THESIS.

Soil Bacteria

J. H. Briley. 1896.

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J. H. Briley.

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THESIS

BACTERIA OF THE SOIL.

When compared with other sciences bacteriology is of recent origin and development. It has but recently appeared upon the stage of action and taken its rank as a separate and distinct science, but not withstanding this it has already taken rapid strides in the march of progress and has accomplished much in its realm of investigation. It has brought to light many truths which, until this star appeared in the horizon, had lain hidden in a maze of ignorance and has in this early period of its active life produced results sufficent to cause humanity to feel thankful for its birth and existence.

Untold suffering has been prevented, an incalcuable number of lives rescued from an untimely grave, and thousands of dollars saved to the industrial world by students in the field of science.

Pasteur and Koch may justly be called the creators of this science for it was they who made the first great discoveries which gained for bacteriology a recognition by men in the previously established fields of investigation. Before their efforts resulted in any material benefit, however, others were at work on the subject and even before their time a few persons had a faint glimmering idea of the little bodies in which bacteriologists are interested.

Kircher in 1640 announced that with a lens of twenty-five diameters he could see living objects before unheard of. In 1680 Leennan Hoeck, a dry goods clerk, discovered living and moving objects in the saliva and was the first to unquestionable distinguish microbes. Following these discoveries the science developed slowly because instruments for examination had not reached nearly as high a degree of perfection as now,and consequently the microbe was allowed to rest in peace or was at least little disturbed by prying investigators. This was not long to be, however, As the instruments for investigation improved the science developed and with the improvement of the microscope in 1833 it received considerable impetus.

From time to time new discoveries were made which mark important steps in bacteriology. The one which most of all established the science on a firm foundation was that of Pasteur in 1853. From a cause then unknown the silk worm industry in France was nearly ruined. Scientific men of every class took up the work to find the cause and if possible suggest a remedy. All were unsuccessful until Pasteur by request of the minister of agriculture made investigations He was so successful that the silk raising industry, so important and so nearly driven from existence, was restored.

About the same time Da Vaile and Koch discovered the germ of anthrax and proved beyond reasonable doubt that this desease was caused by a micro organism.

From that time until the present numerous discoveries have been made, and many men have come into prominence because of their work in this science, among whom are Doctors Vaughn and Novey of our own state.

So much for the history of the science; now, let us consider briefly some of the germs, their functions and manner of growth .

Bacteria today are defined by scientists as unicellular microscopic plants whose chief function is to destroy dead matter and thus reduce compounds to simple elements. In this way they produce putrification, fermentation or decomposition.

A classification of bacteria is rather difficult, but we will describe all the known kinds under three heads, viz:- Micro Coccus, or ~~or~~ spherical bodies, baccilus or rod shaped, and spirillum, or those which grow in the form of a spiral.

o Micro Coccus. — Bacillus. Spirillum.

Farther than this they are classified with regard to their oxygen requirements as aerobic and anaerobic, which are also ~~classified with~~ qualified by other descriptive terms, viz:- obligative and facultative, e.g. an obligative aerobe which can exist only in the presence of free oxygen, while a facultative aerobe can live with or without free oxygen, but does better when there is plenty of oxygen. Other classifications s are made according to

habitat	as	parasitic	or	saprophytic	
movement	"	mobile	"	non mobile.	
color	"	chromogenic	"	"	chromogenic
effect on Media	"	liquifying	"	"	liquifying.
action	"	pathogenic	"	"	pathogenic.

The pathogenic or desease producing germs are no doubt of greater moment to the human race than the opposite class and hence have called forth more investigation, but owing to limited time and experience I was unable to make any study of these, except to a somewhat limited extent with bacillus of tuberculosis, a desease which has and is at the present exciting considerable attention from bacteriologists the world over. There have already been made great advances in the means of diagnosing this desease, and investigations are going on with the hope of discovering a remedy for the dreaded contagion. In my soil bacteria it may be possible that I have a pathogenic germ, but means were not at hand to make the necessary investigations.

The intense heat also prevented a gelatine growth which is necessary to tell whether they are liquifying or not. Neither was sufficient apparatus at hand to investigate the anaerobic microbes.

With the knowledge now at hand upon the subject of soil bacteriology we can explain phenomena hitherto not understood. History tells us how the savages of the South Sea Islands and

other barbaric lands poisoned their arrows by sticking them into the ground, but until the advent of bacteriology into the world of science the effect could be traced to no apparent cause. Now, we know that the deadly poison was a germ either bacillus of tetanous or of malignant odema either of which when introduced into the animal economy nearly always produces fatal results.

Those who have worked along the line of soil bacteriology tell us thatmicrobes are more numerous near the surface, though not in the uppermost layers of the soil, the number decreasing with the depth until at about three meters there are practically no organisms. Just what these minute organisms do in the soil is somewhat of a question. Some, as I have said, produce deseases of a very fatal character, while others are interesting because of their action in nitrification and in the destructive decomposition of organic material by which it is fitted for assimilation by the higher plants.

