1922
M83

STUDIES ON THERMOPHILIC BACTERIA

By

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A.B. University of Illinois, 1919

M.S. University of Illinois, 1921

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
BACTERIOLOGY IN THE GRADUATE SCHOOL
OF THE UNIVERSITY OF ILLINOIS, 1922

URBANA, ILLINOIS

1922
M83

UNIVERSITY OF ILLINOIS

THE GRADUATE SCHOOL

August 15, 1922

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY
SUPERVISION BY Lethe Eleanora Morrison

ENTITLED Studies on Thermophilic Bacteria

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR
THE DEGREE OF Doctor of Philosophy

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Acknowledgement

It is with pleasure that the author takes this opportunity to express her appreciation for much kindly advice and assistance received during the supervision of this work from Professor F. W. Tanner. She also wishes to thank him for encouragement received in all graduate study and teaching done under his supervision.

Thanks are also extended to those others, who by various means, aided in the completion of this investigation.

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I. Introduction

A new interest in thermophilic bacteria is rapidly developing since the investigations of Barlow (1912), Weinzirl (1919), Cheyney (1919), Donk (1920), and Bigelow and Estey (1920), have suggested their significance in the spoilage of canned foods. The wide distribution of these organisms in nature is demonstrated beyond doubt by a review of the literature which has been published describing strains isolated from soil, water, foods, manure, ensilage, hay, cotton, etc. The infection of foods with thermophiles results, probably, from their contact with soil and water. The object of this investigation was to study the characteristics, particularly the temperature relations including thermal death point determinations, of some thermophilic bacteria isolated from different samples of water and soil.

II. Historical

In order to have the present dissertation more complete in regard to the historical review and bibliography, a table summarizing the literature on thermophilic bacteria used in the Master's Thesis by this author is also included. (Table I.)

Since the experimental work of this investigation deals principally with the temperature relations and thermal death points of thermophilic bacteria isolated from various sources, it was deemed advisable to include here a brief review of that literature on thermophilic organisms regarding these points only.

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Inventor	Organism	Source	Temperature	Remarks
Alabert (1879-80)	<i>Bacillus thermophilus</i>	Geism's River water	40° - 70° C.	First isolated thermophilic bacterium
Van Tieghem (1881)	(1) <i>Streptococcus</i> (2) <i>Bacillus</i>	Water in hot springs	70° - 75° C.	First isolated thermophilic streptococcus
Porter and Garrison (1886)	(1) Small rods (2) Filaments	Hot springs at Bismarck	45° - 64° C.	First isolated thermophilic bacilli
Glozier (1887)	Many bacilli (3' kinds)	Geism's River	50° - 70° C.	First isolated thermophilic bacilli
Merrill (1889)	Two bacilli	Slime, Geism's River	40° - 70° C.	First isolated thermophilic bacilli
Belloc (1890)		Stable manure	Up to 70.5° C.	First isolated thermophilic bacilli
Cohn (1893)				Attributed to their own bacteria a role in the so-called "spore" disease of wheat, tobacco leaves, cotton, etc., and made an attempt to isolate any of these cultures
Deleury (1894)	Many bacteria	Sterile milk	40° - 44° C. or 27° - 34° C.	All strongly peptonizing in character; 80° are toxic; all formed spores which were destroyed by water or steam for two hours
Griffiths (1894)	<i>Bacillus valericus</i> <i>Bacillus thermicus</i>	Slime	50° - 66° C. 58° C. optimum 68° C. optimum 65° C. optimum	Functioned in the production of the so-called sweet slime
Deleury (1894)	A bacillus	Slime milk	45° - 50° C.	Produced acid
MacFadyen and Ellsall (1894)	Many bacilli	Earth, river and sea water, river mud, air dust, straw and feces of men, etc.	50° - 65° C.	Their most marked property appears to be the decomposition of proteins which they are able to effect. Most of them possess active fermentation properties
Gorini (1895)		Milk	37° -	Ambroz (1910) suggested that this organism was thermotolerant since it grew at 37° C. also
Korinski (1895)	(1) <i>Bacillus illidensis</i> (2) <i>Bacterium Lubrizi</i>	Hot springs of Illidze in Borsia	50° - 58° C. 55° - 57° C.	Has no explanation to offer as to significance of the presence of these two organisms in hot springs. Suggests that the examination of other hot springs for similar bacteria would greatly increase the knowledge of biology of water bacteria
Rabinowitch (1895)	Eight species of thermophilic bacteria	Many sources; widely distributed in nature	34° - 75° C.	Concluded that peculiar ability of so-called thermophilic bacteria to grow at temperatures so much higher than the optimum temperature for common bacteria is a property of adaptation to environment
Weller (1895)	<i>Bacillus I</i> <i>Bacillus II</i> <i>Bacillus III</i>	"Sterile" milk	22° - 50° C. 23° - 40° C. 25° - 55° C.	Found thermophilic bacteria in eight out of eleven samples of so-called "sterile" milk. Two of the three bacilli formed spores; none liquefied gelatin; all produced hydrogen sulphide
Herdman (1895)	Theophile bacteria	Slime	50° - 60° C.	No attempts made to characterize or identify the organisms
Kedzior (1895)	Cladotrix form	River Spree	35° - 65° C. 35° C. optimum	Facultative anaerobe grows better without oxygen. Spores very resistant to heat and to disinfectants such as 5 per cent phenol
Teich (1895)	<i>Bacillus form</i>	Hot springs of Illidze	44° - 38° C.	Large oval spores formed in one end of the rods make them appear club-shaped
Davis (1897)	Probably a bacillus	Hot springs of Yellowstone Park	Up to 85° C.	Functions in the formation of mineral deposits in hot springs
Harshbarger (1897)	White filamentous bacteria	Hot springs of Yellowstone Park	35.4° C.	Becomes of a sulphur yellow color at 175° F. Yellow growth due to species of Begriatoa, a plant which is classed with the Bacteriaceae, and which, during life, deposits sulphur granules
Koning (1897)	<i>B. tabaci III</i> <i>B. tabaci IV</i> <i>B. tabaci V</i>	Tobacco		Ambroz (1910) speaks of these organisms as real thermophilic bacteria
Hayashi (1897)	Many bacillus forms; mucopolysaccharide mass of bacteria; iron bacteria	Hot springs in Japan	41-69.0° C.	Hot springs of Japan furnish a good medium for thermophilic bacteria
Wittlin (1897)		Hot springs in Switzerland		Ambroz (1910) explains the negative results of Wittlin on the grounds of inadequate methods of research
Laxa (1898)	<i>Clostridium relatiuosum</i>	Fermentation in sugar manufacture	35° - 58° C.	This organism is a facultative anaerobe whose spores are not killed by exposure to steam at 120° C. for 15 minutes
Opreau (1898)	<i>Bacillus thermophilus liquefaciens aerophilus</i> <i>Bacillus thermophilus aerobius</i> <i>Bacillus thermophilus aquatilis</i> <i>Bacillus thermophilus reducens</i> <i>Bacillus thermophilus liquefaciens tyrocyticus</i>	Soil, Berlin Zoological gardens Canal water Spring water, ice Baked serum test glass Requefort cheese	1° - 70° C. 36° - 60° C. 36° - 60° C. 60° - 62° C. Room temperature to 60° C.	Opreau gave rather a detailed description of the five forms studied by him
Poupe (1898)	An organism similar to <i>Clostridium relatiuosum</i> Laxa (1898)	Syrup	45° C.	
Schillinger (1898)	Four types	Soil	60° C. max.	After experiments on bacteria from soil carried on at different temperatures, he came to the conclusion that thermophilic bacteria are not properly so-called; the term thermotolerant should be applied to those organisms which can adapt themselves to high temperatures
Teiklinsky (1898 and 1899)	<i>Thermococcus vulgaris</i> <i>Thermococcus lanuginosus</i>	Soil	48° - 69° C.	Also isolated 6 varieties of bacteria from the hot springs of Island of Ichia which she called strict thermophiles; optimum temperature 60° C.
Knackels (1899)	<i>B. thermophilus aquatilis liquefaciens</i> <i>B. thermophilus aquatilis liquefaciens aerophilus</i> <i>B. thermophilus aquatilis chromogenes</i> <i>B. thermophilus aquatilis lanuginosus</i>	Spring water, Berlin	50° - 60° C. optimum	Knackels said of these organisms that they were not only thermotolerant, but also thermophilic
Vermhout (1899)	<i>Bacillus tabaci fermentationis</i>	Per entire tobacco at 44° - 50° C.	50° C. optimum	Not a true thermophile since its optimum temperature was 25° C.

