THESIS.

THE AMOUNT OF BACTERIA IN MILK.

Loren P. Fimple 1896.

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A Glance at the Field.

A science which is comparatively young, and which to some people might appear uninteresting is bacteriology. But when we consider how Bacteria may effect either to modify or annihulate any farm of organized life, its importance at once becomes apparent.

What geology and minerology are to the miner, what astronomy is to the navigator, what anatomy and zoology are to the physician, such must bacteriology be to him who would be the physician of all organized life.

To those unacquainted with the part which these minute organisms play in nature, the word "bacteria" often brings only visions of desease. This is certainly an incorrect view as soon becomes apparent. When we take into consideration the fact that the number of bacteria capable of producing desease is relatively small--- more than this, the great majority of them are concerned in processes that have no connection with desease, but are directly beneficial to all living members of both the animal and vegetable kingdom In reality they are necessary to life upon the surface of the earth. By their action the highly complicated tissues of dead animals and vegetables are resolved into simple compounds--

carbonic acid, ammonia and water. They also aid the digestion. Admitting as we must that they are often the agents that produce disagreeable tastes and odors, as in butter that "has seen its best days" we must say in their favor that they are as often the cause of agreeable flavors and pleasant odors, besides producing many beautiful colors. Thus we see that while some of them produce sickness or unpleasant sensations, others "appeal to the feelings"in the opposite way of pleasure to the taste, the smell, the sight.

History of, and Definitions.

We have considered somewhat in a general way the part which bacteria play in the role of existence. It may be well to say a few words concerning the history of the develepement of the science, and in defining terms, in order to have a clearer conception of our subsequent investigations.

Bacteriology has only existed as a destinct science for about ten years. The names most intimately connected with its advancement are those of Koch and Pasteur. To the farmer, Robert Koch, of Germany, we are largely indebted for the first investigations along the line of consumption or tuberculosis as caused by bacteria. In the latter, Louis Pasteur of France, we have brought to our attention an example where bacteriology saved millions of dollars to the silk raisers of France. Thus only fulfilling what has been written, "Ye shall know the truth, and the truth shall make ye free." Although bacteriology has been so recently differentiated as a distinct science, yet something was known of it two hundred years ago, whe n a Hollander discovered serpentine looking objects in the mouth.

But now, to be exact, what is a bacterium? It may be defined to be a microscopic, unicellular, vegetable organism, closely allied to fungi. These organisms reproduce themselves by means of simply fusion and by forming spores; and it may be added here, that one of the greatest obstacles to compete sterilization results from the fact that these spores are much more resistant to chemical and thermal influences than the vegetative forms from which they arise, and it is this that frequently requires disinfection of those substances containing spores a matter of more difficulty.

Requisites for Bacterial Growth.

We have seen what bacteria are made of and how reproduced, let us now consider some of the requisites for bacterial growth. These requisites are three in number as follows:

1st, Organic matter.

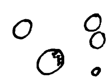
2nd, Moisture

3rd, Suitable temperature.

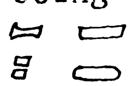
The kind of organic matter varies somewhat with the especial spe. ties of bacteria considered,as also does the degree of heat ans moisture. The temperature required usually varying from 70 to 98 degrees F.

Methods of Classification.

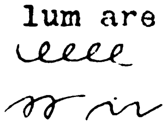
Bacteria may be classified in various ways, depending on the principle of division used. From the standpoint of form they can be divided into three classes, viz: Micrococcus, bacillus and spirillum. The micrococci are circular



) bacteria resembling a billiard ball in general) appearance. The bacilla are the rod like bacteria being longer than wide. They assume a variety of shapes,



) as to details, some have rounded ends, some square) ends, some are many times as long as wide, while again some are only a trifle longer than wide. The spirillum are the spiral like bacteria. These are forms of bac-



) teria that can be grouped in a great variety of) ways, as clusters, chains, in twos and in fours.

Bacteria can also be classified as to their oxygen requirements. Some being only able to live in the presence of oxygen are called anaero bic. Some seem especially favored, being able to live under either condition, although usually haveing a preference for one condition. These are faculties. The classification can be illustrated graphically thus:

- Bacteria
  - Aerobic
    - Obligative
    - Facultative
  - An aerobic
    - Obligative
    - Facultative

On another"principle of division" bacteria may be classified as pathogenic or non pathogenic. The pathogenic are those relating to desease either by entering the body of man directly or by elaborating a poison (toxic plemain) which when intorduced into the body causes desease.

Our investiga*ions from the nature of circumstances under which we have labored, have been confined to the examination of the non pathogenic airobic germs, saver perhaps with the examination of teberculosis from animals subjected to vivi section, which suspected of tuberculosis, Diphtheria, Anthrax and Fever bacteria.

Manner of Procuring Germs.

The way in which germs are procured for investigation depends upon the circumstances, and the especial specie of bacteria under consideration; thus in examining the soil, the water, and the air for bacteria there special ways to be pursued in obtaining germs from each.

My Special Work.

After spending the greater part of our time in getting

our laboratory in running order, getting apparatus placed and stock solution made, and then in a common course of elementary bacteriologicul investigation, as to the different culture media, the various methods of coloring, etc., we have spent the remaining time on separate lines of work. In this each member of the class has examined the work pursued by the others, thus it will be observed that the work has been necessarily almost in common. The subject assigned me was--

The Bacteriology of Milk.

