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Notes on a Thermophilic Bacillus

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NOTES ON A THERMOPHILIC BACILLUS.

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That group of plants capable of growth and reproduction at temperatures high above the normal or ordinary has long been an object of interest and study among scientists. An examination of any systematic treatise on the Schizophyceæ will be rewarded by a considerable list of species that grow in hot springs, etc. In Rabenhorst's "Flora Europa Algarum" for instance, there are no fewer than thirteen genera having species or varieties characterized as "thermalis," while others are known to live in water having a temperature of 80°-93° C. Conspicuous and abundant as the Schizophyceæ are in these thermal waters, yet they are accompanied by many less conspicuous forms, the Bacteria, Schizomycetes. These have been discovered and described only since the introduction of modern laboratory technique.

Although these heat loving or heat tolerant Bacteria were first found in thermal waters, more recent investigations have shown that these organisms have a much wider distribution. In the last few years many of this class have been isolated from a variety of sources, such as from soil even to a considerable depth, sewage, feces from both cold and warm blooded animals, dust, straw, dung, snow, air, milk, syrup, cheese and a variety of others. Of course, there is no development of the organism in these substances at ordinary temperatures, the organisms for the most part being spore producers and very resistant.

A careful study of these forms by Schillinger* has shown that they may be separated into two classes, somewhat

*Hyg. Rundschau 8:568. 1898.

arbitrarily it is true, the thermotolerant and the thermophilic. The optimum temperature of the former lies below 40°C. though growth may occur at much higher temperatures, even above 60°C. The optimum of the latter is above 40°C., this being the minimum for most forms. These temperatures, and favorable conditions for the growth of these forms, may be found in many places in nature besides the thermal springs. Even the soil when exposed to the direct rays of the sun is heated sufficiently to enable them to grow. Then, more important, heaps of decaying or fermenting organic matter such as silage or manure develop enough heat in their interior to give the optimum temperature for many.

In order to determine something as to the thermophilic bacterial flora of the manure heap, the study here presented was undertaken. The study has not been completed, and notes are given on one only of the organisms isolated.

Method of Isolation.—A rapidly fermenting heap of horse manure, with an interior temperature of about 58° C., was chosen for the study. Some of this material was removed in a sterile dish, washed with sterile water, and various dilutions used for pouring plates. These latter were incubated at a temperature of 60° to 65° C. for eighteen hours and examined. A large number of colonies had made their appearance in every instance, and seemed to be quite uniform in character, i. e., probably one species was in preponderance. Cultures were made from these colonies on various media, and these incubated at a temperature from 60° to 62° C. A description of this organism follows.

Careful comparison with published descriptions of various thermophilic bacteria showed that probably this organism was the *Bacillus thermophilus* I. of Sames, isolated by him from earth.

MORPHOLOGY.—Rods, motile when young, 3-5 microns long, .6 microns broad, sometimes single, generally in chains of two to ten or more, ends of organism rather truncate. Stains easily and regularly with aniline dyes,

*Hyg. Rundschau 8: 320.

not acid fast. Spores formed in abundance in twenty-four hours or less, polar in position rarely median, do not distend mother cell, from 1.4–1.8 microns long .6 microns broad, readily stained with double stain. These spores are very resistant to dessication and to heat.

CULTURAL CHARACTERS.

Dung Agar Plate Cultures.—Colonies amœboid, in twenty-four hours, $\frac{3}{10}$ mm. in diameter, flat, thin, smooth, colorless to light gray. Under low power of the microscope colonies may be seen to consist of twisted threads of bacteria, edge of colonies rather indefinite, slightly resembling *B. mycoides*, colony soft. Deep colonies similar but better defined. Very often under favorable conditions a few hours were sufficient to cover the plate with a uniform layer of the organisms. Sames notes this character in particular. "Die Platte bei 62° oft schon nach 6 Stunden uberwuchhern und zu dieser Zeit Sporen enthalten."

Nutrient Agar Plate.—Growth resembling that on young agar, but not nearly so luxuriant.

Gelatine.—Gelatine is not peptonized.

Milk.—No change perceptible, though mounts showed the organism to be present in numbers. Growth was not as luxuriant as noted by Sames.

Litmus Lactose Agar Stab.—At the end of twenty-four hours very faint acid tinge. In forty-eight hours lower half of tube partially decolorized, upper half showing decided increase in alkalinity. Growth almost exclusively on the surface, thin, colorless. Spore production particularly abundant.

Potato.—Very little or no growth. Mounts made from the surface of the potato showed the organism to be present in comparatively small numbers.

Bouillon.—Uniform clouding in twenty-four hours, in forty-eight hours, a sediment.

Agar Stab.—Surface growth as in plates. Practically no growth along the deeper portion of a needle track.

Dunham's Solution.—Little growth. Indol faint.

PHYSIOLOGICAL CHARACTERS. *Dessication.*—The spores are capable of withstanding drying for an indefinite period.

Temperature Optimum.—About 60° C. Minimum about 40° C. Maximum about 70° C.

Relation to Oxygen.—The organism is probably a strict ærobe, though a very slight development takes place in glucose agar stabs prepared according to Wright's method.