1. 10-11-1911

2. 10-12-1911

3. 10-13-1911

4. 10-14-1911

5. 10-15-1911
6. 10-16-1911

7. 10-17-1911

Inventor	Year	Organism	Medium	Temperature	Remarks
Cornell (1860)	1860	Bacterium thermophilum	Milk, cream, etc.	70° - 75° C.	First isolated from milk; called "thermo acidophile"
Germann (1900)	1900	Bacillus thermophilus Griseb.	Milk	50° - 60° C. optimum	Isolated from milk; used for cheese making
Rodwell & Hastings (1901)	1901	Bacillus thermophilus	Milk	50° - 60° C. optimum	Isolated from milk; used for cheese making
Schardner (1903)	1903	Group I Streptococcus thermophilus Group II Streptococcus thermophilus	Milk, etc.	50° - 60° C.	Group I is more acid fast than Group II
McNeil (1902)	1902	Streptococcus lactis	Hot springs of Yellowstone Park	70° - 80° C.	One of the first thermophiles isolated from hot springs
Willsky (1903)	1903	Streptococcus thermophilus	Human alimentary tract	50° C.	Believed the constant appearance of thermophilic bacteria in feces was explained by their wide distribution in nature and their great resistance; thermophiles are probably merely variations of common non-thermophilic organisms
Harter (1904)	1904	Bacterium thermophilus rodatus	Water	50° - 60° C. optimum	Also isolated one organism apparently identical with B. thermophilus IV (Rabinowitch, 1905)
Harter (1904)	1904	Actinomyces thermophilus	Soil	50° - 60° C. optimum	Strict aerobic; liquefied gelatin slowly; coagulated milk
Harter (1904)	1904	Two forms	Hot spring	60° - 65° C. optimum	A mesophilic form positive for catalase produced large central spores
Griffith (1905)	1905	A bacterium	Hot spring	70° - 75° C. optimum	A mesophilic form positive for catalase produced large central spores
Griffith (1905)	1905	Thirteen bacilli, five streptothrix forms	Adult and infant stools	50° - 60° C.	All were strictly aerobic; all gave positive results for catalase
Niehe (1905)	1905	Bacillus thermophilus alpha	Milk	50° - 60° C.	No lactic acid production observed
Anitskov (1906)	1906	Bacillus thermophilus delta	Milk	50° - 60° C.	Only occasionally found thermophiles in human alimentary tract
Pardou (1906)	1906	Bacillus thermophilus alpha Bacillus thermophilus eta Bacillus thermophilus gamma Bacillus thermophilus delta	Milk	50° - 60° C. optimum	Author says Pardou's work on the chemical reactions of thermophiles is fundamental and far surpassed in detail and accuracy other work that had been done. Three of four strains described were desulfurizers; all of them were strongly proteolytic
Lau (1906)	1906	B. cylindricus B. rufus B. testaceus B. calidus	Soil	50° - 60° C. optimum	Thermal death point 100° C. for 20 hours Thermal death point 100° C. for 7 1/2 - 8 hours Extremely resistant spore. Thermal death point 100° C. for 19-20 hours Thermal death point 100° C. for 3 hours
Ratella (1906)	1906				Reported thermophilic bacteria of little significance in water
Rabinowitch (1907)	1907	B. thermophilus I B. thermophilus II B. thermophilus III	Hot spring	70° C. optimum	Concluded that hot spring is favorable media for thermophilic bacteria
Niehe (1907)	1907	B. caldofactor	Milk	50° - 60° C. optimum	Claimed this organism was responsible for heating of milk under orthothermophilic conditions (Maximum above coagulation point of protein, 60° - 70° C.)
Tirelli (1907)	1907	Four rods; four cocci; two thread forms	Drinking water	50° - 60° C. optimum	Believed temperature relations due to particular chemical nature of protoplasm rather than to their adjustment to the circumstances of their environment
Schütze (1908)	1908	Many forms of B. caldofactor (Niehe) type	Point clover hay	50° - 60° C. optimum	Believed that thermophiles grew better under aerobic conditions at high temperature and at lower temperature they grew better under anaerobic conditions
Heffer (1909)	1909				Discussed possibility of spontaneous combustion of different organic materials by thermophilic bacteria
Ammer (1910)	1910				A complete review of literature on thermophiles up to 1910
de Kruffyff (1910)	1910	Ten rod forms	Soil, water, air in tropics	55° - 65° C.	Claimed thermophiles were very abundant in tropical climates. Proved thermophiles were very important in biological changes in nature
Georgievich (1910)	1910	B. thermophilus Transiensis B. thermophilus Viviani B. thermophilus Lomanitche	Hot springs	50° - 60° C. optimum 45° - 45° C. optimum 70° - 75° C. optimum	
Koch & Hoffman (1911)	1911	Two spore-forming bacilli One spore-forming bacterium One thread form	Soil	52° C.	Concluded that the nature of the media had a great influence on the temperature response of these organisms, since they grew in soil at a temperature of 28° - 30° C. and would not grow on artificial media at this temperature
Harlow (1912)	1912		Canned corn		An organism said to be responsible for spoilage of canned corn
Kroulik (1912)	1912				Thought bacteria and actinomycetes forms which decompose cellulose at 60° - 65° C. are widely distributed in nature and occur especially where cellulose is naturally decomposed
Moock (1912)	1912				Review of literature
Ambroz (1913)	1913	Dextrorotatory thermophilum	Soil	37° C. below minimum temperature	Called a true thermophile because it would not grow at room temperature nor at 37° C. Ambroz thinks the importance of thermophilic bacteria in the cycles of nature can not be overestimated since they play such an important role in metabolic processes
Merre (1913)	1913	Numerous forms	Sand of Sahara		
Prinselm (1913)	1913				Studied decomposition of cellulose by thermophilic bacteria
Koesowicz (1912-13)	1912-13				Studied thermophilic bacteria in sugar juices
Gerrey (1913)	1913	Nine different bacteria	Dust, soil, etc.	60° - 80° C. maximum	Suggested separating into two groups, the true thermophiles and the facultative thermophiles
Cheney (1913)	1913	Organisms like I, IV, and VI of Rabinowitch	Canned foods	50° C.	Explained fact that so few thermophiles are found in canned foods on the market by supposing that nearly all cans containing them become spoiled and so are automatically eliminated before reaching the market
Patraschke (1919)	1919	Streptococcus lactis thermophilus	Milk		
Weinsel (1919)	1919	B. thermophilus B. aerothermophilus B. thermophilus	Canned foods	37° C. optimum 50° C. optimum 60° C. optimum	
Donk (1920)	1920	B. aerothermophilus	Canned corn, string beans, etc.	60° C. optimum	Can live at 80° C. in the presence of dark stacks
Miller (1920)	1920				Found that the thermophiles were present in the soil
O'Brien-Smith (1920)	1920	Spore-bearing	Fermenting tank	60° C. optimum	Can live at 80° C. in the presence of dark stacks

Miquel (1888) attributed the ability of Bacillus thermophilus to live and thrive at a temperature of 70° C. and above to the particular character of its protoplasm. He thought, since it grew at a temperature at which egg albumin and blood serum were rapidly coagulated, that this bacterium possessed a protoplasmic structure different from that of animals and higher plants.

Globig (1888) did not attribute the ability of the bacteria which he isolated from soil, to grow at a temperature of 50° - 70° C., to any peculiar characteristic. He thought, because these organisms were found in the superficial layers of the earth, that the rays of the sun furnished the heat necessary for their growth.

Flügge (1894) was probably the first to report thermal death point determinations for thermophilic bacteria. The spores of the different thermophiles isolated from so-called sterile milk were found to withstand 100° C. for different periods of time varying from 3/4 to 5 hours.

No explanation was offered by Karlinski (1895) for the significance of the two thermophilic bacteria isolated from hot springs. He considered it remarkable that while the cultures of Bacterium Ludwigii lost their vitality at a temperature below 50° C. and were killed at room temperature, the original organisms endured transportation in the original water and a

lowering of the temperature to 20° C. for several days. The spores of Bacillus Illidzensis capsulatus were found to withstand flowing steam at 100° C. for 4 minutes.

Rabinowitsch (1895) found that the spores of eight thermophilic bacteria isolated from various sources survived steam at 100° C. for from 5 to 6 hours. However, in spite of this remarkable resistance to heat, she concluded that the ability of the so-called thermophilic bacteria to grow at such high temperatures was a property of adaptation to environment. She thought these organisms were mostly aerobic but that in the absence of oxygen they would grow at ordinary temperatures.

That thermophilic bacteria might find conditions favorable for growth in the temperatures developed during various fermentations in nature, such as the fermentation of manure, ensilage, and moist hay, was suggested by MacFadyen and Blaxall (1896).

The spores of the cladothrix form described by Kedzior (1896) were found to be very resistant to heat and to disinfectants. They were not killed by flowing steam at 100° C. in less than 3 1/2 to 4 1/2 hours.

Laxa (1898) reported the spores of Clostridium gelatinosum to be very resistant to both dry and moist heat. They were not killed by exposure to dry heat at 150° C. for 15 minutes nor by exposure to moist heat at 100° C. for 75 minutes.

In the same year Opreescu (1898) concluded from his work on thermophilic bacteria that their biological properties showed wide variations in relation to temperature, these variations depending upon the media used. This was the first reference made in the literature to the fact that the type of media used governed the temperature for the growth of thermophiles.

About the same time, Schillinger (1898) concluded from the results of his experiments that in spite of the fact that these bacteria grew at such a high temperature, this temperature could not be called the optimum. He also thought they were improperly called thermophilic and that the name thermotolerant should be applied to them.

The next year Michaelis (1899), in opposition to Schillinger, stated that the term thermophilic should be retained and that the high temperature for the growth of the organisms studied by him was in all cases the optimum temperature.

Mlle. Tsiklinsky* (1899) encountered two forms in soil

* There seems to be some confusion in the literature with regard to the spelling of this investigator's name. The two papers published in the Annals l'Institute Pasteur bear the name with two different spellings. The names of some of the other investigators are also found with two different spellings. This accounts for a few mistakes in the names of authors in another publication by the present authors on this subject.

which she called Thermoactinomyces vulgaris and Thermomyces lanuginosus. The spores of the first of these were not killed after 20 minutes at 100° C. in an autoclave. The spores of the

second one were killed in 1 minute at 100° C. but withstood dry heat at 80° C. for 3 hours.

The most extensive work on the thermal death points of thermophilic bacteria up to this time, was done by Sames (1900). He found that the temperature at which the spores were formed influenced their resistance to flowing steam at 100° C. The period of time for which the spores of the eight Bacilli described by him resisted steam at 100° C. ranged from 1 minute to 120 minutes. The nature of the medium used was also found, as in the work by Oprescu, to influence the temperature for the growth of these organisms; separating them into thermophiles and thermo-tolerants, although the line between them was not closely drawn.

In an account describing a Micrococcus found in pasteurized milk, Russell and Hastings (1902) stated that this organism was able to resist the action of heat at 76° C. for 10 minutes. This fact seemed to indicate that the protoplasm of the organism was markedly different from that of most bacterial protoplasm and suggested a "degree of tolerance toward heat that was somewhat analogous to that possessed by the thermophilic organisms in their capacity to develop at abnormal temperatures".

Schardinger (1903) studied one group of bacteria in foods and milk which grew between room temperature and 55° C. The spores of two forms in this group withstood an exposure to boiling water for 2 hours, while the spores of two other forms

were killed in 1 hour by a like exposure.

In the same year Setchell (1903) carried out an investigation of the life of hot springs. He found that bacterial forms endure the highest temperature observed for living organisms, being abundant at 70° - 71° C., and found in some considerable quantity at 82° C. and at 89° C. In trying to explain this phenomenon, Setchell stated that there was nothing to indicate that the protoplasm of these thermophilic bacteria contained so little water as to render it incoagulable by the high temperatures which they endure. To him it seemed rather that there might be some important difference in the essential proteids of the mixture or in the nature of the constitution of the substance which rendered it less coagulable.

Again in 1903 Mlle. Tsiklinsky contributed to our knowledge of thermophilic bacteria by an extensive report of these organisms isolated from human feces. She found that the spores of four Bacilli, isolated from infant feces, resisted heating for 5 minutes at 100° C. in an autoclave. As a result of her researches she concluded that thermophiles were merely variations of common non-thermophilic organisms, having adapted themselves slowly to high temperatures. She thought that the length of time during which they had adapted themselves to high temperatures made them either facultative thermophiles or obligate thermophiles. She explained their constant presence in feces on the basis of their wide distribution in nature and their

great resistance. Her results seemed to show that all obligate thermophilic bacteria were obligate aerobes and that the facultative thermophiles were facultative aerobes. In this respect her results agreed with those of Rabinowitsch.

