed over the pots. Six plants were inoculated with each organism, and the same number of untreated plants showing tip-burn were kept under the same conditions for control. This experiment was repeated twice. Results are shown in Table VIII. Since the results were the same with all six organisms they are not shown separately in the table.

TABLE VIII

Relations of the Disease to Tip-Burn.

| Organism | Condition of plants | Method of inoculation. | Results. | |
|---|---|---|---|---|
| | | | 2 days | 12 days. |
| 1-6 inc. | Tip-burn | Sprayed with a 1-100 diluted bouillon culture. | Rotted areas 1 mm broad showing beneath tip-burn on sides leaves. Spots have typical soft-rot appearance | Plants all dark brown and pulpy |
| 1-6 inc. | Healthy | Do | No signs of disease | No signs of disease |
| Controls | Healthy and Tip-burn | no treatment | no signs of disease | no signs of disease. |

In this experiment it is shown that the disease starts within two days after inoculation where the plants are affected with tip-burn. The organism after gaining entrance into the host tissue, progresses in the typical manner, soon causing the entire plant to be broken down. The controls and healthy plants that were inoculated did not show any signs of the disease at the end of two weeks.

Differences in Pathogenicity from Previously Described Diseases.

In the foregoing experiments it is shown that the organisms causing the disease under consideration only attack lettuce plants following injury or some other disease. This disease differs in these respects from any of the previously described diseases, as the organisms causing the latter have been reported as attacking healthy lettuce plants. The Kansas pathogene described by Miss Brown (2) was characterized by attacking the margins of leaves on healthy plants while the South Carolina and Louisiana pathogenes and also the pathogenes described by Burger (9) were described as attacking healthy plants at various points.

## TEMPERATURE RELATIONS OF THE DISEASE.

That the temperature is a factor in development of the disease is shown in Table IX Lettuce heads were placed in moist chambers and inoculated with a diluted bouillon culture of the organism by placing a drop of the culture on the leaf and then rupturing the epidermis with a needle. Three sets were prepared. One set was incubated at room temperature 23° to 25° C. A second set was incubated at 10° to 12°C, and a third set was incubated at 5° to 7° C

The results were identical for all six organisms

TABLE IX

Effect of Temperature: Test of Head Lettuce in Moist Chambers.

| Organism no. | Temperature | Results. | | | |
|---|---|---|---|---|---|
| | | 2 days | 3 days | 4 days | 5 days |
| 1-6 inc. | 23°-25°C | + | ++ | +++ | +++++ |
| 1-6 inc. | 10°-12°C | - | + | ++ | +++ |
| 1-6 inc. | 5°-7°C | - | - | +<br>- | + |
| Controls | Each of above cases | - | - | - | - |

This experiment shows that infection takes place much more quickly and the progress is more rapid at temperature from 23°to 25°C. This agrees closely with the temperature for growth.of the organisms in bouillon and on agar cultures. It was found that the organisms still maintain their vitality after being frozen in broth cultures for 24 hours.

DISTRIBUTION OF SOFT ROT ORGANISM IN THE SOIL.

Samples of soil were collected from several fields at or near the Michigan Agricultural College; from the Horticultural Gardens, where diseased lettuce had grown; and from woodland that had never been under cultivation. Small bits of these samples of soil, inoculated upon carrot disks,

produced rot in from 2 to 4 days; with the exception of samples taken from compost consisting of muck, sand and manure that was ready for use in the greenhouse from which the results were negative. The organisms were isolated from these rotted carrots, and tests were made on lettuce in moist chambers also with positive results.

On making a brief study of these organisms, two of the organisms isolated from the soil from the Hortigultural Gardens proved to be identical with organisms four and six isolated from diseased lettuce on the same plot. An organism identical with No. 6 was also isolated from one of the samples of field soil. Several years back the particular area had been used for garden purposes. This experiment shows that the soft rot producing organisms are rather widely distributed in the soil, and makes it quite evident that the disease comes from soil infections.

## DESCRIPTION OF THE PATHOGENES.

### Organism No. 1

The organism is a small rod with rounded ends, occurring singly, in pairs and rarely in short chains. The measurements of single rods vary from 0.46 to 0.79 microns for the length and 0.37 to 0.58 microns for the width, the average size being 0.58 x 0.40 microns. The organism seems not to be motile.

Capsules were stained from old bouillon cultures with Welch's capsule stain. The absence of endospores was tested by staining and by boiling old bouillon for three minutes.

No growth took place from transfers after boiling. Pseudozoogloeae occur in old bouillon cultures and on potato, and are composed of several rods in chains attached together by gelatinous threads. No inoculation forms were noted.

Behavior Toward Stains.

The organism stains well with all anilin and aqueous, basic dyes such as fuchsin and genetian violet, also carbol fuchsin. It is Gram-negative and is not acid fast.

Cultural Characters.

Potato and bouillon proved to be very favorable media for prolonged growth.

Beef-Agar Plates:- The colonies on peptonized beef-agar plates (Ph 6.6) are visible in 24-36 hours, at temperature of 22° to 25°C, when poured from young bouillon cultures. They are at first bluish-white, translucent, smooth, thin, round with entire edges. When 2-6 days old they are ½ to 2mm in diameter, cream-white, round, entire edges, flat internal structure finely granular, and opaque.

Beef-Agar Stroke:- Moderate growth in 24hours at 23°-25°C filiform, flat, verrucose, glistening, translucent, opaque, with age and gradually spreading. Decided odor of decaying organic matter.

Agar Stab: In 2 days there is moderate growth on the surface of the stab. Feeble growth along line of puncture, which is filiform, Growth on surface is cream-white.

Beef Bouillon:- Peptonized beef bouillon (Ph. 6.8) is densely clouded, turbed with a pellicle in 24 hours at room temperature 22° to 25° C. In five days there occurs in some cases a veronese green (a) ring at top of medium about 1 cm broad which gradually disappears in ten to twelve days. In 5-8 days the pellicle begins to break up and hangs down through the medium in flakes; also a viscid sediment appears. In 3 weeks there is a viscid white sediment, the medium above clear and almost white. No.further change in 5 weeks.

Nutrient Gelatin:- Colonies appear in peptonized gelatin (Ph. 6.5) in 36-48 hours at 11° to 12° C . They are very small, never reaching over one-half to 2 mm in diameter. Are round, smooth, flat, glistening, white with entire edges. No liquefaction.

There is no liquefaction in stab cultures kept in water bath at 15° to 17° C. Growth appears on the surface of the stab in two to three days. Growth along line of puncture faint and filiform.

Uskinsky's Solution:- There is heavy clouding of the medium in 24 hours with white pellicle. Veronese green ring appears at top of medium in 2 days, extending throughout the medium in 8 to 10 days. In 2 weeks there is a heavy pellicle, cream yellow and sediment at bottom. Solutions start changing

(a) The colors in this paper are given according to Ridgway, Robert-Color Standards and Color Nomenclature 43 p. Col. pl. Washington, D. C. 1912

color in three weeks, pellicle disappears, heavy sediment five weeks; medium is clear above dull green yellow color with white sediment.