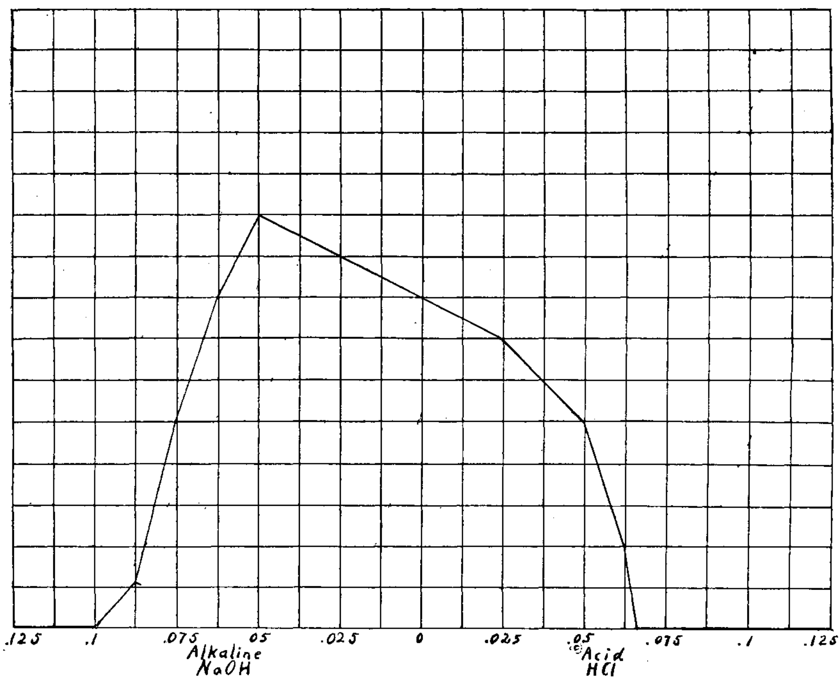

FIG. 8. Curve showing relation of growth to acidity or alkalinity of the medium.

Pigment.—No pigment is produced.

Gas Production.—No gas is produced in dextrose, lactose or saccharose media.

Relation of Growth to Acidity, Alkalinity of Medium.—Bouillon tubes each containing 10 c.c. of Bouillon, exactly neutral to phenol-phthalein were treated with quantities of normal hydrochloric acid and sodium hydrate varying from $\frac{1}{80}$ c.c. to .3 c.c. The above table shows the results in the form of a curve. The amount of growth in the tube containing the most growth was used as a standard, fixed arbitrarily at 10, and the remainder was scaled carefully with this as a basis.

Indol Production.—Negative or very weak. Odor very little if any.

Various media were prepared in order to determine the forms in which nitrogen and carbon are available to the organism as food. As a basis the Stickstoffreie "Mineralische Nährlosung" of Meyer was used.

KH_2PO_4	.1g
$CaCl_2$	.1g
$MgSO_4$-$7H_2O$	.3g
NaCl	.1g
Fe_2Cl_6	.01
H_2O	1000g

To this solution was added various sources of nitrogen and carbon, as is shown, with results, in the following table:

Number.	Source of Nitrogen.	Source of Carbon.	Growth.
1	1 per cent Asparagin	1 per cent Asparagin	None.
2	1 per cent Asparagin	1 per cent Glycerin	None.
3	.05 per cent Asparagin	1 per cent filter paper	None.
4	1 per cent Urea	1 per cent peptone	Good.
5	1 per cent peptone	1 per cent dextrose	Good.
6	1 per cent peptone	1 per cent dextrose	Good.
7	1 per cent peptone	1 per cent lactose	Good.
8	1 per cent peptone	1 per cent saccharose	Good.

It is probable that this organism is incapable of assimilating its nitrogen from any less complex compound than the peptones.

Mikroscopisches Practicum II. 15.

PLATE XXI.

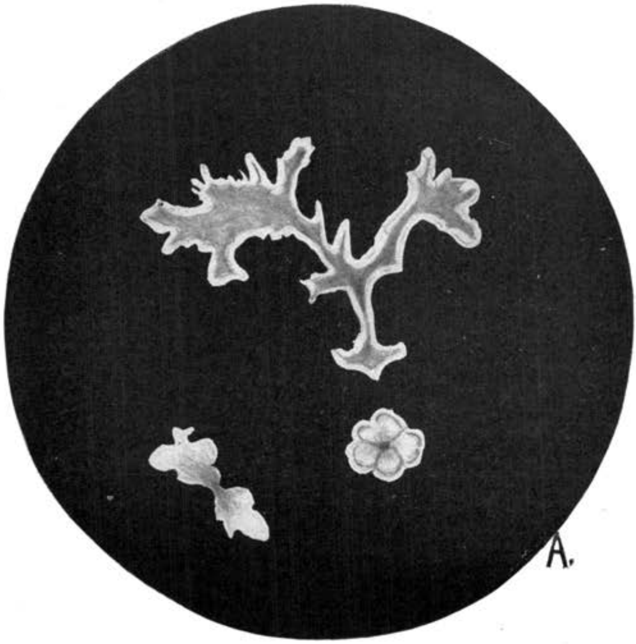

FIG. 1. Colonies in agar plate, 18 hours. Natural size.

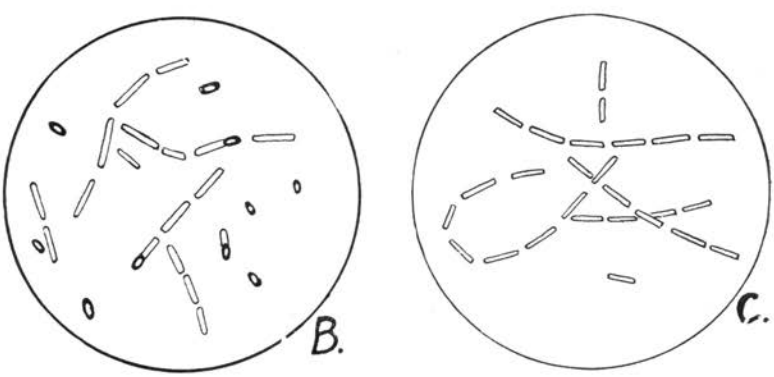

FIG. 2. *B*, Bacilli and spores from agar, 24 hours. *C*, Bacilli, showing chains from bouillon, 18 hours. [Charlotte M. King.]