Bruini (1905) offered two possible explanations for the existence of thermophilic bacteria in the intestinal tract. Perhaps they were merely transitional forms of ordinary bacteria from air and foods and had no part in the fermentation and putrefaction in the intestines, or perhaps thermophiles found the proper growth conditions in symbiosis with other organisms so that they could grow at temperatures below their optimum.

The thermal death points at 100° C. of the spores of the four thermophilic bacteria that Blau (1906) isolated from soil were found to vary from 7 to 20 hours. From the results of his work, Blau reached the conclusion that varying the kind and concentration of the media changed to some extent both the morphological and physiological characteristics of these organisms.

During investigations on the spontaneous heating of hay, Miehé (1907) compared thermophilic bacteria with ordinary bacteria, and concluded there were many transitional stages between the two types, the main groups being distinguished by their minimum temperatures. Organisms that did not grow at 25° C. were thermophiles according to Miehé's classification; these he again divided into (1) orthothermophiles with the maximum temperature

above the coagulation for albumin (60° - 67° C.) and (2) thermotolerants with a maximum temperature of 50° - 55° C., but which also grow well at ordinary temperatures. The organisms which grew best at higher temperatures but also grew, though feebly, at lower temperatures, he termed psychrotolerant.

In accordance with the findings of Miquel, Russell and Hastings, and Setchell, Tirelli (1907), from the results of his work, concluded that the ability of thermophiles to withstand such high temperatures must be attributed to the particular nature of their protoplasm, rather than to their adjustment to the circumstances of environment.

In work on thermophilic bacteria in the tropics de Kruffy (1910) tested the resistance of the spores of ten forms isolated from soil and found that they were killed in boiling water in 4 1/2 to 8 hours. He also found that thermophilic bacteria grew better aerobically at the higher temperatures and anaerobically at the lower temperatures. The same results were obtained by Rabinowitsch and Tsiklinsky.

Ambroz (1910) claimed that if the high temperatures at which thermophiles would grow was not the optimum, as stated by Schillinger, then thermophilic bacteria should grow better at ordinary temperatures than they do at the high temperatures. This is not the case.

The conclusions drawn by Oprescu, Sames, and Blau were confirmed by Koch and Hoffman (1911). The organism which they isolated from soil would grow on artificial media at 52° C., but

not at 25° - 28° C.; in soil they would grow at 28° - 30° C. but not so well as at 52° C. From these results they also concluded that the nature of the medium used had a great influence on the temperature demanded for the growth of thermophiles.

Some spores of the thermophiles found in soil by Kroulik (1912) were quite resistant to heat. The spores of two of these organisms withstood sterilization with flowing steam for 2 hours, while those of two others were a little less resistant.

Noack (1912) emphasized the importance of the fact that the resistant forms of thermophilic bacteria would stand many changes of temperature below as well as above their optimum temperature. He believed that this was the reason they can be isolated from soil almost any time, whereas, except in the tropics, the temperature of soil seldom rises to the optimum temperature for these organisms. This might also account for their occurrence in decomposing organic matter in which the temperature, due to spontaneous heating seldom reaches the optimum temperature for their growth.

Although the thermophiles studied by Negre (1913) were isolated from the sands of the Sahara, they were less resistant than many of the forms which have been described. The spores of these forms resisted heating at 100° C. for 15 to 20 minutes.

Bergey (1919) divided thermophilic bacteria into two groups, namely, the true thermophiles, and the facultative thermophiles. In his studies on many organisms in these two groups he found that the spores of true thermophiles resisted a temperature

of 100° C. for periods of time varying from 5 to 400 minutes, while those of the facultative thermophiles were killed in 15 to 60 minutes at 100° C. In the light of this it appeared probable to Bergey that the optimum temperature for growth was related to the heat resisting powers of the spores. He considered that the phenomenal characteristic of thriving at temperatures above those at which egg and serum albumin coagulate, exhibited by thermophilic bacteria, might be due to the reaction of the medium in which the bacteria were grown, or to the mineral content of their own protoplasm.

While investigating the use of a biorizator for pasteurizing milk Patzschke (1919) discovered an organism which he named Streptococcus lacticus thermophilus. This organism resisted a temperature of 75° C. for 3 minutes but was killed at 85° C. in 10 seconds.

The organism isolated by Donk (1920) from canned corn was very resistant because it was found in corn that had been processed at 118° C. for 75 minutes.

The experiments of Bigelow and Estey (1920) constitute the first very extensive work on the thermal death points of thermophilic bacteria. They described a new method of determining the thermal death points of spores, at a temperature of 100° C. and above, under definite and well controlled conditions. The longest length of time that any of the spores of the organisms which they studied were found to resist the action of heat under the described conditions was 1320 minutes at 100° C. This is a

greater degree of resistance than has been reported by any other investigator.

The spores of an organism found to cause the fermentation of spent wattle-bark in the corrosion of white lead by Grieg-Smith (1920) were very difficult to destroy. This was especially true when the spores were contained in the pores of the bark. They lived after an exposure to 186° to 205° C. for 2 1/2 hours.

Although Buchanan (1922) gives no authority for his remarks, he makes the statement that thermophilic bacteria "are so resistant that they will withstand boiling water literally for days before being destroyed".

Thermal death point determinations and temperature relations reported by the investigators included in this survey are summarized in the following table.

Table II

Investigator	Reference	Organism Described	Source	Temperature for Growth	Thermal Death Points
(1) F. M. (1897)	Ann. de l'Inst. Pasteur, 17, 372	Bacillus pasteurii	Sterilized milk	74° - 44° C. optimum - 54° C.	Spores killed at 100° C. for 3 to 4 hours
(2) F. M. (1897)	Ann. de l'Inst. Pasteur, 17, 372	Bacillus pasteurii I	Soil; excreta; grains; milk; soil; manure; excreta; grains; milk.	34° - 75° C.	Spores resisted steam at 100° C. for 3 to 6 hours
		" " II	Soil; excreta; grains; milk.	"	"
		" " III	Soil; excreta; grains; milk.	"	"
		" " IV	Soil; excreta; grains; milk.	"	"
		" " V	Excreta; grains; milk.	"	"
		" " VI	Excreta; grains; milk.	"	"
		" " VII	Excreta; grains; milk.	"	"
		" " VIII	Excreta; grains; milk.	"	"
(3) Korlinaki (1895)	Hyg. Revue, 1, 308	Bacillus illudensis Capriolus	Hot springs of Illud in Russia	50° - 58° C.	Spores withstood flowing steam at 100° C. for 4 minutes.
(4) G. G. (1896)	Ann. de l'Inst. Pasteur, 17, 328	A thermophilic coccobacillus form	River Sree	55° - 65° C.; 55° C. optimum	Spores killed by flowing steam at 100° C. in 3 1/2 to 4 1/2 hours.
(5) Lenz (1898)	Centralbl. f. Bakteriologie, 11, 3, 362	Clostridium relatuense	"Füllmaße" in sugar manufacture	25° - 58° C.	Spores not killed by exposure to dry heat at 150° C. for 15 minutes or moist heat at 100° C. for 75 minutes.
(6) Taitelinsky, M. P. (1899)	Ann. de l'Inst. Pasteur, 13, 507	Thermophilomyces vulgaris Thermomyces lanuginosus	Soil	42° to 62° C.; 57° C. optimum 42° to 60° C. 54° to 55° C. optimum	Spores not killed after 20 minutes at 100° C. in an autoclave. Spores killed in one minute at 100° C.; withstood dry heat at 80° C. for 3 hours.
(7) S. (1900)	Ztschr. f. Hyg., 33, 313	Bacillus I Bacillus II Bacillus III Bacillus IV Bacillus V Bacillus VI Bacillus VII Bacillus VIII	Earth Pus from nose injected with earth and immunized against tetanus Vaginal mucus during pregnancy Raw milk Aqueous litmus solution Same as Bacillus II Air Earth	56° - 70° C. optimum 70° - 74° C. maximum 56° - 70° C. optimum 73° C. maximum 56° - 70° C. optimum 56° - 60° C. optimum 56° - 70° C. maximum 56° - 62° C. optimum 63° - 66° C. maximum 56° - 70° C. optimum 66° - 70° C. maximum 50° - 60° C. optimum 66° - 70° C. maximum 50° - 60° C. optimum 66° - 70° C. maximum	At 750.2 mm. pressure spores resisted live steam for 3 hours and 10 minutes. Spores were formed at 62° C. Spores resisted steam at 743.7 mm. pressure for 2 hours and 10 minutes. Spores were formed at 62° C. Resistance of spores formed at 62° C. at 740. mm. pressure = 25 minutes. Resistance of spores formed at 56° C. to steam at 745.1 mm. pressure = 15 minutes. Resistance of spores formed at 62° C. to steam at 745.5 mm. pressure = 210 seconds. Spores formed at 62° C. at 745.5 mm. pressure = 60 seconds. Resistance of spores formed at 62° C. to steam at 745.1 mm. = 13 minutes. Spores formed at 37° C. at 745.3 mm. = 2 minutes. Resistance of spores formed at 56° C. to steam at 743.3 mm. = 100 minutes. Spores formed at 37° C. at 749.3 mm. = 60 to 70 minutes. Resistance of spores formed at 56° C. to steam at 745.1 mm. = 120 minutes. Spores formed at 62° C. resistance to steam at 748.9 mm. = 60 minutes.
(8) Russell & H. (1901)	Centralbl. f. Bakteriologie, 11, 6, 336	A thermophilic form	Pasteurized milk	70° - 25° C. optimum 76° C. maximum	Thermal death limit - 76° C. for 10 minutes.
(9) Taitelinsky (1903)	Ann. de l'Inst. Pasteur, 17, 217	Bacille No. 7 Bacille No. 8 Bacille No. 9 Bacille No. 10	Infant feces " " " " " "	24° - 57° C. 37° C. to 60° C. 37° C. optimum 42° to 45° C. optimum 37° C. optimum Grew also at 20° C.	Spores resisted heating for 5 minutes at 100° C. in an autoclave. Spores resisted heating for 5 minutes at 100° C. in an autoclave. Spores resisted heating at 100° C. in an autoclave for 5 minutes. Spores resisted heating for 5 minutes at 100° C. in an autoclave.
(10) Schardiner (1903)	Ztschr. f. Untersuch. d. Nahrungs-u. Genussmittel, 6, 865	Group I No. 1 " No. 2 " No. 5 " No. 6	Feeds etc. " " "	Room temperature - 55° C.; 56° C. optimum " " "	Spores withstood 3 hours exposure to boiling water. " " " " " " " " " " " " Spores killed within 1 hour in boiling water. Spores did not withstand exposure of 1 hour in boiling water.
(11) E. (1906)	Centralbl. f. Bakteriologie, 11, 15, 97	B. cylindricus B. rotundus B. tostus B. salidus	Soil " " "	60° - 70° C. optimum 55° - 60° C. " 60° - 70° C. " 60° - 65° C. "	Thermal death point 100° C. for 13 to 20 hours " " " " " " 7 1/2 to 3 hours " " " " " " 19 to 20 hours " " " " " " 7 to 8 hours
(12) de Krayff (1910)	Centralbl. f. Bakteriologie, 11, 26, 65	Bacterium No. 1 " " 2 " " 3 " " 4 " " 5 " " 6 " " 7 " " 8 " " 9 " " 10	Soil " " " " " " " " "	37° - 70° C.; 60° C. optimum 40° - 73° C.; 65° C. " 40° - 70° C.; 60° C. " 40° - 72° C.; 63° C. " 39° - 67° C.; 60° C. " 35° - 73° C.; 65° C. " 35° - 70° C.; 55° C. " 39° - 70° C.; 58° C. " 33° - 67° C.; 60° C. " 38° - 59° C.; 60° C. "	Spores killed in 5 1/2 to 6 hours in boiling water. Spores killed at 100° C. in 6 1/2 to 7 hours " " " " " " 6 1/2 hours " " " " " " 9 hours " " " " " " 6 1/2 hours " " " " " " 4 1/2 hours " " " " " " 5 1/2 to 6 hours " " " " " " 7 1/2 hours " " " " " " 7 hours " " " " " " 5 hours
(13) K. (1912)	Centralbl. f. Bakteriologie, 11, 36, 339	Bacillus I 1 Bacillus I 2 Bacillus II 1 Bacillus II 2	Soil " " "	60° C. optimum 60° C. optimum 30° - 58° C. 55° - 60° C. optimum Same as for B. II 1	Spores withstood 2 hours sterilization with flowing steam Spores withstood sterilization with flowing steam for 2 hours Resistance of spores greater than that of B. II 2 Spores did not withstand the action of flowing steam for 2 hours.
(14) H. (1913)	Compt. rend. Soc. de Biol., 74, 344	Bacillus 3 " 4 " 6	Sands of the Sahara " "	70° C. max. 50° C. opt. " "	Spores resisted heating at 100° C. for 15 to 20 minutes.