Cohn's Solution: There is a faint cloudiness in three days. five days,a pellicle which soon breaks and leaves small amount of white sediment. In 6 weeks the medium is still clouded, no further change.

Fermis' Solution: Medium is slightly clouded in 24 hours, densely so in two days and water green in color with pellicle. There is a viscid sediment in two weeks. In four weeks there is a heavy scum on top, mortius yellow. In five weeks the scum has all settled to the bottom. In six weeks there is a cream yellow viscid sediment, the medium above being still clouded and of pinard yellow in color.

Sterile Milk: No noticeable change in medium in five weeks. In six weeks the medium begins to clear up, and settles out in a fine curdlike percipitate.

Litmus Milk:- An alkaline ring appears at the top of the medium in two days, which gradually extends downward until the entire tube shows an alkaline reaction in ten days, with white pediment at bottom. In six weeks the medium is dark tyrian blue.

Steamed potato cylinders: There is an abundant growth filiform in two days, which gradually becomes spread, glistening, raised contoured, and slimy. The growth is first at dirty cream yellow, then changing to a cinnamon-buff and finally in four weeks to clay color. The medium is changed to a dark greyish brown. No diastatic action on the starch.

Further tests were made for diastatic action on starch, by adding 0.2 per cent soluble potato starch to nutrient agar. Plates were poured and streak cultures were made on the plates after the agar had hardened. At 5,7 14, and 17 days tests were made by pouring Lugol's iodine solution over the plates. No light areas appeared near the growth. Checks were run with organisms known to decompose starch, in each case light areas appeared around the growth.

Other Cultural Features of the Organism.

Ammonia Production — Abundant.

Indol — Indol is not produced.

Nitrate Reduction: Nitrates are not reduced.

Tests were made when nitrate bouillon cultures were 7, 14, and 17 days old.

Hydrogen Sulphid: Hydrogen sulphid is not produced. Tests were made by hanging pieces of lead acetate paper in mouths of tubes of bouillon, potato and milk cultures.

Toleration of Acids.

The organism is very sensitive to citric and malic acids. Growth occured in neutral beef bouillon with o.0 per cent of tartaric acid added, titrating +13 on Fuller's scale. No growth occurred in a o.2 per cent solution of the same acid, nor in 0.1 per cent solution of citric and malic.

Toleration of Sodium Hydroxide.

The organism does not tolerate sodium hydroxide above -15 Fuller's scale. Tests were made in neutral beef bouillon reading -15, -20 and -25 Fuller's scale. No growth occurred

at this reaction.

Temperature Relations.

Thermal Death Point. When 24 hour bouillon cultures were kept at 54°C for ten minutes in capillary tubes in water bath, no growth occurred when transfered to bouillon. Sometimes growth occurred after heating at 53° C. Therefore, the thermal death point of the organism is between 53° and 54°C.

Minimum Temperature. The minimum temperature for growth is bwlow 0°C. Tests were made by keeping bouillon culture in crushed ice and salt for two days, at a temperature below 0°C.

Optimum Temperature: The optimum temperature for growth is between 23° and 25° C.

Maximum Temperature: The maximum temperature for growth is 40°C.

Gas Formation.

The organism is a facultative anaerobe and does not form gas. Tests were made in fermentation tubes 1 per cent peptone water and bouillon to which 1 per cent of dextrose, lactose, saccharose, maltose, glycerin and mannit respectively were added. Growth occurred in the closed ends of all tubes. No change in PH.

Future Tests for Anaerobism.

Deep culture agar plates were poured and inverted. 1 gm of pyrogallic acid was placed on the cover on a small piece of cotton 10 cc of 20 per cent NaOH was added and the plates

were easled with nodelling clay. Also slants were inoculated with Giltuer H tubes and oxgen taken up in the same manner. Colonies appeard in 24 to 36 hours, and growth on the slants was visible in 24 hours.

Relations to Light.

The organism is not particularly sensitve to sunlight. Thinly seeded agar plates were poured, and half of each plate was covered with black paper, The plates were exposed to direct sunlight in April and May. No growth appeard on the exposed side of the plate after being exposed for 50 min. 15 colonies on dark side. On the plates exposed 45 min. 1 colony appeard on the exposed side while there were ten colonies on the unexposed side.

Relation to Moisture.

The organism is not killed readily by drying. Drops of 24 hour bouillon culture of the organisms were placed on cover slip in petri dishes, and were kept in the dark at room temperature 23° to 25° C. On making transfers of pieces of the slips to bouillon, No growth occured after the third day. Growth occured in those tubes reciving the slip after being incubated for 2 days.

Loss of Virulence.

No loss of virulence of the organism was noted when inoculations were made on lettuce 18 months after isolation.

Pathogenicity.

The organism is pathogenic to lettuce, carrot, onion and cabbage, only in case of injury or following some other disease of the plants.

Group Number.

According to the descriptive chart of the Society of American Bacteriologists the group number is 222,3333033

Brief Technical Description of the Organism.

The organism is a short rod with rounded ends; non-motile; capsules; pseudozooglocae; no spores; no involution forms noted; faculatative anaerobe; agar colonies, cream white; clouds bouillon very heavily in 24 hours, with a veronese green ring at top in six days; growth on potato cylinders is abundant, growth first a dirty cream yellow later changing to common-buff and finally to clay color. The potato darkens slowly; no diastatic action; alkaline reaction in litmus milk does not liquefy gelatine; produces ammonia; does not reduce nitrates; does not produce hydrogen sulphid nor indol; non-fluoriscent; grows well in Uskinky; and Fermi's solutions; but very feebly in Chon's solution. Thermal death point 53° to 54° C (under conditions stated); Optimum temperature 23° to 25° C; Maximum 40° C; minimum below 0° C Stains well with basic anilin and aqueous dyes; is Gram-negative; not acid-fast; tolerates tartaric acid in 0.1 per cent; tolerates sodium hydroxide only -15 Fuller's scale; is not very sensitive to sunlight; is not killed readily by drying; retains its virulence for more than eight months.

Organism No. 2

The organism is a slightly curved rod with rounded ends, occurring singly, in pairs and occasionally in short chains.

The single rods vary from 1.04 to 1.50 microns long by 0.41 to 0.70 microns wide, the average size being 1.25 x 0.47 microns The organism is motile by means of flagella at one pole, varying from 1 to 3, more commonly one. They were stained with Loeffler's flagellar stain.

The absence of endospores was tested by staining and by heating. Capsules were stained with Welch's Capsule Stain. Pseudozoogloeae occur in old broth cultures and on potato, and are composed of short chains, attached by gelatinous threads. Few involution forms were noted, in acid and alkali media, as very small and swollen cells.

Behavior Toward Stains.

The organism stains readily in all basic anilin and aqueous dyes, carbol fuchsin and methylene blue. It is Gram-negative, and is not acid fast.

Cultural Charcters.

