THESIS.

BACTERIOLOGY OF THE SOIL.

W. T. Barnum. 1896.

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W. T. Barnum.

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THESIS

BACTERIOLOGY OF THE SOIL.

The giving of students of this college an opportunity to pursue the study of bacteriology, clearly shows that the subject is gaining a place of equal importance as the other sciences. It has been but little more than a decade since the subject has been considered of much importance, but in that time its rapid developement and its recognized importance seem to have overwhelmed the slower progress of the other sciences.

The confidence placed in bacteriology seems to be fully justified. It has already accomplished much, but more can be expected from it. In the medical world it has explained many mysteries. The cause of many diseases can now be pointed out.

It has also been shown that bacteriology is intimately connected with agriculture, and it is in this connection that we are most interested in it. It has been shown that the oxidation and reduction which takes place in nature --- such as the resolution of dead animal and vegetable matter, the breaking up of nitrogenous materials into the nitrates, the specific fermentation so important in articles of diet, and the many other purposes--- are caused by the growth of microbes. We can not appreciate the importance

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of bacteria on the farm. Until recently some of us never thought of the farm as being stocked with bacteria, but they are there. They are in the wells, the brooks, and the rivers, in the milk,the butter, and the cheese; and in the soil, the air, and the compost heap.

Among most people bacteria are looked upon as something to be dreaded. The reason for this seems to be that the earliest investigations showed that bacteria caused disease. While it is true that certain bacteria cause disease, it is equally true that other bacteria are of great benefit to us.

Take for example the changing of cider to vinegar. The cider contains alcohol and to become vinegar it must be converted into acetic acid by taking up oxygen. This is done by the growth of bacteria (mother of vinegar.) The bacteria in some way take the oxygen from the air and gives it to the alcohol.

Again, it is to bacteria that the farmer owes that article of food produced by the silo. If the correct bacteria growth is secured, the ensilage will become a very desirable article of food for stock.

The butter-maker owes something to bacteria. They act as decomposing agents. As a result of this decomposition of the cream, products are produced, which at first have a pleasant odor and taste. If the cream is churned at this period the butter becomes flavored with these products of decomposition. The lfavor is the aroma of good butter and it is the gift of bacteria. In cheese making it bacteria that breaks down the casin and create products of a pleasant taste, without which the cheese would be worthless.

A tree falls to the gound and the trunk becomes softened into a mass of brown powder which after a time sinks into the ground and dissapears. A bird dies, after a time the flesh undergoes putrefaction, gases are given off, and the rest sinks into the gournd and dissapears. It is through the agency of bacterial organisms that the tree dissapears and that the tissues of the bird are decomposed. It is through their agency that the soil is inriched and the continuance of life rendered possible.

An organism of special importance, because of its power to enrich the soil, is the, "nitrifying organism." Plants can not feed upon such things as albumen. They must have nitrates. The "nitrifying organism" converts the album enous compounds into nitrates by further decomposition, which the ordinary putrefying germs will not accomplish. Then toq it is through bacteria that certain plants are able to derive nitrogen from the air. By the growth of these bacteria we may hope for a continuance of the supply of nitrogen to the soil.

Theremight be mentioned other processes that would show the benefit of the growth of bacteria to man, but these are sufficient to show some of the things that bacteria do and that they are not altogether producers of evil.

Definition.

Bacteria may be defined as single celled microscopis plants, living on higher organisms.

Classification.

Bacteria are classified as to form into:

micrococcus or spherical
bacillus or rod-shaped
spirillum or screw-shaped

as to action into

pathogenic or disease producing.
non-pathogenic or not disease producing.

Multiplication.

The principal way in which germs multiply is by division. The cell becomes elongated and then divides into two by a transverse groove. The two parts may separate or remain together. Bacteria under certain conditions contains spores. These possess a greater power of resistance than the germ. The spores are freed by the destruction of the cell and, under favorable conditions, they again produce bacteria.

Movement.

Some bacteria have the power to move about in liquids. This is done by the developement of whip-like appendages which, by their vibrations, propel the germ.

Action of Microbes upon Media.

Media are the materials in which the microbes are grown. In general, the microbes reduce the media into its inorganic compounds. In gelatin the microbes may rapidly liquefy it, as a result of the developement of soluable ferments; they may slowly liquefy it; or they may not grow in gelatin at all. The same may be true of agar.

Identification of Germs.

Germs may be identified by watching they action in the different media. Observe whether or not they liquify gelatin; coagulate milk; grow exposed to air; are pathogenic or non-pathogenic; and whether or not they produce gases, acids, alkalies, colors, spores, odors, or phosphoresence. Also notice the temperature at which they grow, rapidity of growth, action of the anilin dyes upon them, and the form of growth in the media. These observations applies to the description of the germ and its characteristics, as given by the text-book, will enable the worker to make the identification .