Investigator	Real Death Points.
(15) Bergey (1919)	<p>Journal Death Point at 100° C. - 400 minutes</p> <p>" " " " - 300 minutes</p> <p>" " " " - 200 minutes</p> <p>" " " " - 180 minutes</p> <p>" " " " - 120 minutes</p> <p>" " " " - 60 minutes</p> <p>" " " " - 5 minutes</p> <p>" " " " - 5 minutes</p> <p>" " " " - 120 minutes</p> <p>" " " " - 120 minutes</p> <p>" " " " - 15-60 minutes</p>
(16) Patzschke (1919)	<p>27 1 death point - 75° C. for 3 minutes</p> <p>22 ed temperature of 85° C. for 10 seconds.</p>
(17) Donk (1920)	<p>Journalled by processing at 118° C. for 75</p> <p>es</p>
(18) Bigelow & Esty (1920)	<p>Journal necessary to destroy a known suspension of</p> <p>27 in medium of known hydrogen-ion concentra-</p> <p>decreases as the temperature increases.</p> <p>hydrogen-ion concentration influences the</p> <p>necessary to destroy a known suspension of</p> <p>at a given temperature.</p> <p>initial concentration of spores influences</p> <p>me necessary to sterilize a medium of</p> <p>hydrogen-ion concentration at a given</p> <p>ature.</p>
(19) Grieg-Smith (1921)	<p>not killed after exposure to 186° to 205° C.</p> <p>1 1/2 hours.</p> <p>I</p>

Investigator	Reference	Organisms Described	Source	Temperature for Growth	Thermal Death Points.
(15) Bergey (1919)	Jour. Bact., 4, 301	Type 1. Type 2 Type 3 Type 4 Type 5 Var. a. Type 5 Var. b. Type 6 Type 7 Type 8 Var. a. Type 8 Var. b. Type 9	Dust; contaminated milk media Dust; soil Dust; soil; horse manure. Same as Type 3 Dust; g. pig feces, horse manure Dust; cheese; g. pig feces. Horse manure Same as Type 6 Rabbit's stomach Contamination on agar Same as Type 3	50° - 60° C. minimum 75° - 80° C. maximum 37° C. minimum 70° C. maximum 37° - 50° C. minimum 70° - 75° C. maximum 50° C. minimum 75° - 80° C. maximum 37° C. minimum 60° - 70° C. maximum 37° C. minimum 50° C. maximum 37° C. minimum 60° C. maximum 37° C. minimum 60° C. maximum 60° C. minimum 70° C. maximum 20° - 37° C. minimum 50° - 60° C. maximum	Thermal Death Point at 100° C. - 400 minutes " " " " " - 300 minutes " " " " " - 200 minutes " " " " " - 180 minutes " " " " " - 120 minutes " " " " " - 60 minutes " " " " " - 5 minutes " " " " " - 5 minutes " " " " " - 120 minutes " " " " " - 120 minutes " " " " " - 15-60 minutes
(16) Patzschke (1919)	Ztschr. f. Hyg., 31, 226	Streptococcus lacticus thermophilus	Milk		Thermal death point - 75° C. for 3 minutes Resisted temperature of 85° C. for 10 seconds.
(17) Donk (1920)	Jour. Bact., 5, 373	B. stearothermophilus	Canned corn	45° - 76° C.	Not killed by processing at 118° C. for 75 minutes
(18) Bigelow & Esty (1920)	Jour. Infect. Dis., 27, 602	19 thermophilic microorganisms			Time necessary to destroy a known suspension of spores in medium of known hydrogen-ion concentration decreases as the temperature increases. The hydrogen-ion concentration influences the time necessary to destroy a known suspension of spores at a given temperature. The initial concentration of spores influences the time necessary to sterilize a medium of known hydrogen-ion concentration at a given temperature.
(19) Grieg-Smith (1921)	Proc. Linnean Soc. New South Wales, 46, Part I, 76.	A rod-shaped bacterium	Fermenting wattle-bark		Spores not killed after exposure to 186° to 205° C. for 2 1/2 hours.

III. Experimental

Sources of Cultures

Cultures 53 to 87 inclusive, used in this investigation, were isolated from various samples of soils and hog and cow feces. Part of these samples were used in an investigation on the distribution of Clostridium botulinum in nature by Tanner and Dack (1922). Some of the soils were taken from fields that had been manured recently, some from fields that had been treated with commercial fertilizers, and some from fields that so far as could be determined, had received no treatment for the last five years. All of the soils tested were found to contain thermophilic bacteria. Cultures 88 and 89 were isolated from "Ever Fresh Milk", a commercial product. The cultures 1 to 52 were isolated from different samples of water and were used in the master's thesis by the author.

Methods of Isolation

Ten cubic centimeter portions of water suspensions of the different soils and feces to be tested were inoculated into large test tubes, each containing 20 cc. of dextrose broth. These tubes were incubated at 55° C. for 24 hours; from these mixed cultures, dextrose agar plates were poured in the usual manner and incubated at 55° C. for 24 hours. By using several dilutions for plating it was possible to get well isolated colonies and one thermophilic bacterium was isolated from each sample of soil or feces tested. The two cultures from "Ever Fresh Milk" were isolated from different bottles of

this product by plating in different dilutions on dextrose agar. The method of isolation used for the thermophilic bacteria from water (cultures 1 - 52) was described in the master's thesis.

Methods of Study

Inoculations into the different media used in this work were made either from 24 hour agar slant cultures or 24 hour broth cultures. The Descriptive Chart of the Society of American Bacteriologists indorsed by them in 1920 was used in the study of these thermophiles. A copy of this chart is shown on the next page. The index number for each culture was determined under as uniform conditions as possible. Some of the characteristics of the cultures 1 - 52 as determined for the group numbers of these organisms were used in the index numbers of these cultures.

Media and Technic

With one or two exceptions, the media used in this study were those recommended by the Committee on the Descriptive Chart in their report, "Methods of Pure Culture Study", presented to the Society of American Bacteriologists (1919). All media were tested for sterility by incubation at 55° C. for 12 to 24 hours before being used; the cultures were all grown at 55° C. Due to the rapidity with which thermophiles grow it was not found necessary to incubate test cultures longer than 4 days, except in the case of milk cultures which were incubated for 7 to 10 days. Two per cent agar was used throughout this investigation because it seemed to be more suitable

Name of organism..... Studied by..... Culture No.....
Date of isolation..... Number of transfers..... Length of each incubation..... days. Series No.....
Medium used..... Temperature.....

MORPHOLOGY

NOTE—Underscore required terms.

VEGETATIVE CELLS. Medium used..... temp..... age..... days..... Form..... spheres, short rods, long rods, filaments, com- mas, short spirals, long spirals, curved, Arrangement, single, pairs, chains, flocs, clusters, cubical packets. Limits of Size..... Ends, rounded, truncate, concave. CAPSULES, present on..... How stained.....		SPORANGIA, present, absent. Medium used..... temp..... age..... days..... Form..... elliptical, short rods, spindled, clavate, drum- sticks. Limits of Size..... Size of Majority..... ENDOSPORES, present, absent. Location of Endospores, central, polar. Form, spherical, elliptical, elongated. Limits of Size..... Size of Majority..... Wall, thick, thin. Sporangium wall, adherent, not adherent.	
MOTILITY In broth..... On agar..... FLAGELLA, No..... Attachment, polar, bipolar, peritrichate. How stained.....		IRREGULAR FORMS. Present on..... in..... days..... at..... °C. Form spindled, cuneate, filamentous, branched, or.....	
STAINING REACTIONS. Gram..... Acid fast..... Special stains.....			