Beef bouillon and potato proved to be very favorable media for prolonged growth of the organism.

Beef-Agar Plates: The colonies appear on peptonized beef agar (PH 6.6) in 24 hours at room temperature 23° to 25°C when poured from young bouillon cultures. They are at first, round, small white and smooth. At three days they are cream-white, flat to slightly raised, verrucose, opaque, irregular with lobate edges, internal structure reticulate, and from 2 to 5 mm in diameter.

Beef-Agar Stroke: Growth 18 to 24 hours, abundant, filiform then spreading, flat, glistening, smooth, translucent

with decided odor.

Agar Stab: Moderate growth in 2 days on surface of stab. Growth feeble along line of puncture, filiform, surface growth cream-white,

Beef Bouillon: Medium is strongly clauded in 18 to 24 hours, with pellicle. In two days the medium is veronese green. The pellicle disappears and green color disappears in five to seven days, with flocculent sediment. Decided odor. The medium is still clouded in six weeks and has a colonial buff color.

Nutrient Gelatin:- Colonies appear on peptonized gelatin (PH 6.5) in two days at 11° to 12° C are very small, white flat, entire and glistening. The colonies are 1-3 mm in diameter. No liquefaction.

Gelatin stab cultures show growth at surface in 36 hours at 15° to 17° C. Growth along line of puncture is very feeble, filiform. No liquefaction.

Uskinsky's Solution:- Slight cloudiness in 24 hours, becoming dence with pellicle in 2 days. Light green-yellow ring at top in 3 days. The pellicle begins to break up and hang down through the medium in stringy masses. Yellowish green sediment in 2 weeks. In 6 weeks the color of medium is dull yellow green, with viscid cream yellow sediment.

Cohn's Solution. No growth.

Fermi's Solution: Slight cloudiness in 24 hours. Yellow pellicle in 2 days, which becomes a dense martius yellow scum in 2 weeks, the medium being clea. Five weeks the scum has disappeared and the medium is clear, and chartreuse yellow

with a cream yellow viscid sediment.

**Sterile Milk;** No change noted in medium in five weeks. In 6 weeks there is a pinkish scum on the surface, with fine curdlike particles floating and sediment.

**Litmus Milk:** An alkaline ring appears at the top of the medium in 24 hours. All the medium shows alkaline reaction in 8 to 10 days with a white sediment at bottom. The medium gradually changes in color, until at the end of 6 weeks it is pale windsor blue.

Steamed Potato Cylinders: There is abundant growth in 2 days, filiform then spreading slightly raised and contoured, glistening and slimy. In 10 days the growth is clay color, changing to tawny-olive then to sayal brown and is slimy. The medium is gradually browned. There is no diastatic action on the starch.

Further test for diastatic action on starch with streak cultures on o,2 per cent soluble starch agar. Tests with Lugol's iodine solution were negative.

Other Cultural Features of the Organizm.

| | |
|---|---|
| Ammonia Production | Abundant |
| Indol | No indol is produced. |
| Hydrogen Sulphid | Hydrogen sulphid is not |

produced. Tests were made with lead acetate paper with bouillon, milk and potato cultures, no blackening occurred.

**Nitrates.** Nitrates are not reduced. Tests were made on nitrate bouillon and peptone water after 7, 14, and 21 days by adding 1 cc of potato starch solution to each culture; then 1 cc of freshly prepared potassium

iodide solution (1:250), after which 5 drops of dilute sulphuric acid (2:1) were added. No blue color appeard.

Toleration of Acids: The organism will not grow in nutrient bouillon, with a 0,1 per cent of citric, malic acid, tartaric acid added.

Toleration of Sodium Hydroxide: The organism will not grow in neutral beef bouillon with sodium hydroxide added, titrating -20 Fuller's scale. Faint growth in -15 culture.

Temperature Relations.

Thermal Death Point: When 24 hour bouillon cultures were kept at 52° C for 10 minutes in capillary tubes in water broth, no growth occurred, when transferred to bouillon tubes. Growth occured occasionally when kept at 51° C Thus the thermal death point lies between 51° and 52° C.

Maximum. The maximum temperature for growth is 34° C.

Optimum Temperature. The Optimum temperature for growth is between 23° and 24° C.

Minimum Temperature. The minimum temperature for growth is below 0° C.

Gas Formation.

The organism is aerobic and does not form gas. Tests were made with fermentation tubes with peptone water containing 1 per cent of dextrose, lactose, saccharose, maltrose, mannit and glycerin respectively. Growth occurred in the open arms of the tubes but no growth in closed arm. There was a change in the Ph. reading of dextrose solution from 6.5 to 5.8, indicating slight change to acid. No change in any other solution. No gas.

Further Tests for Anaerobism.

Deep culture plates and Giltner H. Tubes were deprived of Oxygen with pyrogallic acid and sodium hydroxide. No growth occurred.

Relations to Light.

The organism is not particularly sensitive to sunlight. Plates were poured from young bouillon culture, and half of each plate covered with black paper and exposed to direct sunlight at noon day in April and May inverted on crushed ice. Plates that were exposed for 30 minutes showed from 10-15 colonies on uncovered side and 60-70 on covered side. Forty minute plates show 5-7 colonies on uncovered side and 50-60 colonies on covered side while on plates exposed for 50 minutes, no colonies appeared on uncovered side 70-80 on the covered side.

Relation to Moisture.

The organism is not killed very readily by drying. Drops of 24 hour bouillon culture were placed on cover slips in petri dishes and allowed to dry in the dark at 23° - 25° C, No growth occurred when slips were transferred to bouillon after 3 days. Growth occured after 2 days.

Loss of Virulence.

When lettuce plants were inoculated 18 months after isolation of the organism, no loss of virulence was noted.

Pathogenicity.

The organism rots lettuce, carrots, onions and cabbage, when entry is gained throughout diseased areas.

Group Number.

According to the descriptive chart of the Society of American Bacteriologists the group number is 212.2333033.

Brief Technical Description of the Organism.

It is a short rod with rounded ends; flagella 1 to 3, at one pole; capsule; pseudozooglocae; no spores, few involution forms noted; aerobic; Agar colonies first white; then cream white; clouds bouillon very heavily in 24 hours at 22° to 26° C, and in 2 days the medium is veronese green, growth on potato cylinders abundant, and in ten days is clay color which gradually changes to sayal brown; the potato darkens slowly. There is no diastatic action on potato starch; does not liquefy gelatin; alkaline reaction in litmus milk; produces ammonia; non fluorescent; does not reduce nitrates; does not produce hydrogen sulphid nor indol; grows well in Uskinsky's and Fermi's Solutions but not in Cohn's solution; Optimum temperature 23° to 24° C; maximum 34° C; minimum below 0° C, and thermal death point 51° to 52° C (under conditions stated). Stains well with basic anilin and aqueous dyes; not acid-fast; Gram-negative; does not tolerate acids very well nor sodium hydroxide; is not killed very readily by drying; not very sensitive to sunlight; retains its virulence for over 18 months.