In my first attempt along this line I procured a sample directly by having the herdsman milk directly into the test-tube. After thus procuring the milk I heated three tubes containing gelatin by placing them in a bracer containing warm water and thus melting the geletin. It may be said here that the gelatin was made as prescrubed in Novey's Bacteriology, by taking

Nutrient | gelatin x water x salt x peptom x
Gelatin | egg- abbumen - Which was beaten and rendered slightly alkaline as prescribed. When finally prepared the gelatin wa poured into test-tubes to the depth of an inch. The test-tubes, of course, were previously sterilized. After the gelatine was intorduced into the tubes they were sterilized fifteen minutes a day, for three successive days. After having heated three of these tubes containing nutrient gellatin, 1 C. C. of milk was introduced

into the first by means of a sterilized pipette, into the second 1/2 C. C. and into the third one drop. Then the gelatin was poured into the sterilized petri dishes, due precaution being taken to sterilize the mouth of the test tube before inverting it. The object of growing in a petri-dish is to isolate and separate colonies that they can be counted and pure culture finally obtained. After having thus made three petri dish cultures. I put them where water could trickle under them, inorder to prevent their melting and thus allow colonies to coalese.

My first attempts were unsuccessful, because in owing to slowness in colonies to appear I removed the dishes from the water and as the weather was very warm the nutrient gelatin melted. But I pursued the same general line of treatment in my next experiment save that I procured the milk used, from a can in the milk room (in the N.E. corner of the barn) a few minutes after it had been milked. This was done July 15th, and no growth was observed until July 17th, when I counted colonies in the petri dish culture containing one drop of milk with following results as noted by squares and square divisions on the

1	one	of	least	squares	no.	of	colonies	20
2	"	"	"	"	"	"	"	9
3	"	"	"	"	"	"	"	8
4	"	"	"	"	"	"	"	7

5	one of least squares no. of colonies	4
6	" " " " " " "	18
7	" " " " " " "	17
8	" " " " " " "	15
9	" " " " " " "	5
10	" " " " " " "	11
		114

I	Minimum of 10 observations	4
II	Maximum " " "	20
III	Average " " "	11.4

Nine of the above little squares equal one of the larger ones. Number of larger squares on bottom of petri dish equals sixty-six. Hence 66 x 9 x 11.4=6571.6, which is the estimated number of colonies obtained from one drop of milk. Examination of petri dish containing 1/2 C.C. of milk showed an average of, at least, 30 colonies for each little square, which would give us 17820 as the total number in1/2 C.C. of milk. Simple stains were made from both of these and also permanent mounts Bacilli and micrococci were found, the micrococci greatly predominating. July 20th after an elapse of five days these petri dishes had liquified, this indicating that there were liquifying bacteria present. The dishes were, we may add, subjected to the same conditions of temperature at first.

Agar Agar Cultures.

July 16th, I made petri dish cultures of milk which had been milked directly into the test-tube. The nutrient solution used was glycerine agar (See 95 and 136 Novey)

Ordinary agar formula	(	500 ordinary bouillon.
	(	1 1/2 - 2 % finely chopped agar.

Glucose agar = 100 C.C. ordinary agar x 2 dram glucose.

Glycerine agar = 100C.C. ordinary agar x 6-11 C.C. glycerine.

This is poured to a depth of three fourths of an inch into the test-tubes. These are turned on their sides in a sloping manner while agar is melted, to allow agar to spread in a sloping manner on one side of the test-tube. After a lapse of four days I found several (6) colonies had formed in the petri dish culture made from agar, as above prescribed. From the largest of these colonies some of the bacteria from the largest one of the colonies, were introduced, by means of a sterilized platenium wire into some milk which had been milked from the cow into a test-tube and then sterilized three successive days at 100 C. From one of the small test tubes I similarly inoculated another specimen of sterilized milk. I regret to add that up to this time, July 24th, have detected no change in the milk, this seeming to point in the direction that milk may have germicide power. (See Bulletin No. 25 U. S. Dept Agr'l.) This was merely suggested to me, but I hope some one in the future may be able to obtain more definite data on this matter.

Pure Cultures.

From the agar petri dish culture I made pure cultures from two different colonies by intorducing some of material of each into an agar test-tube. A simple stain was made from the first of those the day following and a small bacilli found--- about one and a half times as long as wide, with rounded ends and very often arranged in pairs. Come, of course, being disturbed were floating around. This I suspected of being bacilli acidilactice. The colonies from which this was obtained were irregular in outline.

This was some of the work performed and in general the methods which one would pursue in a more exhaustive study of the non pathogenic bacteria of milk.

Means of Identifying Bacteria.

The means of identifying bacteria are interesting. They may be classed somewhat as follows:

1st, By pure culture and studying characteristics.

2nd, by relation to gelatin-- liquifying or not.

3rd, By relation to oxygen.

4th, By kind of chemical products.

5th, By form of colonies.

6th, By motility and lastly by the peculiar stain.

We are thus led to see that the identification may be comparatively easy in some cases and difficult in others, yet to the lovers of science, what science presents a broader field? What study more interesting and useful to man?