CULTURAL CHARACTERISTICS

Underscore required terms.		SKETCHES		Underscore required terms.	
Agar Stroke..... Incubation Temperature..... °C Age..... d	Growth, scanty, moderate, abundant, none. Form of growth, filiform, echinulate, beaded, spread- ing, arborescent, rhizoid. Elevation of growth, flat, effuse, raised, convex. Lustre, glistening, dull. Topography, smooth, contoured, rugose. Optical Characters, opaque, translucent, opalescent, iridescent. Chromogenesis..... Photogenic, Fluorescent. Odor, absent, decided, resembling..... Consistency, butyrous, viscid, membranous, brittle. Medium, grayed, browned, reddened, blueed, greened.		Nutrient Broth Temperature..... °C Age..... d	Surface growth, ring, pellicle, flocculent, membranous, none. Clouding, slight, moderate, strong, tran- sient, persistent, none, fluid turbid. Odor, absent, decided, resembling..... Sediment, compact, flocculent, granular, flaky, viscid on agitation, abundant, scant, none.	
Gelatin Stab Temperature..... °C Age..... d	Growth, uniform, best at top, best at bottom. Line of puncture, filiform, beaded, papillate, villous, arborescent. Liquefaction, none, crateriform, napiform, infundi- buliform, sacate, stratiform; begins in..... d. complete in..... d. Depth of liquefaction in tube of 10 mm. diameter evenly inoculated at 20° C. for 30 days..... mm. Medium, fluorescent, browned.		Medium (liquid) Temperature..... °C Age..... d		
Medium (solid) Temperature..... °C Age..... d			Medium Temperature..... °C Age..... d		

Index No.*
BRIEF CHARACTERIZATION

As each of the following characteristics is determined, indicate in proper marginal square by means of figure, as designated below:

PRIMARY CHARACTERISTICS	Microscopic Features	Form: 1, streptococci; 2, diplococci; 3, micrococci; 4, sarcinae; 5, rods; 6, commas; 7, spirals; 8, branched rods; 9, filamentous Spores: 1, central; 2, polar; 3, absent Flagella: 1, peritrichic; 2, polar; 3, absent Gram stain: 1, positive; 2, negative
	Miscellaneous Biochemical Reactions	Pathogenicity, etc.: 1, for man; 2, for animals; 3, for plants; 4, parasitic but not pathogenic; 5, saprophytic; 6, autotrophic Relation to oxygen: 1, strict aerobe; 2, facultative anaerobe; 3, strict anaerobe Gelatin liquefaction: 1, positive; 2, negative In nitrate media: 1, nitrite and gas; 2, nitrite but no gas; 3, neither nitrite nor gas Chromogenesis: 1, fluorescent; 2, violet; 3, blue; 4, green; 5, yellow; 6, orange; 7, red; 8, brown; 9, pink; 0, none
	Carbohydrate Reactions	Diatstatic action: 1, positive; 2, negative From dextrose: 1, acid and gas; 2, acid without gas; 3, no acid From lactose: 1, acid and gas; 2, acid without gas; 3, no acid From sucrose: 1, acid and gas; 2, acid without gas; 3, no acid
	Vegetative Cells	Diameter: 1, under 0.5μ; 2, between 0.5μ and 1μ; 3, over 1μ Length: 1, less than 2 diameters; 2, more than 2 diameters Chains (4 or more cells): 1, present; 2, absent Capsules: 1, present; 2, absent
	Spores	Shape: 1, round; 2, oval to cylindrical Diameter: 1, less than diameter of rod; 2, greater than diameter of rod
SECONDARY CHARACTERISTICS	Cultural Features	Abundance: 1, abundant; 2, moderate; 3, slight; 4, absent Lustre: 1, glistening; 2, dull Surface: 1, smooth; 2, contoured; 3, rugose Agar colonies: 1, punctiform; 2, round (over 1 mm. diameter); 3, rhizoid; 4, filamentous; 5, curled Gelatin colonies: 1, punctiform; 2, round (over 1 mm.); 3, irregular; 4, fila- mentous
	Milk	Acid: 1, sufficient for curdling; 2, insufficient for curdling; 3, no acid Rennet curd: 1, present; 2, absent Peptonization: 1, present; 2, absent

*Recording the "Index Number" here is optional: but its use will be found convenient if the charts are to be filed according to the salient characteristics of the organisms. The Index Number consists of the first thirteen figures from the margin (primary characteristics) copied down in the order of their occurrence in the margin, placing a dash wherever a heavy rule occurs in the margin. Thus, B. coli belongs to the group 5312-41220-1111.

for incubation at 55° C. than the 1 or 1 1/2 per cent agar as ordinarily used.

Microscopic Features

The microscopic features were determined by use of carbol fuchsin and Gram stains. For the staining of flagella the new method proposed by Plimmer and Paine (1921) was used, with certain modifications, and proved to be a very quick and efficient method. Since it was found that the thermophilic bacteria under observation grew very rapidly at 55° C., 8 - 12 hour agar slant cultures were used. Some of the growth was removed carefully and put into tubes containing sterile water which had been held at a temperature of 55° C. for about an hour. These water suspensions were kept at 55° C. for 30 - 60 minutes and then 2 or 3 small drops of the suspensions were placed on slides that had been prepared according to the description of Plimmer and Paine and maintained at 55° C. for 15 - 60 minutes. It was found that by keeping the suspensions and slides at 55° C., thus avoiding any change in temperature during the preparation of the smears, that the motility was not interfered with and the flagella were then easily demonstrated by staining. The smears were allowed to dry at 55° C. over night and then fixed and stained by the method as described by the above mentioned authors.

Miscellaneous Biochemical Reactions

Just one of the cultures (No. 4) was used to test its pathogenicity for guinea

pigs. This culture was grown on blood agar slants for 18 hours, the growth removed and a suspension of it in physiological salt solution made. Guinea pigs were given injections of this suspension intravenously and interperitoneally with no effect. Bruini (1905) is the only investigator who attributed a pathogenic effect to a thermophile. A zero is used for this characteristic in all of the index numbers simply to indicate not that it was not pathogenic, but that this characteristic was not studied on all of the cultures.

For the determination of gelatin liquefaction the provisional method was used. It is designed to distinguish "true liquefiers" (organisms producing ecto-enzymes) from organisms that produce endo-enzymes of proteolytic action that are released from the cell after death and cause liquefaction of the gelatin if incubated for a long period. The cultures were given a preliminary cultivation for 24 to 48 hours (according to the rapidity of growth) in a 1 per cent solution of gelatin at 55° C.; then the surface of gelatin in test tubes was inoculated and incubated at 55° C. for 4 days. By this method all the cultures except 78, 83, and 84 were found to be gelatin liquefiers.

To test for the production of nitrites and gas in nitrate media both nitrate broth and nitrate agar slants were used.

Carbohydrate Reactions

The method of Baker (1922) was used, with certain modifications, to determine the production of acid and gas in dextrose, lactose, and sucrose broths. Brom thymol blue was added to these carbohydrate broths before the media was tubed and sterilized. It was found that the addition of 15 cc. of a 0.04 per cent alcoholic solution of the indicator to every liter of carbohydrate broth made a good concentration for the detection of acid production, and was not strong enough to inhibit growth.

For the determination of diastatic action, starch agar plates were used and dot inoculations were made in the center on the surface of the agar in the plates instead of the usual streak inoculations.

Other Characteristics

Tests for indol were made on nutrient broth and Dunham's peptone solution cultures; Witte's peptone was used in these media since it seemed to be better suited for the production of indol by bacteria. Both the nitroso-indol test and Ehrlich's test were used. Ehrlich's reagent was prepared according to the method described by Norton and Sawyer (1921). It was found that this test gave more satisfactory results when the tubes were heated slightly. All the cultures studied, except No. 81, produced indol. In general the conclusions of Norton and Sawyer were confirmed.

For the determination of the production of hydrogen sulfide, nutrient broth made with Witte's peptone, over which a strip of lead acetate paper was suspended by means of the cotton plug, was used. The blackening of the paper indicated hydrogen sulfide formation. Streak cultures on "Bacto Lead Acetate Agar" plates were also used. All but cultures No. 83 and 84 formed hydrogen sulfide.

Litmus milk and sterile milk to which brom cresol purple had been added, were used to determine the reactions of the thermophiles in milk. These milk cultures were incubated 7 - 10 days. Most of the cultures grew well in milk; only two cultures, Nos. 54 and 59 produced no apparent change in the milk. Cultures No. 83 and 84 produced alkali with no other apparent change. The majority of the rest of the cultures showed coagulation and peptonization with an alkaline reaction, some of them having shown a slight preliminary acidity. A few cultures showed slight acidity in milk and a few showed distinct acidity with coagulation but no digestion of the casein.

Temperature Relations

Of the 89 cultures used in this investigation 18 cultures were chosen for use in a more intensive study of temperature relations and thermal death point determinations. The choice of these 18 cultures was made to include organisms from as many sources as possible. These cultures were grown on agar slants, in gelatin tubes, and on agar plates, at

the five different temperatures available for incubation in these laboratories. The agar slants, 5 for each culture, were inoculated by means of a small wire loop from a 24 hour broth culture of the organism to be tested, and these agar slants incubated simultaneously at the five given temperatures. The gelatin was inoculated by placing a loopful of 24 hour gelatin solution cultures on the surface of gelatin in tubes. Five tubes were prepared for each organism, and these tubes incubated at the five given temperatures. Large petri dishes were used for the agar plate cultures, dot inoculations being made in the center of the plate on the surface of the hardened agar. A small wire loop was used to make these inoculations from 24 hour broth cultures of the organisms.

All of these inoculated media were incubated at the five given temperatures for 24 hours in order to have a definite period of incubation; a 24 hour incubation period was used because the agar streak cultures and plate cultures seemed to reach their maximum growth within such a period of incubation. The comparative amount of growth attained in gelatin and on agar slants at the different temperatures was judged as carefully and as accurately as possible with the naked eye; the diameter in mm. of the giant colonies produced on the agar plates at these same temperatures was measured.

The data secured are given in the following table (Table III). Figures are used to indicate the amount of growth,

1 indicating the greatest amount of growth at the stated temperatures, 2 the next best, etc. This method is used for the lack of a better one.

The method, proposed by Bigelow and Estey (1920), for the determination of thermal death points of typical thermophilic organisms, with a few modifications, was followed in this study.