Organism No. 3

The organism is a large rod with rounded ends, occurring singly, in pairs, and occasionally in short chains. The

single rods vary from 1.45 to 2.50 long by 0.66 to 1.25 wide, the average size being 1.88 x 0.83. Motile by means of 1 to 2 flagella at one pole, more commonly one. Flagella stained with Van Ermengen's flagella stain.

Capsules were stained with Welch's capsule stain. The absence of endospores was tested by staining and by heating. Pseudozooglocae occur in old bouillon cultures, and on potato. They are composed of short chains attached by gelatinous threads. Only few involution forms noted; in slightly acid and alkali medium, swollen cells and very small cells were seen.

Behavior Toward Stains

The organism stains well with all basic anilin and aqueous dyes. It is Gram-negative and not acid-fast.

Cultural Characters

Beef-agar Plates:- On peptonized beef-agar (Ph 6.6) colonies are visible in 24 hours at 22°- 25°C. The colonies are round, smooth, slightly raised, entire, white, opaque, and have a thick dense center. The colonies vary 1-4 mm in diameter.

Beef-Agar Stroke:- Growth occurs in 24 hours, abundant, filiform then spreading, flat, dull opaque and slightly verrucose. Ocassionally the medium is changed to viridiue green, This occurred in about one out of ten trials.

Agar Stab:- There is moderate growth in 2 days on the surface of stab. Feeble beaded growth along line of puncture. Surface growth is cream white.

Beef Bouillon:- Peptonized beef bouillon (Ph. 6.8) kept at 22° to 25° C is densely clouded, turbid in 24 hours with pellicle. In 2 days the medium is veronese green. In 5 days the medium is veronese green, pellicle broken up, with a flaky sediment. In 6 weeks the medium is cream white and clear, with a viscid sediment.

Nutrient Gelatin:- Colonies appear on peptonized gelatin (Ph. 6.5) in 2 days at 11° to 12° C. They are very small 1-3 mm in diameter, flat, entire, glistening, and no liquefaction.

The organism does not liquify gelatin in stab cultures kept at 15° to 17° C. There is a good growth on surface, contoured, and white. The medium in some cases is changed to viridine green for 1 cm at top, which gradually disappears. The growth along line of puncture is beaded.

Uskinsky's Solution:- There is a faint cloudiness in 24 hours. In 3 days the e is a white pellicle at top of medium. In 5 days the medium is greenish-yellow, with sediment at bottom. Pellicle begins to break up in 8-10 days. In 6 weeks the medium is still cloudy, water green in color, with a viscid white sediment.

Cohn's Solution:- The organism does not grow in Cohn's solution.

Fermi's Solution: The organism clouds Fermi's Solution in 24 hours, with a pellicle occurring in 3 days. In 5 days the medium has a pinkish tinge, In 2 weeks there is a pink scum on surface of medium, which changes to a citron yellow in 3 weeks. In 6 weeks the medium is still clouded, of a livid pink color with a pinkish white sediment.

Sterile Milk:- There is no noticeable change in sterile milk until 4 weeks, there occurrs a fine curdlike suspension, which settles out. In 6 weeks the medium is clear with a curdlike sediment.

Litmus Milk:- The organism produces an alkaline ring at top of medium in 24 hours; the medium gradually changes to alkaline throughout in 10 days, with a white sediment. The medium is still turbid in 6 weeks, dark madder blue in color.

Steamed Potato Cylinders:- There is an abundant growth on steam potato in 24 hours. The growth is first filiform, then spreading, contoured, glistening, slimy and of a dirty cream color which changed to fawn color in 10 days. In 5 weeks the growth is wood brown with a pinkish tint. The medium is gradually browned. There is no diastatic action on the starch.

Further tests were made for diastatic action on starch, with streak cultures on o.2 per cent starch agar plates. The tests were negative.

Other Cultural Features of the Organisms.

| | |
|---|---|
| Ammonia Production:- | Abundant. |
| Indol:- | Indol is not produced. |
| Nitrate Reduction:- | Nitrates are not reduced. |
| Hydrogen Sulphid | Is not produced. |

Toleration of Acid:- The organism clouds bouillon in 24 hours, when o.1 per cent tarteric acid is added (titrating + 13 Fuller's Scale). Does not grow in 0.1 per cent bouillon solutions of malic and citric acids.

Toleration of Sodium Hydroxide: Tests were made in meutral bouillon with -20 -25 and -30 Fuller's Scale sodium

hydroxide. Faint cloudiness occurred in the -20 solution in 7 days. The medium is viridine yellow, which gradually disappears. No growth beyond -20.

Temperature Relations:

Thermal Death Point:- When 24 hour bouillon cultures are kept in water both in capillary tubes at 54° C for 10 minutes, no growth occurs when transfers are made to bouillon. Sometimes growth occurred at 53° C. Therefore, the thermal death point lies between 53° C and 54° C.

Minimum Temperature:- The minimum temperatere for growth is below 0° C.

Optimum:- The optimum temperature for growth of the organism is 24° to 25° C.

Macimum Temperature: The maximum temperature for growth is 40° C.

Gas Formation.

The organism is a facultative anaerobe and does not produce gas. Tests were made in fermentation tubes with peptone water and bouillon to which 1 per cent of lactose, dextrose, saccharose, maltose, mounit and glycerine were added respectively. Growth occurred in both open and closed ends of tubes, but no gas. In the bouillon solutions of dextrose, lactose and maltose the medium is changed to a rhodnite pink color, which gradually changes to a dull pinkish brown in the open end of the tube. There is no change in the Ph of the medium.

Further tests for anaerobism were made by depriving agar plate cultures and Giltner H tubes of Oxygen by the use of sodium hydroxide and pyrogallic acid. Growth occurred in 24 to

36 hours.

Relations to Light.

The organism is not particularly sensitive to sunlight. Thinly seeded plates poured from young bouillon cultures and half of each plate covered with black paper, did not show any growth after being exposed to direct sunlight for 50 minutes on the uncovered side, while there were 35-50 colonies on the covered side of plates. There were 1-5 colonies on uncovered side of plates exposed for 45 minutes and 50-60 colonies on covered side.

Relation to Moisture.

The organism is not killed very readily by drying. Drops of 24 hour bouillon culture were placed on sterile cover slips in petri dishes in the dark at room temperature 25° to 25° C. No growth occurred from transfers made of pieces of the cover slips into bouillon, after five days, while there was growth in those reciving slips after 4 days drying.

Loss of Virulence.

Inoculations were made on head lettuce in moist chambers 18 months after isolation, and no loss of virulence was noted.

Pathogenicity.

The organism rots, lettuce, carrots, onions and cabbage after gaining entrance to the host tissue through some injury or following other diseases.

Group Number.

According to the descriptive chart of the Society of American Bacteriologists the group number of the organism is 222.3333033.

Brief Technical Description of the Organism.