How germs were grown.

As culture media, galatin was first used. It was made by boiling 500g. of chopped lean beef in 1000C.C. of water, for 15 minutes. This material was then filtered and to the filtrate then was added 100g. of gelatin, 10 g. of dry peptone, and 5 g. of common salt. This mixture was then heated in a water bath and filtered. The solution was then made neutral and nearly clear. Twenty-five sterilized test-tubes were then filled to a depth of about 1 1/2 inches. The gelatin hardened upon cooling.

A sample of road dust was then procured. It was pulverized and 5 c. of it weighed out. The gelatin in one of the test tubes was then melted by steam heat. When sufficiently cooled, but still liquid, the 5 c. of soil was poured in, this inoculated the gelatin. An "Esmarch Roll-tube Culture," was then made of the inoculated gelatin by rotating the tube in a nearly horizontal position under a stream of cold water. This solidified the gelatin in a thin layer over the inside of the tube. This was placed in a cool place for developement. In 48 hours colonies were deveolped, but the hot weather melted the gelatin and no results were obtained.

With another sample of soil, from the freshly plowed garden, another test-tube of gelatin was inocultaed. With this a "Petri-dish Culture" was made by pouring the contents of the test-tube into a sterilized petri-dish.

The gelatin was solidified by placing the dish into a very shallow current of water. In 48 hours colonies had developed. The surface of the gelatin appeared pitted. From above many of the colonies were white, circular in form, and had entire edges. By the aid of the "Colony Counter" it was estimated that there were from 6,000 to 7,000 colonies growing. Each colony is said to represent one germ, so there must have been six or seven thousand germs in the 5 c. of soil.

An examination of these colonies was made by the aid of the microscope. In order to bring out the shape of the microbes more distinctly,"Simple Stains"of them were made This was done by the use of the anilin dyes, gentian violet being a favorite. Several different examinations of these colonies were made and it was found there were different kinds of germs present. One moment showed a fine exhibit of micrococcus. Three others showed bacilli, two of which were much alike. The others grew in the form of long bending threads. During the next 24 hours this gelatin was liquified by the growth of the germs. Pure cultures of these germs were not made.

Gelatin is not a satisfactory media to work with during hot weather. A more convenient form is agar. To make this boil 500 g. of chopped lean beef in 1000c.c. of

water. Filter and add 10 g. of dry peptone and 5 g. of common salt. Heat this in water bath for one hour and filter. The result is "Ordinary Bouillon." To this add 2 % of agar. Place in test-tubes and sterilize for 15 minutes per day for three days. This should give a semi-transparent solid which will not melt at ordinary temperature Glycerine agar was made by adding 100c.c. of the ordinary agar, 6 or 7% of pure glycerine.

The next sample of soil was obtained from the celery muck bed. Glycerine gaar was inocultaed with 5 g. of the soil in the same manner as was the gelatin. In 48 Hours colonies were developed. At least twelve temporary mounts were made from the colonies, but as far as the microscopical examination was concerned, no difference could be seen in the germs. They were all bacilli and looked just alike. A "Streak Culture" was made of this germ in glycerine agar. It was done by drawing an inoculated wire along the surface of the agar. On the surface the growth showed as white spots of a cream like nature. While below the slender branches were developed which grew out into the agar A "Stick Culture" was made from the former by passing an inoculated platinum wire into the agar. This produced a growth similar to that below the surface in the "Streak Culture." The greatest growth was near the surface.

In the soil examination no pathogenic germs were found. As they do not grow exposed to air, the conditions in the media were not favorable for their growth.

It may be said that the surface soil contains numerous bacteria most of which are bacilli of the non-pathogenic class. In some localities the soil contains pathogenic bacteria, such as germs of tetanus, malignant aedma, and symptomatic anthrax. These germs growing in the animal economy produce a posion which may prove fatal. These products have been used by some savage tribes to poison their arrow heads. Writers say that tetanus bacillus may be inhaled or ingested without harm. The most common way for an animal to become inocultaed with tetanus is through a wound, and especially a punctured wound, as the germ will not grom when exposed to the air. Long exposures to sunlight and air, or to a temperature of 100c. for fifteen minutes will kill both germs and spores.

As can be seen, the object of this thesis work was to secure a somewhat general knowledge of the forms and methods of bacteriological investigations. However, incomplete the work may have been, we consider the knowledge gained of much value, as it has opened up to us a field for investigation that was entirely unknown before.