Nutrient agar slants were inoculated with pure cultures of the organism to be tested and grown at 55° C. for 48 hours. The growth from two agar slants of each culture was brought into suspension by pouring 10 cc. of sterile nutrient broth onto the slants and emulsifying. These suspensions were then transferred to flasks containing 100 cc. of sterile broth and incubated for 7 days at 55° C. At the end of the incubation period the flasks were placed in a refrigerator for 24 to 48 hours, when they were heated to 85° C. for 15 minutes to kill all vegetative forms, cooled immediately, and placed again in the refrigerator to prevent the germination of spores. These were the stock suspensions used for the determination of the thermal death points of the spores. The concentration of spores in the suspensions was determined by plating in different dilutions. The relative ability of the 18 cultures to form spores under these conditions is shown by the counts. The number of spores per cc. of suspension for the 18 cultures varied from 390 to

Table III

GROWTH OF THERMOPHILES ON 3 DIFFERENT MEDIA AT 5 TEMPERATURES (CENTIGRADE)

Culture Number	Agar Slant					Gelatin					Agar Plate				
	25°-30°	37°	42°-45°	55°	60°	25°-30°	37°	42°-45°	55°	60°	25°-30°	37°	42°-45°	55°	60°
1	5	4	3	1	2	3	2	1	3	-	4	4	2	1	3
10	-	3	2	1	-	4	3	2	1	-	5	4	1	2	3
19	4	3	2	1	3	5	2	1	3	4	5	2	1	3	4
21	-	4	3	1	2	5	2	1	3	4	5	3	1	2	4
26	-	4	2	1	3	-	3	2	1	4	4	4	2	1	3
33	5	4	2	1	2	4	3	1	2	-	5	4	2	1	3
37	5	4	2	1	3	5	3	1	2	2	4	4	2	1	3
39	5	4	2	1	2	-	3	1	2	3	4	4	2	1	3
46	-	4	3	1	3	5	2	1	3	4	5	4	2	1	3
47	-	4	3	1	2	5	2	1	3	4	5	4	2	1	3
51	-	4	3	1	1	5	2	1	3	4	5	4	2	1	3
52	-	4	3	1	1	5	2	1	3	4	5	4	2	1	3
53	-	4	3	1	1	5	2	1	3	4	5	4	2	1	3
59	4	3	2	1	2	-	3	1	2	1	4	3	2	1	3
64	4	3	2	1	3	-	3	1	2	1	5	4	2	1	3
67	-	4	2	1	4	-	3	1	2	1	-	-	2	1	3
80	-	4	2	1	1	-	3	1	2	1	-	-	2	1	3
89	-	4	2	1	1	5	4	1	2	1	-	-	2	1	3

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73,800,000.

The tubes used for the determinations were hard glass tubes 5 mm. in diameter and 250 mm. in length; they were prepared for use by soaking over night in weak hydrochloric acid solution, rinsing thoroughly with distilled water, draining, wrapping in heavy brown paper in packages of 15 each and sterilizing. These tubes were inoculated with 1 cc. of the suspensions of spores, sealed off to within 40 to 50 mm. of the surface of the liquid, and held in the refrigerator until ready to be heated.

The thermal death points of the spores at 100° C., 105° C., 110° C., 115° C., and 120° C. were determined by immersing the sealed tubes in an oil bath adjusted to the desired temperature. A DeKhotinsky electric bath containing "Crisco" was used to maintain a constant temperature, and a Wasserman test-tube rack for suspending the sealed tubes in the bath. Before immersing the sealed tubes in the oil bath the temperature was increased one degree in order to compensate for the loss in temperature due to the immersion of the tubes in the oil and 30 seconds were allowed for the heat to reach the center of the tubes and for the temperature to drop to that at which it was previously adjusted, before recording the time.

A series of tubes was exposed to the desired temperature for definite periods of time, a tube being removed at the end of each period and immediately placed in a bath of ice water

in order to prevent further action of the heat on the spores of the bacteria. When cold, the tubes were placed in the refrigerator and held until the sterility of the medium could be determined.

Sterility was determined by inoculating agar plates with the contents of the heated tubes and incubating for 2 to 4 days. In most cases, when spores had survived the heating, growth occurred within 24 hours, and in no case did growth occur after 48 hours.

The hydrogen-ion concentration of the suspensions was determined at the beginning and end of each period of heating by the colorimetric method. The color chart of indicators showing the colors of Clark and Lubs' indicators in solutions of known pH was used to determine the pH of the different suspensions.

Charts were prepared to show the thermal death points of these 18 thermophiles at the five given temperatures. These follow.

Table IV, following the charts, summarizes the work of the thermal death point determinations on these organisms and also indicates the change in pH of the broth suspensions due to the heating.

Temperature Centigrade	Time In Minute															
	0	10	20	30	40	50	60	70	80	90	100	120	140	160	180	200
100	_____															
105	_____															
110	_____															
115	_____															
120	_____															

Culture No. 1
3,580,000 spores per cc.

Temperature Centigrade	Time In Minutes															
	20	40	60	80	100	120	140	160	180	200	220	240	260	280	300	320
100																
105																
110																
115																
120																

Culture No. 10
390 spores per cc.

Temperature Centigrade	Time In Minutes															
	20	40	60	80	100	120	140	160	180	200	220	240	260	280	300	320
100	<div style="text-align: center;"> <p>Culture No. 19</p> <p>75,000 spores per cc.</p> </div>															
105																
110																
115																
120																

Temperature Centigrade	Time In Minutes																
	20	40	60	80	100	120	140	160	180	200	220	240	260	280	300	320	
100																	
105																	
110																	
115																	
120																	

Culture No. 21
5,560,000 spores per cc.

Temperature Centigrade	Time In Minutes															
	20	40	60	80	100	120	140	160	180	200	220	240	260	280	300	320
100	_____															
105	_____															
110	_____															
115	_____															
120	_____															
<p style="text-align: right;">Culture No. 53 184,000 spores per cc.</p>																

Temperature Centigrade	Time In Minutes																
	20	40	60	80	100	120	140	160	180	200	220	240	260	280	300	320	
100
105
110
115
120

Culture No. 59
144,000 spores per cc.

Temperature Centigrade	Time In Minutes															
	20	40	60	80	100	120	140	160	180	200	220	240	260	280	300	320
100
105
110
115
120

Culture No. 64
30,000 spores per cc.

Temperature Centigrade	Time In Minutes															
	20	40	60	80	100	120	140	160	180	200	220	240	260	280	300	320
100	_____															
105	_____															
110	_____															
115	_____															
120	_____															

Culture No. 67
1,644,000 spores per cc.

Temperature Centigrade	Time In Minutes
100	10 15 20 30 40 50 100 150 175 200 225 250 300 350 400 450
105	
110	
115	
120	

Culture No. 80
500,000 spores per cc.

Temperature Centigrade	Time In Minutes															
	20	40	60	80	100	120	140	160	180	200	220	240	260	280	300	320
100	—————															
105	—————															
110	—															
115	—															
120	.															

Culture No. 89
11,000 spores per cc.

[illegible][illegible]

Table IV.

Time Required to Destroy the Spores of Thermophiles at Stated Temperatures.

Culture Number.	Spores per cc.	Time Required to Destroy and Changes in pH at											
		100°C.		105°C.		110°C.		115°C.		120°C.		Min.	pH
		Min.	pH	Min.	pH	Min.	pH	Min.	pH	Min.	pH		
1	3,580,000	110	7.8-8.0	45	7.8-7.8	7	7.8-7.8	2.50	7.8-7.8	2.00	7.8-7.8	2.00	7.8-7.8
10	390	15	7.6-7.6	10	7.6-7.6	3	7.6-7.6	2.00	7.6-7.6	1.00	7.6-7.6	1.00	7.6-7.6
19	75,000	40	7.8-7.8	20	7.8-7.8	5	7.8-7.8	2.00	7.8-7.8	1.50	7.8-7.8	1.50	7.8-7.8
21	5,560,000	150	7.6-7.8	60	7.6-7.6	5	7.6-7.6	2.00	7.6-7.6	1.50	7.6-7.6	1.50	7.6-7.6
26	8,900,000	80	7.8-7.8	30	7.8-7.8	3	7.8-7.8	2.00	7.8-7.8	1.50	7.8-7.8	1.50	7.8-7.8
33	27,000	60	7.6-7.6	50	7.6-7.6	3	7.6-7.6	2.00	7.6-7.6	1.25	7.6-7.6	1.25	7.6-7.6
37	800,000	80	7.6-7.6	60	7.6-7.6	4	7.6-7.6	2.00	7.6-7.6	1.50	7.6-7.6	1.50	7.6-7.6
39	123,000	190	7.6-7.8	40	7.6-7.6	4	7.6-7.6	2.00	7.6-7.6	1.75	7.6-7.6	1.75	7.6-7.6
46	40,000	90	7.4-7.6	50	7.4-7.4	5	7.4-7.4	2.00	7.4-7.4	1.00	7.4-7.4	1.00	7.4-7.4
47	176,000	120	7.8-7.9	30	7.8-7.8	5	7.8-7.8	2.00	7.8-7.8	1.75	7.8-7.8	1.75	7.8-7.8
51	275,000	70	7.8-7.8	20	7.8-7.8	5	7.8-7.8	2.50	7.8-7.8	1.50	7.8-7.8	1.50	7.8-7.8
52	73,000,000	60	8.0-8.0	30	8.0-8.0	7	8.0-8.0	2.00	8.0-8.0	1.25	8.0-8.0	1.25	8.0-8.0
53	184,000	35	8.0-8.0	25	8.0-8.0	7	8.0-8.0	2.00	8.0-8.0	1.00	8.0-8.0	1.00	8.0-8.0
59	144,000	70	8.0-8.0	40	8.0-8.0	7	8.0-8.0	3.00	8.0-8.0	1.50	8.0-8.0	1.50	8.0-8.0
64	30,000	70	7.8-7.8	50	7.8-7.8	5	7.8-7.8	2.50	7.8-7.8	1.50	7.8-7.8	1.50	7.8-7.8
67	1,644,000	90	7.8-7.9	60	7.8-7.8	5	7.8-7.8	2.00	7.8-7.8	1.25	7.8-7.8	1.25	7.8-7.8
80	500,000	220	7.8-7.9	70	7.8-7.8	9	7.8-7.8	3.00	7.8-7.8	1.50	7.8-7.8	1.50	7.8-7.8
89	11,000	180	7.6-7.8	60	7.6-7.6	9	7.6-7.6	3.00	7.6-7.6	1.75	7.6-7.6	1.75	7.6-7.6

Index Numbers

Table V. includes the source and "Index Number" for each organism studied. On page 29 is found a key to be used to determine what characteristics of these thermophiles are indicated by the figures in the given index numbers.

Another table (Table VI) shows the separation of the thermophiles studied into classes according to the index numbers.