The organism is a large rod slightly bent with rounded ends, motile by means of 1 to 2 flagilla, mostly on, capsules; pseudozoogloeve; no spores, involution forms rare and few types; facultatiye anaerobe; agar colonies, white smooth round with thick center. Growth on potato cylinder is abundant, fawn color then wood brown, no diastatic action on starch; produces alkaline reaction in litmus milk; does not liquefy gelatin; produces ammonia; does not produce hydrogen sulphid nor indol; does not reduce nitrates; grows well in Uskinsky's and Fermi's solution; does not grow in Cohn's Solution; thermal death point 53° to 54° C; maximum temperature for growth 40° C; minimum temperature for growth below 0° C. Is not acid-fast; is Gram-negative; and stains well with basic anidin's and aqueous dyes. Not killed readily by drying; not very sensitive to sunlight; slight toleration of tartaric acid and sodium hydroxide; retains its virulence for more than 18 months.

Organism No. 4.

The organism is a medium sized rod with rounded ends, occurring singly, in pairs and occasionally in short chains. The single rods vary from 1.52 to 2.50 microns long, and .45 to .91 microns wide, the average size being 1.75 x .66 microns.

The organism is only slightly motile; motility being demonstrated best in young agar cultures. The flagella are found at one pole, varying from one to three more commonly one.

They were stained with Loeffler's flagellar stain.

The absence of endospores was tested by staining and also heating old live bouillon cultures. The tests were negative capsules were stained from old bouillon cultures by Welch's Capsule Stain.

Pscudozoogloeae occur in potato and in old bouillon cultures, and are composed of masses of short chains attached together by gelatinous threads. No irregular or involution forms were noted, except in case of alkali media, swollen cells and cells much reduced in size were noted.

Behavior Toward Stains.

The organism stains readily in all common aqueous, anilin and alcoholic stains, such as gentian violet, fuchsin,, and carbol fuchsin.. It is not acid-fast and is Gram-negative.

Cultural Characters.

Beef bouillon proved to be a very good medium for this organism for prolonged growth.

Beef-Agar Plates:- The colonies on peptonized beef-agar plates (PH 6.6) are visible in 20-24 hours at room temperature $22^{\circ}$ to $25^{\circ}$ C, when poured from young bouillon cultures. They are at first almost white, smooth, thin with entire edges. When 2-6 days old they are cream-white opaque glistening edges lobate, slightly raised, verruoose, radiate, internal structure somewhat areolate, varying in size from 1 to 4 mm in diameter.

Agar Stroke:- In 24 hours there is an abundant growth, filiform, at $24^{\circ}$ to $26^{\circ}$ C. 2 days the growth is spreading, cream-white, thin, smooth, opaque, and irregular margin. Agar does not change color.

Agar Stab:- There is moderate growth on surface in 2 days, faint filiform growth along line of puncture. The surface growth is cream color and becomes verrucose on the surface.

Beef Bouillon:- Peptonized beef bouillon (Ph. 6.8) is densely clouded in 24 hours, with a pellicle, at room temperature 23° to 26° C. In 4-6 days the pellicle is interrupted with long flaky filaments hanging down in the medium with a viscid sediment. There occurs in some cases a veronese-green ring at the top of the medium about 1 cm broad, which gradually disappears in 8-12 days. In 20 days there is a viscid, white sediment at the bottom of tube, the culture being clear above, and of a deep colonial buff color. In 40 days there is no further change.

Uskinsky's Solution:- There is a moderate clouding of the medium in 24 hours, with cream-yellow colored pellicle and the upper half of the medium is veronese green. All the medium is veronese green in 7 days with a cream yellow sediment. In 5 weeks the medium has changed to a dull green-yellow, clear above with heavy cream yellow sediment.

Cohn's Solution:- There is a very faint cloudiness in 3 days, with a very thin pellicle in 5 days, which disappears in 2 weeks, with small amount of sediment at bottom. In 2 weeks the upper part of the medium is pale green-yellow, which has extended throughout the medium in 4 weeks. No further change in 6 weeks.

Fermi's Solution: Faint cloudiness appears in 24 hours. In 3-5 days the medium is water-green color, with pellicle which become cream yellow. There is a viscid sediment in 12

days, the pellicle breaking up, settling out to bottom. The culture is all clear above of a citron-yellow color, in 3 weeks with a heavy white viscid sediment.

Sterile Milk:- In 6 weeks there is no change in the medium that can be detected. In 7 weeks there occurs clearing with a fine curdlike percipitate at bottom.

Litmus Milk: There occurs an alkaline ring at the top of the medium in 24 hours. The entire tube shows an alkaline reaction in 10 days. In 6 weeks the medium has gradually changed to a tyrian blue color with a grayish curdlike percipitate at the bottom.

Nutrient Gelatin:- The colonies appear in peptone gelatin (Ph. 6.5) in 2-3 days, at 12° to 15° C. They are small, never reaching over 1-2 mm in diameter, round flat, shining, with entire edges. No liquefaction.

There is no liquefaction in stab cultures, kept at a temperature of 15° to 17° C. Growth appears on the surface at from 2- 3 days. Growth is very faint and filiform along line of puncture. No liquefaction.

Steamed Potato Cylinders:- There is abundant growth in 2 days at a temperature of 24° - 26° C. The growth is first cream yellow, filiform, then spreading slightly raised and contoured, and shining. In 14 days the growth is tawny-olive in color, which changes in 20 days to orchraceous, tawny.

The medium is grayish brown. No diastatic action on starch. Tests for diastatic action on 0.2 per cent starch agar were negative.

Other Cultural Features of the Organism.

| | |
|---|---|
| Ammonia Production | Abundant |
| Indol | Indol is not produced. |

Nitrates. Nitrates are not reduced. Tests were made when nitrate bouillon cultures were 7, 14 and 21 days old.

Hydrogen Sulphid:- Hydrogen Sulphid is not produced. Tests were made by hanging lead-acetate paper in tubes of beef bouillon, milk, and potato cylinders. Paper did not blacken.

Toleration of Acids.

The organism is very sensitive to acids. No growth occurred with neutral beef bouillon with 0.1 per cent of malic, citric or Tartaric acid added.

Toleration of Sodium Hydroxide.

The organism tolerates sodium hydroxide to -20 Fullers' Scale. Tests were made in beef bouillon -15, -20, -25, -30 and -35. Claudiness appeared in -15, culture in 3 days and -20 in 5 days. The culture was viridine yellow, which gradually disappeared. No growth beyond -20.

Temperature Relations.

Thermal Death Point :- When cultures were made from a 24 hour bouillon culture of the organism kept at 51° C for 10 minutes in capillary tubes no growth occurs. Growth occurs sometimes after heating at 50° C. This test was repeated several times. Therefore, the Thermal Death point of the organism lies between 50° and 51° C.

Maximum Temperature. The maximum temperature for growth is 34°C.

Minimum Temperature. The minimum temperature for growth is below 0° C.

Optimum Temperature:- The optimum temperature is 24° to 26° C.

Gas Formation.