The method which I pursued in my investigation was as follows: I secured at different times three samples of soil; the first from the road in front of the silo, the second from freshly plowed garden soil and the third from the plot of lathyrus sylvestrus. I took five grams of the earth and thoroughly mixed this with a gelatine culture, turned the whole into a steralized petri dish and let stand in running water until the gelatine hardened. I then set it aside and awaited developements for a couple of days.

At the end of this time numerous little specks which when viewed from a nearly horizontal position appear like funnel shaped depressions, indicated colonies of microbes. Each colony, it is assumed, grows from a single microbe and thus if the work is perfectly done the number of colonies will represent the number of microbes in the original five grams of earth and an estimate can be made of the number in any given amount of soil. This calculation necessitates the growth of all the microbes, aerobic and anaerobic.

Culture Media.

Liquid media is made sometimes from lean beef but more commonly from Leibig's meat extract. Of the latter 2 1/2 grams to 1000 C.C. of water are the relative amounts. After heating and filtering this,10 grams of peptone and five grams of common salt are added. Make this neutral or very slightly alkaline by sodium carbonate and the solution is ready for use. I had no occasion to use this as a media for the growth of germs but simply as a basis for the production of the other medias as will be seen.

Gelatine.

This is prepared by adding to the liquid media or bouillon, as it is called, a portion of the gelatin. This is heated, brought to the proper condition of alkalinity, poured into steralized test-tubes and allowed to cool. It maintains its solidity not exceeding 720 F., but above this

breaks down and is useless as a culture media.

Agar.

For hot weather such as we have experienced this season and where there are not facilities for controlling temperature in the laboratories, it becomes necessary to substitute something for the gelatine. Agar answers this purpose. It is made by adding to the bouillon a prescribed amount of agar instead of gelatin as in the last. This is boiled for fifteen minutes each day for three successive days and then like the gelatineis poured into steralized test-tubes and at once becomes a soil which will not liquify at the highest temperature of summer. This is known as ordinary agar.

Glycerine Agar,

Is made by adding to the above about six percent of pure glycerine and heated as for the ordinary.

Glucose Agar,

Is made by adding glucose to the ordinary agar in the proportion of two grams to 100 C.C. of agar. Heat as for the others.

Another solid culture media much used and easily prepared is the potato. It is made ready for use by washing in a solution of bichloride of mercury, 1 to 1000C.C. of water,

which is a disinfectant and steam heating for one hour. These are halved and placed in a moist chamber and are ready for inoculation.

Steralization.

Test-tubes, petri dishes and all glassware used in the growth and handling of bacteria are steralized by keeping at a temperature of 150-175 degrees C. in a dry air steralizer for one hour. Tubes before steralization are plugged with cotton wadding in the proper manner.

Microscopic Examination.

When the germs have grown and formed colonies a microscopic examination may be made.

Hanging drop.

Place a drop of steralized water on a clean cover glass. Stab , as it were, a colony with a steralized platinum wire and then stir the drop. Now, invert the cover glass on a hollow slide and place on stage of microscope for examination In this way, the movement, if any, can be detected.

STAINED SPECIMENS are made by inoculating the drop of water as before and then holding over the flame until perfectly dry. Then pass through the flame three times to fix. Cover this with analine dye for about twenty seconds. Invert on slide and examine.

PERMANENT MOUNTS differ from the last described only in

the fact that both coverglass and slide must be perfectly dry and then place the coverglass on a drop of balsom specimen side down and let dry for a couple of days.

Following out the line of work I have just described I found in the soil five germs, four of which are bacilli.

From my first inoculation I secured a micro coccus and a bacillus. The colonies of both of these were circular in outline, but the colony of the micro coccus appeared to be the smaller of the two. Both stained readily with analine dyes. The micro coccus seemed to have a tendency to unite in pairs, thus giving them somewhat the appearance of bacilli. The bacilli were rather square at the ends and united, several in a string and multiplied by division and had a slight movement.

The second inoculation resulted in the finding of bacillus. This time a very thin slender one about three times as long as broad. They join together and form long thread like strings. They grew very readily at the room temperature and colonies rather larger than any of the previous ones.

The third inoculation gave me a growth of two bacilli of which I made pure cultures. One of these was a short thick rod about once and a half times as long as broad and also showed the spore very nicely. The other was a very large

microbe and about twice as long as broad; and seemed to have a tendency to unite in twos or threes.

My limited investigatinn thus shows that the bacilli largely predominate. The experience of others has also shown this to be the fact.

These experiments show plainly that the soil is loaded with bacteria even if no more were present than here described, but we must remember that there may be numerous species in the deeper strata of the earth which grow only under anaerobic conditions.

This rather limited investigation may mean, on the part of those who choose to labor through the substance of this thesis, comparatively little but to me the work has opened up a new world of knowledge and research, interesting and instructive, and has revealed methods of investigation hither to unknown to me and has given quite an extended view into the methods pursued in searching for the micro organisms.