Index No.*.....
BRIEF CHARACTERIZATION

As each of the following characteristics is determined, indicate in proper marginal square by means of figure, as designated below:

PRIMARY CHARACTERISTICS				
Microscopic Features	Form:	1, streptococci; 2, diplococci; 3, micrococci; 4, sarcinae; 5, rods; 6, commas; 7, spirals; 8, branched rods; 9, filamentous		
	Spores:	1, central; 2, polar; 3, absent		
	Flagella:	1, peritrichic; 2, polar; 3, absent		
Miscellaneous Biochemical Reactions	Gram stain:	1, positive; 2, negative		
	Pathogenicity, etc.:	1, for man; 2, for animals; 3, for plants; 4, parasitic but not pathogenic; 5, saprophytic; 6, autotrophic		
	Relation to oxygen:	1, strict aerobic; 2, facultative anaerobe; 3, strict anaerobe		
	Gelatin liquefaction:	1, positive; 2, negative		
	In nitrate media:	1, nitrite and gas; 2, nitrite but no gas; 3, neither nitrite nor gas		
Carbohydrate Reactions	Chromogenesis:	1, fluorescent; 2, violet; 3, blue; 4, green; 5, yellow; 6, orange; 7, red; 8, brown; 9, pink; 0, none		
	Diastatic action:	1, positive; 2, negative		
	From dextrose:	1, acid and gas; 2, acid without gas; 3, no acid		
	From lactose:	1, acid and gas; 2, acid without gas; 3, no acid		
SECONDARY CHARACTERISTICS	Vegetative Cells	From sucrose:	1, acid and gas; 2, acid without gas; 3, no acid	
		Diameter:	1, under 0.5 μ ; 2, between 0.5 μ and 1 μ ; 3, over 1 μ	
		Length:	1, less than 2 diameters; 2, more than 2 diameters	
		Chains (1 or more cells):	1, present; 2, absent	
	Spores	Capsules:	1, present; 2, absent	
		Shape:	1, round; 2, oval to cylindrical	
	Cultural Features	Agar Stroke	Diameter:	1, less than diameter of rod; 2, greater than diameter of rod
			Abundance:	1, abundant; 2, moderate; 3, slight; 4, absent
			Lustre:	1, glistening; 2, dull
		Milk	Surface:	1, smooth; 2, contoured; 3, rugose
Agar colonies:			1, punctiform; 2, round (over 1 mm. diameter); 3, rhizoid; 4, filamentous; 5, curled	
Gelatin colonies:			1, punctiform; 2, round (over 1 mm.); 3, irregular; 4, filamentous	
Milk	Acid:	1, sufficient for curdling; 2, insufficient for curdling; 3, no acid		
	Rennet curd:	1, present; 2, absent		
	Peptonization:	1, present; 2, absent		

*Recording the "Index Number" here is optional: but its use will be found convenient if the charts are to be filed according to the salient characteristics of the organisms. The Index Number consists of the first thirteen figures from the margin (primary characteristics) copied down in the order of their occurrence in the margin, placing a dash wherever a heavy rule occurs in the margin. Thus, *B. coli* belongs to the group 5312-41220-1111.

Table V.

Culture Number.	Source	Index Number.
1	Dug well, Champaign	5111-01120-1232
2	Raw water, Hamilton	5111-01120-1232
3	Deep well, Sears Roebuck	5211-01120-1232
4	"Boneyard", Urbana	5111-01120-1232
5	18 ft. dug well, Odin	5211-01120-1232
6	Raw water, Pontiac	5111-01120-1232
7	City supply, Joliet	5211-01120-1232
8	I.C. Railroad	5211-01120-1232
9	Raw water, Alton	5111-01120-1232
10	Raw water, Kankakee	5111-01120-1232
11	Raw water, Decatur	5211-01120-1232
12	20 ft. dug well, Newman	5211-01120-1232
13	Raw water, Quincy	5111-01120-1232
14	City supply, Lake Forrest	5111-01120-1232
15	Raw water, Moline	5111-01120-1232
16	30 ft. dug well, Macomb	5111-01120-1232
17	City supply, Waukegan	5111-01120-1232
18	City supply, Oak Park	5111-01120-1232
19	145 ft. driven well, Dickson	5111-01120-1232
20	200 ft. drilled well, "	5111-01120-1232
21	25 ft. well, Chicago	5211-01120-1232
22	Tap water, DeKalb	5211-01120-1232
23	75ft. drilled well, Peoria	5211-01120-1232
24	22 ft. dug well, Carmi	5111-01120-1232
25	40 ft. dug well, Newman	5211-01120-1233
26	Raw water, Danville	5111-01120-1232
27	Well, C.&A. Railroad, Bloomington	5211-01120-1232
28	Raw water, Herrin	5111-01120-1232
29	Spring, Bloomington	5121-01120-1233
30	19 ft. dug well, Waverly	5211-01120-1232
31	35 ft. dug well, Cardiff	5111-01120-1232
32	Well, Champaign	5111-01120-1232
33	City supply, Belleville	5111-01120-1232
34	Well water, Streater	5111-01120-1232
35	Well, Bloomington	5111-01120-1232
36	Well, Bloomington	5111-01120-1232
37	Well, Bloomington	5111-01120-1232
38	Raw water, Carlinville	5111-01120-1232
39	City supply, "	5111-01120-1232
40	City supply, Quincy	5111-01120-1232
41	City supply, Alton	5111-01120-1232
43	Spring, Harristown	5111-01120-1232
44	Galesburg, well	5111-01120-1232
45	Mississippi river, Moline	5111-01120-1232
46	City supply, Moline	5111-01120-1232
47	Raw water, Rock Island	5111-01120-1232
48	30 ft. dug well, Forest	5111-01120-1232
49	20 ft. dug well, Matoon	5111-01120-1232
50	Raw water, East St. Louis	5111-01120-1232

C.I.

Table V.
(Continued)

Culture Number.	Source	Index Number.
51	City supply, East St. Louis	5111-01120-1232
52	30 ft. dug well, Winchester	5111-01120-1232
53	Hog feces	5111-02120-1232
54	Hog feces	5111-02120-1232
55	Hog feces	5111-01120-1232
56	Soil from old hog lot	5111-01120-1232
57	Soil from dry hog lot	5111-02120-1232
58	Soil from vegetable garden	5111-02130-2333
59	Soil from city garden	5111-02120-1232
60	Soil from garden	5111-02120-1232
61	Soil from corn field. Not manured with- in last 5 years.	5111-02120-1232
62	Soil from corn field. Manured	5111-02120-1232
63	Soil from corn field. Manured	5111-02120-1232
64	Soil from corn field. Not manured with- in last 5 years.	5111-02120-1232
65	Cow feces.	5111-02120-1232
66	Cow feces.	5111-02120-1232
67	Cow feces.	5111-02120-1232
68	Soil from experimental plot.	5111-02120-1232
69	Soil from vegetable garden.	5111-02120-1232
70	Garden soil.	5111-02120-1232
71	Garden soil.	5111-02120-1232
72	Soil from city garden.	5111-02120-1232
73	Soil from garden (new ground).	5111-02120-1232
74	Garden soil.	5111-02120-1232
75	" "	5111-02120-1232
76	" "	5111-02120-1232
77	Soil from corn field.	5111-02120-1232
78	Soil from corn patch. Manured.	5111-02220-1232
79	Soil from land next to railroad. Sown to oats.	5111-02120-1232
80	Soil from pea field, Northern Illinois	5111-01130-2333
81	Soil from corn breeding plots. Land formerly in cattle feeding lots.	5212-01130-2333
82	Soil from oat field.	5111-02120-1232
83	Soil from corn field.	5111-02230-1222
84	Soil from oat field.	5111-02230-2222
85	Soil from corn field.	5121-02120-1232
86	Soil from pea field, Northern Illinois	5111-01120-1232
87	" " " " " "	5111-02120-1232
88	"Ever Fresh Milk"	5111-01120-1232
89	" " " "	5111-02120-1232

I

Table VI.

Class	Index Number	Culture Numbers	Number of Cultures in each Class.
I	5111-01120-1232	1,2,4,6,9,10,13-20,24, 26,28,31-41,43-52,55, 56,86,88.	42
II	5211-01120-1232	3,5,7,8,11,12,21-23, 27,30.	11
III	5211-01120-1233	25.	1
IV	5121-01120-1233	29.	1
V	5111-02120-1232	53,54,57,59-77,79,82, 87,89.	26
VI	5111-02130-2333	58.	1
VII	5111-02220-1232	78.	1
VIII	5111-01130-2333	80.	1
IX	5212-01130-2333	81.	1
X	5111-02230-1222	83.	1
XI	5111-02230-2222	84.	1
XII	5121-02120-1232	85.	1

1 2 3 4 5 6 7 8 9 10 11 12

13 14 15 16 17 18 19 20 21 22 23 24

25 26 27 28 29 30 31 32 33 34 35 36

37 38 39 40 41 42 43 44 45 46 47 48

49 50 51 52 53 54 55 56 57 58 59 60

61 62 63 64 65 66 67 68 69 70 71 72

73 74 75 76 77 78 79 80 81 82 83 84

85 86 87 88 89 90 91 92 93 94 95 96

97 98 99 100 101 102 103 104 105 106 107 108

109 110 111 112 113 114 115 116 117 118 119 120

121 122 123 124 125 126 127 128 129 130 131 132

133 134 135 136 137 138 139 140 141 142 143 144

145 146 147 148 149 150 151 152 153 154 155 156

157 158 159 160 161 162 163 164 165 166 167 168

169 170 171 172 173 174 175 176 177 178 179 180

181 182 183 184 185 186 187 188 189 190 191 192

193 194 195 196 197 198 199 200 201 202 203 204

205 206 207 208 209 210 211 212 213 214 215 216

217 218 219 220 221 222 223 224 225 226 227 228

229 230 231 232 233 234 235 236 237 238 239 240

241 242 243 244 245 246 247 248 249 250 251 252

253 254 255 256 257 258 259 260 261 262 263 264

265 266 267 268 269 270 271 272 273 274 275 276

277 278 279 280 281 282 283 284 285 286 287 288

289 290 291 292 293 294 295 296 297 298 299 300

301 302 303 304 305 306 307 308 309 310 311 312

313 314 315 316 317 318 319 320 321 322 323 324

325 326 327 328 329 330 331 332 333 334 335 336

337 338 339 340 341 342 343 344 345 346 347 348

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361 362 363 364 365 366 367 368 369 370 371 372

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625 626 627 628 629 630 631 632 633 634 635 636

637 638 639 640 641 642 643 644 645 646 647 648

649 650 651 652 653 654 655 656 657 658 659 660

661 662 663 664 665 666 667 668 669 670 671 672

673 674 675 676 677 678 679 680 681 682 683 684

The organisms in Class I differ from those in Class II only in the location of the spore. Those in Class I had central spores and those in Class II had polar spores. This differentiation is made on a character of minor importance in classification and analysis of bacterial groups. Consequently, the bacteria in Classes I and II may be regarded as belonging to the same class when merely spore formation itself is considered. Bushnell (1922) has called attention to the difficulties arising when only one characteristic such as location of the spore in the rod is considered. The two classes (I and II) are left separated in this summary since the separation is called for on the latest Descriptive Chart and because some of the recent reports on anaerobic spore formers indicates this to be a fairly constant characteristic. Hall (1922) stated that three distinct groups of anaerobic spore formers could be differentiated on location of the spore,--

- I. Bacteria with central spores which do not swell the rods.
- II. Subterminal or clostridial spores.
- III. Terminal or plectridial spores.
 - a. round spores.
 - b. elongated spores.