The organism is a facutative anaerobe and does not form gas. Tests were made in 1 per cent peptone water to which 1 per cent of each of the following carbon compounds were added. Dextrose, lactose, saccharose, maltose, glycerin and mannit. Growth accurred in the open ends of all tubes and in the closed ends of the lactose, dextrose and saccharose solutions. There occurred a slight change in the reaction to the acid side of dextrose, point Ph 6.5 to Ph 5.4. There was no change in any other solutions.

Further Test for Anaerobism.

Cultures were made in plates and tubes deprived of oxygen with pyrogollic acid and sodium hydroxide. Colonies appeared in the plates in 36 to 48 hours. Growth occurred on the slants in Giltner H. Tubes in 24-36 hours.

Relation to Light.

The organism is not particularly sensitive to light. Plates were exposed to direct sunlight on crushed ice in April and May, one-half of plate being covered with black culture. No growth occurred on uncovered side of plate after exposure for 50 minutes. 1 or 2 colonies appread on the exposed side of 45 minute exposure there being 10-30 colonies on unexposed side.

Relation to Moisture.

The organism is not killed readily by drying. Drops of 24 hour bouillon culture were transferred to sterile cover slips, in petri dish and the dish was placed in the dark at a temperature of 25° C. When kept for 2 days and a piece of the cover slip dropped in tubes of bouillon growth occurred; but no growth occurred in tubes which received covers which the organism had been drying for 3 days.

Loss of Virulence.

No loss of virulence was noticed when inoculations were made 8 months after insolation.

Pathogenicity.

The organism is not pathogenic to plants unless injured in some way or attacking the plants following some other disease It rots carrots, onions, lettuce and cabbage.

Group Number.

According to the dexcriptive chart of the Society of American Bacteriologists, the group number is 222.2333033.

Brief Technical Description of the Organism.

The organism is a large rod with rounded end; flagella 1- 3 at one pole; capsules; pseudoloogloene, no spores; few involution forms noted; facultative anaerobe; Agar colonies are cream-white; clouds bouillon very heavily in 24 houfrs at 22° to 28° C; and in four to six days the medium is veronese green; growth on potato cylinder is abundant, first cream-yellow, later it is tawny-olive. The potato is darkened slowly no diastatic action; does not liquefy gelatin; produees ammonia; does not reduce nitrates; does not produce hydrogen

sulphid nor indol; no fluorescence; grows well in Uskinsky's and Fermi's solution; but only feeble growth in Cohn's solution; optimum temperature 24° to 26° C; maximum 34° C; minimum below 0° C. Thermal death point between 50° and 51° C (under conditions stated); stains readily with basic anilin and aqueous dyes; is Gram-negative; not acid-fast; tolerates sodium hydroxide -20 Fuller's scale; does not tolerate acids to any marked degree; is not killed very readily by drying nor by sunlight; retains virulence for more than 8 months.

Organism No. 5

The organism is a small rod, non-motile, occuring singly and in long chains. The single rods vary from 1.0 to 1.44 microns long by 0.40 to 0.63 microns wide, the average size being 1.12 x 0.53 microns.

Capsules were stained from old bouillon culture with Welch's Capsule Stain. The absence of endospores was tested by staining and heating. Pseudozoogloeae occur in old bouillon and potato cultures, and are composed of masses of long chains attached together by gelatinous threads. No involution forms noted.

Behavior Toward Stains.

The organism stains well in all basic anilim and aqueous and alcoholic dyes. It is not acid-fast and is Gram-negative.

Cultural Characters.

Beef-Agar Plates:- Colonies appear on peptonized beef-agar plates (ph. 6.6 ) in 24 hours at room temperature, 23°

to 25° C. The colonies are first cream color and round. Later they are cream-yellow and have irregular margins, almost rhizoid, glistening, flat, smooth, 2-5 mm in diameter with a violet fluorescence.

Beef Agar Stroke:- Growth occurs in 18 to 24 hours at room temperature 23° to 25° C. The growth is spreading, flat slightly contoured, glistening, opaque and of a cream yellow color.

Agar Stab:- Yellow growth occurs on the surface of the stab in 24 hours. Growth along line of puncture is feeble and filiform.

Beef Bouillon:- Peptonized beef bouillon (Ph. 6.8) is densely clouded in 24 hours. No other change in medium noted, until in 6 weeks the medium is clear with little sdeiment.

Nutrient Gelatin:- Small round cream colored colonies appear in 2 days on peptonized gelatin (Ph. 6.5) kept at 11° to 12° C. They are flat, entire and glistening, Liquefaction begins in 4 days and is cup-shaped.

Liquefactinn in gelatin stabs kept at 15° to 17° C begins rapidly in 2 days. First it is crateriform, and then strateform The entire tube is liquefied in 10 to 12 days.

Uskinsky's Solution:- The organism does not grow in Uskinsky's solution.

Cohn's Solution:- No growth.

Fermi's Solution:- No growth.

Sterile Milk:- Clearing begins without coagulation in 7 days. Curdlike sediment in bottom. In 6 weeks there is curd-like, flaky mass hanging through the medium. The clear medium is yellow ocher color.

Litmus Milk:- In 5 days an acid reaction is shown at top of medium. The medium shows acid reaction through out in 10 days. In 6 weeks the medium is liver-brown in color, with a white sediment at bottom.

Steamed Potato Cylinder:- There occurs a scanty to moderate growth on potato. The growth is spreading, flat, glistening, and of a mustard yellow color. The potato is slowly changed to a light brown color. No diastatic action on the starch.

Further tests for diastatic action were made by making streak cultures, of the organism on 0,2 percent starch agar, Tests with Lugal's iodine solution in 5, 7 and 14 days did not show light areas near growth.

Other Cultural Features.

Ammonia:- Test for ammonia with Nessler's test solution were made in old bouillon cultures. These tests showed abundant production of ammonia.

Hydrogen Sulphid:- Is not produced.

Indol:- Is not produced.

Nitrate Reduction:- Nitrates are not reduced.

Toleration of Acids:- The organism does not tolerate acids very well. No growth occurred in bouillon to which o.l per cent of citric, malic or tartaric acids were added.

Toleration of Sodium Hydroxide.

Tests were made in neutral bouillon to which was added sodium hydroxide titrating -20, -25, -30 and -35 on Fuller's scale. Growth occurred in -20 and -25, but no growth in -30 and -35 colution.

Gas Formation

The organism is a facultative anaerobe and does not produce gas. Tests were made in peptone water and bouillon to which was added 1 per cent dextrose, lactose, saccharose, maltose, mannit and glycerine respectively. Growth occurred in both open and closed ends of tubes, but no gas was formed. There was no change in the Ph. of the media.

Further tests for anaerobism were made by depriving plates and tubes of oxygen with pyrogallic acid and sodium hydroxide. Growth occurred in each case in 36 to 48 hours.

Temperature Relation.

Thermal Death Point:- The thermal death point of the organism is between 52° and 53° C. for 10 minutes using the capillary tube method.

Minimum Temperature:- The minimum temperature for growth is below 0° C.

Optimum Temperature:- The optimum temperature for growth is 23° to 24° C.

Maximum Temperature:- The maximum temperature for growth is 39° C.

Relations to Light.

The organism is not very sensitive to sunlight. Thinly seeded plates were poured from bouillon cultures, and half of

each plate was covered with black paper. The plates were exposed to direct sunlight on crushed ice, at noon-time in April and May. Plates exposed for 50 minutes, showed no growth on uncovered side, while there were 70-80 colonies on uncovered side. Plates exposed for 40 minutes had 4-6 colonies on uncovered side while there were 80-90 colonies on covered side.

Relations to Moisture.

The organism is not very readily killed by drying. Drops of 24 hour old bouillon culture were placed on sterile cover slips in petri dishes, and allowed to dry in the dark at room temperature. No growth occurred in bouillon tubes receiving pieces of these slips after 3 days. Growth occurred in those kpet for 2 days.

Loss of Virulence.

There is no loss of virulence of the organism 8 months after isolation.

Pathogenicity.

The organism rots, lettuce, carrots, onions and cabbage, in case of injury or following other disease.

Group Number.

According to the descriptive chart of the Society of American Bacteriologists the group number if 221.3333633.

Brief Technical Description of the Organism.

The organism is a short, non-motile rod with rounded ends; capsules, pseudozoogloeae; no spores, involution forms not noted; facultative anaerobe; agar colonies cream yellow

with violet fluorescense, irregular margins. Growth on potato cylinders is scanty, mustard yellow; produces acid reaction in litmus milk; liquefies gelatin rapidly; produces ammonia; does not produce indol nor hydrogen sulphid; does not reduce nitrates; no diastatic action on potato starch; does not grow in Uskinsky's, Cohn's nor Fermi's Solutions; thermal death point 52° to 53° C. Maximum temperature for growth is 39° C minimum below 0° C, Optimum 23° to 24° C. Is not acid-fast; is Gram-netative; and stains well with all basic anilin dyes. Not very sensitive to sunlight; is not killed very readily by drying; slight toleration of alkali and tartaric acid; retains its virulence more than 8 months.

Organism No. 6

The organism is a rod, with rounded ends, occasionally slightly curved, motile by means of 1 to 2 flagella at one pole, more commonly one. Average size 1,37 x 0.65 microns varying from 1.03 to 1.76 microns long and 0.42 to 0.79 microns wide. Flagella were stained with Van Ermengen's flagellar stain.

Capsules were stained with Welch's Capsule Stain and also with Van Ermengen's flagellar stain. The absence of endospores was tested by staining and by heating old bouillon cultures. Pseudozoogloeae occur insome media, and are composed of masses of short chains attached together by gelatinous threads. Various shaped organisms were noted in alkaline media, and also swollen and very small.

Behavior Toward Stains.

The organism stains well with all basic anilin dyes; such as fuchsin and gentain violet. It is Gram-negative and not acid-fast.

Cultural Characters.

Beef-Agar Plates;- Colonies appear on peptone beef agar plates (Ph. 6.6) in 24 hours at 23° to 26° C. They are first round bluish white. The colonies spread rapidly having irregular auriculate margins, becoming 3 mm to 2 cm in diameter, are flat, translucent glistening, finely granular and bluish-white.

Beef-Agar Stroke:- Growth occurs in 24 hours at room temperature. The growth is filiform, flat, glistening, smooth and translucent, with a decided odor.

Beef Bouillon:- Peptonized beef bouillon (Ph. 6.8) is densely clouded in 24 hours at room temperature with a thin white pellicle. In 3 days the medium is veronese green, which disappears in 7 to 10 days. The pellicle breaks up in 5-7 days, with white sediment. In 6 weeks the medium is clear cream white, with a viscid white sediment.

Nutrient Gelatin:- Colonies appear on peptonized gelatin (Ph. 6.5) plates in 2 days at 11° to 12° C. Are very small one-half to 2 mm in diameter, flat, entire, glistening, white and translucent. No liquefaction.

Gelatin stabs are not liquefied when kept at 15° to 17° C in water bath.. Growth is best at surface, appearing in 2 days. The growth along line of puncture is feeble, and beaded

Uskinsky's Solution:- Uskinsky's solution is densely clouded in 24 hours, with white pellicle in 2 days. In 3 days there is a veronese ring at top of medium, which extends throughout the medium in 7 to 8 days. There is noted a change in the color of the medium in 4 weeks to a dull-green yellow. Pellicle has disappeared and a heavy viscid, white sediment at bottom. No further change in 6 weeks.

Cohn's Solution:- There is faint cloudiness in Cohn's solution in 3 days. In 5 days flake like particles are floating in medium. In 2 weeks there is a flaky sediment, the medium being water green. In 5 weeks the medium is still clouded and pale-green yellow.

Fermi's Solution:- Slight cloudiness occurs in 24 hours, medium has a sky blue tinge. There is a white pellicle in 3 days, which becomes a heavy scum in 3 weeks and martius yellow. In 2 weeks there is a flocculent cream colored sediment. In 6 weeks the medium is clear, citron yellow, dense cream color sediment.

Sterile Milk:- No change detected in 6 weeks except a little finely curdlike sediment.

Litmus Milk:- Alkaline ring appears at the surface of medium in 24 hours, which extends through-out in 8 to 10 days. In 10 days there is a white sediment. 6 weeks the medium is Windsor blue.

Steamed Potato Cylinders:- There is an abundant growth on steamed potato cylinders, spreading, first thin, then slightly raised and contoured and glistening. The growth is Orchraceous tawny in 10 days, changing to tawny-olive in 5

weeks and slimy. The medium is slowly browned. No diastatic action on potato starch.

Further tests for diastatic action on potato starch were made by making streak cultures on 0.2 per cent agar plates. Tests were made with Lugol's iodine solution after 5, 7 and 14 days and no light color was visible near the growth.

Other Cultural Features.

Indol:- Tests for indol were made with cultures of bouillon and Dunhan's Solution with Erlich's indol test solution 5 c c of solution, I and 5 cc of colution II were added to 10 cc of liquid culture, and no red color appeared showing that the organism does not produce indol.

Ammonia Production:- Abundant.

Hydrogen Sulphid:- Is not produced.

Nitrate Reduction:- Nitrates are not reduced.

Toleration of Acids.

The organism does not tolerate citric, malic and tartaric acids, when 0.1 per cent are added to bouillon.

Toleration of Sodium Hydroxide.

The organism tolerates sodium hydroxide to -20 in bouillon Fuller's scale. Tests were made in -15, -20 and -25 and -30 bouillon. Growth occurred in -15 in -20 in five days, the medium is changed to viridine green. No growth occurred in -25 and -30.

Gas Formation.

The organism is a facultative anaerobe and does not produce gas. Tests were made in fermentation tubes in peptone water and bouillon to which was added 1 per cent of lactose,

dextrose, saccharose, maltose, mannit and glycerine, respectively. Growth appeared in the closed arms of lactose and sacchrose, and in the open arms of all tubes. No gas was formed. There was a change in the Ph. of dextrose, from 6.5 to 5.6 indicating slight acid reaction.