Combining Classes I and II a total of fifty-three strains are included. This combined class is separated from Class V on the basis of relation to combined oxygen. The strains in Classes I and II showed no growth in the closed arm of the fermentation tube in the presence of dextrose. Those in Class V

showed growth under such conditions. The other classes (III, IV, VI, VII, VIII, IX, X, XI, and XII) contains only one strain each, these being separated from the other larger groups on the basis of characteristics of minor importance, such as action on starch, location of flagella, etc.

IV. Discussion of Results

In general the thermophilic bacteria constitute a homogeneous group of bacteria, having the important characteristics of bacterial characterization in common.

The results obtained by growing some of these thermophiles in different media and at different temperatures agree with those obtained by Opreescu (1898), Sames (1900), Blau (1906), and Koch and Hoffman (1911), in that the nature of the medium seemed to influence the temperature demanded for growth. The examination of Table III shows that the organisms included here grew better at the higher temperatures on agar slants and plates than in gelatin. Some of those which showed no growth at all, or only slight growth, on agar slants after 24 hours incubation at 25° - 30° C., were found to grow well in gelatin at this temperature, causing some liquefaction. Of course, the time must be taken into consideration in judging the growth of thermophiles at any temperature. It was found that some strains which showed no growth on agar slants at room temperature in 24 or 48 hours, would show slight growth at this temperature after a longer incubation. Koch and Hoffman (1911) found that the organisms they studied would not grow in artificial media at 25° - 28° C. but did not mention the length of the incubation period. Probably other investigators who claimed that real thermophilic organisms would not grow at room temperature did not take into consideration the time required for growth to appear

at this temperature.

Of the investigators who reported thermal death points for thermophilic organisms, Russell and Hastings (1902) and Bigelow and Esty (1920) are the only ones who described their method for determining them. There has been no uniformity in the methods used for determining the thermal death points of thermophilic bacteria. Hence, the results obtained by the different investigators cannot very well be compared because so many factors influence thermal death points.

In comparing the results obtained in this investigation with those obtained by Bigelow and Esty (1920), several points deserve mention. The strains used in this study seemed to be less resistant to moist heat than those used by the above mentioned authors under the conditions which obtained in this investigation. Several probable explanations can be offered for this. The pH of the broth suspensions of spores used in this investigation ranged from 7.4 to 8.0, while the different suspensions used by Bigelow and Esty ranged from 6.0 to 6.3. No attempt was made to adjust the pH of the broth after the spores had been grown in it, and no change was noted in pH during the heating of the suspensions, except in a few cases where the exposure at 100° C. was prolonged beyond 70 minutes. Probably if the pH had been adjusted to 7.0 or even 6.0 it would have lengthened the time required to destroy the spores at the stated temperatures. This probability has suggested to the author another research problem in connection with thermal death point

determinations of thermophilic bacteria. She hopes to be able to begin work on this problem in the near future.

While the initial concentration of spores per cc. of suspension determines somewhat the time required for their complete destruction, thermophiles do vary in the degree of their resistance to heat. This is demonstrated by the data given in Table IV. The suspension for culture No. 10 contained the least number of spores per cc. (390) and these were killed by a 15 minute exposure at 100° C. Culture 89 (11,000. spores per cc.) required an exposure of 180 minutes at 100° C. for complete destruction while culture 52 (73,000,000 spores per cc.) only required 60 minutes at 100° C. At the higher temperatures of 115° C. and 120° C., the difference in time required to kill the spores of the different cultures varied only 0.5 to 1.0 minute and 0.25 to 1.0 minute, respectively.

The spores tested by Bigelow and Esty were spores of thermophilic bacteria isolated from cases of spoilage in a variety of canned foods. The presence of these organisms in the canned foods was due to their great heat resistance and ability to withstand the usual sterilizing processes. This, no doubt, explains the fact that these spores were found to be more resistant to heat than any of those described in the literature on thermophiles. The spores used in this study were spores of thermophilic bacteria isolated from water, soil, and feces, and had been grown on laboratory media for 12 months or more. This calls to mind a statement made by Grieg-Smith (1921) to the effect

that when bacteria have been recently isolated from what may be called their natural habitat, they may be, and probably are, more vigorous than after a spell of sub-cultivation in the laboratory.

Sames (1900) found that the temperature at which spores of thermophilic bacteria were produced influenced their resistance to flowing steam at 100° C. For example, the spores of one organism studied by him, when produced at 56° C. resisted steam at 100° C. for 120 minutes, while those of the same organism, produced at 37° C. withstood the same conditions only for 60 minutes. The spores used in this investigation were produced at 55° C. It seems quite probable that the temperature at which the spores are produced has an influence on their resistant properties.

Rahn (1921) says it is well known that temperature is one of the most important factors of life. It is so important that the higher animals protect themselves by a very complicated mechanism of regulation against changes in temperature; the life processes of such animals takes place at a temperature nearly constant from birth till death. This causes the growth of warm-blooded animals to be different from all other organisms. All other organisms, cold-blooded animals as well as bacteria, have the temperature of their environment, and the decrease in temperature decreases the intensity of growth. There are, of course, limits to the favorable influence of high temperatures. Growth

of bacteria will increase with rising temperature to a certain point, called the optimum temperature. The highest temperature at which growth will take place is called the maximum temperature. Correspondingly, the minimum temperature of an organism is the lowest point at which growth can take place.

The relationships of bacteria to temperature are varied. Buchanan (1921) divided bacteria into three groups dependent on variations in their optimum temperatures:--

- I. Thermophilic bacteria
Optimum usually above 45° - 50° C.
- II. Mesophilic bacteria
Optimum 20° - 37.5° C.
- III. Psychrophilic bacteria
Optimum usually below 10° C.

He says of thermophilic bacteria, that the minimum of most true thermophiles is above 40° C., and the maximum temperature of some of thermophilic bacteria is nearly 80° C.

A review of the thermophilic bacteria described in the literature shows that the range at which these organisms have been found to grow is very great. It is evident then, that some division must be made in this group of bacteria. Some of the terms used by the different investigators for the gradations of organisms generally included in this group, are, thermophiles, orthothermophiles, thermo-tolerants, psychrotolerants, obligate thermophiles, true thermophiles, facultative thermophiles, and strict thermophiles. Some divisions have been made on the basis of the minimum temperature, some on the basis of the maximum

temperature, and some on the basis of the optimum temperature.

The question then arises as to whether it is necessary to regard these bacteria as constituting a separate group, if the rapidity of their growth at a certain temperature is the only characteristic separating them from other bacteria which grow more rapidly at the same temperature. This question would not arise in the case of the strict or true thermophiles -- those which grow best at 55° C. and above. In the case of the intergrading forms, however, some confusion might arise. Similar situations exist in other phases of the science, and it is the intergrading form which has caused trouble in the systematic arrangement and characterization of all forms of life. Pathogenicity is a function of bacteria which has received much study and upon which bacteriologists have much definite information. Still, there are many semi-pathogenic bacteria acting as connecting links between the pathogenic members and the non-pathogenic members of a group. For instance, in the colon-typhoid group there is the pathogenic Bacterium typhosum on one end and the ordinarily non-pathogenic Bacterium coli on the other. A similar situation may exist for the thermophilic function, if it may be discussed as such for the moment.

It would seem that the best basis for a division is the optimum temperature for growth, since it has been proved that by acclimatization, both the minimum and maximum temperatures for an organism can be either raised or lowered. The author

proposes the following division of bacteria into groups, using the optimum temperature as the basis for the division.

- I. Strict thermophiles
Optimum temperature above 55° C.
- II. Facultative thermophiles
Optimum temperature 50° - 55° C.
- III. Thermotolerant bacteria
Optimum temperature 40° - 50° C.
- IV. Mesophilic bacteria
Optimum temperature 25° - 40° C.
- V. Psychrotolerant bacteria
Optimum temperature 10° - 25° C.
- VI. Facultative psychrophiles
Optimum temperature 0° - 10° C. (?)
- VII. Strict psychrophiles
Optimum temperature below 0° C. (?)

It is evident from the statements in the text-books on bacteriology that the psychrophilic bacteria should receive the same critical analysis that the thermophilic bacteria are receiving. It is probable that many of the statements concerning psychrophilic bacteria have been handed on from text-book to text-book and that they do not rest upon adequate experimental data. It is interesting to note that the halophilic bacteria are beginning to receive analysis and that it is probable that our information on these bacteria will be greatly amplified in the near future.

Since the growth of thermophilic bacteria at ordinary temperatures seems to involve a time element, it is probable that canned foods that contain thermophilic bacteria might spoil

after being stored for a long time at ordinary temperatures. Of course, if stored at higher temperatures it is well known that spoilage occurs very promptly.

Several other problems in connection with thermophilic bacteria have been suggested to the author by the present investigation. These will be taken up in the near future.

V. Summary

1. Eighty-nine strains of thermophilic bacteria, studied according to the index number as expressed on the descriptive chart of the Society of American Bacteriologists, fell into twelve classes. All of these separations are based upon unessential characteristics of classification. In the light of this fact, the thermophilic group seems to be a homogeneous one, the members having in common all of the important characteristics used in classification.

2. The "Index Number" is a distinct improvement over the old "Group Number", however, some of the points in the "Index Number" would be difficult to determine. For instance, the function of pathogenicity could not be determined without a great deal of experimental work.

3. The thermal resistance of eighteen strains of thermophilic bacteria was studied. At the higher temperatures, 115° C. and 120° C., the thermal death points of these organisms fell within narrower time limits than those at the lower temperatures, 100° C., 105° C., and 110° C.

4. Thermal death points of bacteria were influenced by the number of spores in the suspension.

5. The character of the media influenced the temperatures for the growth of thermophiles.

6. A tentative grouping of bacteria, according to temperature optimums, has been proposed.

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Biographical Sketch

Lethe Eleanora Morrison received her common school education in the common schools of Waterloo, Illinois. She was graduated from Waterloo High School in 1910. She taught in the common schools of Illinois from 1911 to 1914. In the fall of 1914 she entered the University of Illinois, Urbana, Illinois, and in June, 1919, received the degree of Bachelor of Arts, having taken a scientific course with home economics as the major study.

She entered upon duties as half-time assistant in bacteriology in the Bacteriology Department, University of Illinois in 1919. In the fall of the next year she accepted a full time assistantship in the same department. During the tenure of these two assistantships, graduate work was taken in the Graduate School of the University. The degree of Master of Science in Bacteriology was received from this institution in June, 1921. From 1921 to 1922 she was a fellow in Bacteriology in the University of Illinois.

She is a member of the Society of American Bacteriologists and the American Public Health Association. She is also a member of Omicron Nu, Iota Sigma Pi, and an active member of Sigma Xi.

Publications

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