Further tests for anaerobism were made by depriving deep culture plates and Giltner H. tubes of Oxygen with pyrogallic acid and sodium hydroxide. Growth occurred in each case in 2 days.

Temperature Relations.

Thermal Death Point: The thermal death point of the organism lies between 50° and 51° C.

Minimum Temperature:- The minimum temperature for growth is below 0° C.

Optimum Temperature:- The optimum temperature for growth is 24° to 25° C

Maximum Temperature:- The maximum temperature for growth is 34° C.

Relation to Light.

The organism is not very sensitive to sunlight. Plates that were half covered with black paper were exposed to direct sunlight on crushed ice at noon time in April and May. After 55 minutes exposure, there were from 65-70 colonies on covered side of plates and no growth on uncovered side. There were 2-5 colonies on uncovered side of plates exposed 50 minutes, and 40-50 colonies on covered side. There seemed to be no differences in plates exposed for 40 minutes.

Relation to Moisture.

The organism is not very readily killed by drying. Tests were made by placing a drop of 24 hour old bouillon culture on cover slips in petri dishes and allowing them to dry in the dark at 22° to 25° C. No growth occurred in bouillon when slips were transferred that had been drying for 3 days. Growth occurred in those tubes reciving slips which had been drying for 2 days.

Loss of Virulence.

There is no loss of virulence 8 months after isolation. Tests were made by inoculating head lettuce in moist chambers.

Pathogenicity.

The organism is pathogenic to lettuce, carrots, potatoes, onions and cabbage after injury or other diseases.

Group Number.

According to the descriptive chart of the Society of American Bacteriologists the group number is 222.2333033

Brief Technical Description of the Organism.

The organism is a motile rod, slightly curved with rounded ends, flagella at one pole; capsules; no spores; pseudozoogloeae; involution forms few types; facultative anaerobe; agar colonies; bluish white, large irregular, thin and smooth. Growth abundant on potato, tawny-olive, produces alkaline reaction in litmus milk; does not liquefy gelatin; produces ammonia; does not produce indol nor hydrogen sulphid; does not reduce nitrates no diastatic action on potato starch; grows well in Uskinsky's and Fermi's solutions; feeble growth in Cohn's solution; thermal death point 50° to 51°C. Minimum temperature below 0° C

Optimum 24° to 25° C; maximum 34° C; does not produce gas, is Gram-negative and is not acid-fast. Stains well with all basic anilin dyes; is not killed very readily by dying; is not very sensitive to sunlight; does not tolerate acid at 0.1 per cent, tolerates sodium hydroxide to -20 Fuller's scale; retains virulence for mor than 8 months.

## RECOMMENDATIONS FOR CONTROL OF THE DISEASE.

Through field experiments Levin (11) states that lettuce rot can be prevented and also that rot already in progress will be checked by spraying plants with formaldehyde, 1 pint to 30 gallons of water. Various strenths of formaldehyde have been tested at this station, and it was found that solutions strong enough to prevent the disease, will cause burning of the leaves. Under these conditions the use of formaldehyde as a control method is not advised until more thorough tests have been made.

Since the disease under consideration ordinarily attacks plants troubled with tip-burn it seems safe to say that when a control measure is discovered for tip-burn there will be a minimum amount of this type of soft rot under field conditions. Rotation of crops, sanitary cultivation methods and fertilizing with well decomposed manure are strongly advised. And where irrigation is used, the over head method is recommended.

More care should be exercised in harvesting and packing to prevent bruising since the disease causes the maximum damage in transportation and storage. The pathogenes are invariably present on the leaves and are ready to cause infection

at the first injury of the host tissue.

If plants in the field show any considrable amount of disease it is a generally known fact that it is useless to harvest, as very slightly diseased heads will rot rapidly under transportation conditions. Care should be taken to select a perfectly sound product for market purposes, and in harvesting it is advisable to remove the lower older leaves.

## SUMMARY.

1. The bacterial soft rot disease of lettuce is very widely distributed and causes large losses in the lettuce crop each year. The maximum damage being done in transportation.

Diseased specimens were examined from East Lansing and Kalamazoo, Michigan and lettuce shipped from California. Six apparently new pathogenes were isolated and found to be infectious to the host only in case of injury or following some other disease. These pathogenes are described in this paper.

The progress of the disease reaches its height at fairly warm temperature from 22° to 28° C.

Pathogenes causing soft-rot were found present in a large number of soil samples collected in the near vicinity of the Michigan Agricultural Experiment Station fields.

It is recommended that clean cultivation methods, the cleaning up and burning of old diseased leaves in the field, rotation of crops, fertilizing with well rotted manure and overhead irrigation should be employed for control of the disease. For market it is advised to select heads perfectly free from disease and to avoid bruising in packing the crates.

## LITERATURE CITED.

(1) Brown, Nellie A.
1915 A Bacterial Disease of Lettuce. Journal Agr. Research 4: 475-478

(2)
1918 Some Bacterial Diseases of Lettuce. Journ. Agr. Research 13: 367-388 (14 Pls.)

(3) Jones, L. R.
1893 A Bacterial "Stem-rot" of Lettuce. Vt. Agr. Exp. Sta. 6th ann. report 1892 87-88

(4) Voglino, Pietro.
1904 Sulla Batteriosi Delle Lattughe Ann. R. Accod, Agr. Torino 46: 25-33 4 fig.

(5) Stone, G. E.
1907 Bacterial Disease of Lettuce, Mass Agr. Exp. Sta. 19th ann. report, 1906. 163-164

(6) Stevens, F. L.
1908 A.Bacterial Disease of Lettuce. N. C. Agr. Exp. Sta. 30th ann. report 1906 107:29-30

(7) Fawcett, H. S.
1908 Lettuce Disease. Fla. Agr. Exp. Sta. Rept. 1907/08: LXXX-LXXXVII, pl. IV-V

(8) Burger, O. F.
1912 Lettuce Rot; Fla. Agr. Exp. Press. Bul 200 2p.

(9)
1912 A Bacterial Lettuce Disease. Fla. Agr. Exp. Rpt. 1911/12 XCVIII-C

(10) Crapenter, C. W.
1916 Rio Grande Lettuce Disease. Phytopathology 6: 303-305 1 fig.

(11) Levin, Ezra
1917 Control of Lettuce Rot. Phyotopathology. 7:292-293

Plate I. Soft rot on New York head lettuce collected in Chicago, showing rotting on the tips of the leaves. (Photo by Ray Nelson.)

Plate II. Crate of Western New York head lettuce showing soft rot on the heads. (Photo by Ray Nelson.)

Plate III. Heads of California Iceberg lettuce showing soft rot on the outer leaves. Specimens collected at Lansing, Michigan. (Photo by Ray Nelson).

Plate IV. Head of Iceberg lettuce showing soft-rot following tip-burn, at the tips of leaves forming the head. From inoculation experiments in greenhouse.. (Photo by Ray Nelson).