THERMOPHILIC BACTERIA

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True thermophilic bacteria are those that grow at temperatures above the maximum temperature for the great majority of bacteria, especially the pathogenic forms. The maximum temperature for the pathogenic bacteria is about 45°C. Their optimum temperature is about 37.5°C. The true thermophiles show no growth, or only very feeble growth, below 40 to 45°C. Their development requires temperatures above 50°C., and some are able to develop at a temperature of 80°C., though most abundant growth is shown at 60 to 70°C.

A group of facultative thermophilic bacteria has been discovered which develop at room temperature, about 20°C., and have their optimum temperature at about 50°C., and their maximum temperature at about 60°C. In this group belong some of the well-known spore-forming soil organisms.

Two distinct types of true thermophilic bacteria have been found in the laboratory, viz., spore-forming and non-spore-forming rods, and thread forms belonging in the class of *Nocardia*.

Most of the thermophilic bacteria studied belonged to the spore-forming class and these varied in size of rods, size and location of the spores, and to a less degree in their biological characters.

The sources from which these bacteria are obtained indicate that this group is widely distributed in nature. They were found in the dust collected on apparatus in the laboratory, on clothes, in soil, in sewage, in the gastro-intestinal contents and feces of rabbits and guinea-pigs, on grain and in horse manure.

The isolation of these bacteria from the different materials is an easy matter, using the plate method with nutrient agar medium, the plates being incubated at a temperature above 50°C. Under these conditions the colonies developing on the agar medium are certain to be thermophilic and can be obtained in pure culture by transplanting on to tubes of slanted agar.

The biological characters of the 60 cultures studied showed marked variation, especially with regard to their action on gelatin, milk, starch solution and nitrate solution. Eleven cultures failed to show any liquefaction of gelatin others showed only slight liquefaction, while others showed complete liquefaction in three or four days. The action on the gelatin was ascertained by cultivating at 51° for three or four days, and then placing the tubes at a low temperature to note whether liquefaction had occurred.

Most of the true thermophilic cultures showed only slight acidity in litmus milk, though several showed distinct acidity with coagulation but no digestion of the casein. Most of the facultative thermophilic cultures showed a primary acidity with coagulation followed by a slightly alkaline reaction and digestion of the casein.

The action on starch varied in degree, as nearly all cultures appeared to have some action in splitting starch. A number of the cultures changed the starch to dextrin, maltose and glucose, though in many cultures no other change than the dextrin stage could be detected.

The reduction of nitrate showed marked variation in the different cultures. Some showed only slight evidence of nitrite formation, others showed marked nitrite formation, while some of the cultures reduced the nitrate to ammonia.

The true thermophiles belonging in the group of spore-forming bacteria differed in their morphologic and biologic character to such a degree that satisfactory classification was not possible.

An attempt has been made to classify the different types of thermophilic bacteria and a comparison of the results obtained showed that amongst the more than sixty cultures studied, at least seven different rod forms and one thread form were

encountered that may be classed as true thermophiles, while one type of rod was encountered that may be regarded as a facultative thermophile.

The characters of the nine different types studied are given in tabular form on page 304.

The facultative thermophiles studied appeared to be more closely related to each other in that they formed short chains of rods with rounded ends having small oval central spores. These cultures all liquefied gelatin, fermented starch, and reduced nitrate to ammonia. Their action on milk showed at first a slight acidity followed by a slight alkalinity and slow peptonization of the casein. These cultures appeared to belong to a single species.

The true thermophiles resisted a temperature of 100°C., to an unusual degree varying from two to eight hours, so that it appears probable that the optimum temperature for growth is related to the heat resisting powers of the spores.

The facultative thermophiles with their lower optimum temperature for growth also showed a lower resistance to the boiling temperature. This group of bacteria was killed in from one-fourth to one hour at 100°C.

Two cultures of thread-form organisms were studied which had thermophilic properties. These two cultures represented two distinct varieties in their biological activities. On agar they showed a distinct white, mealy growth after several days and this fact has led to their classification with *Nocardia*. These two cultures differed in their action on milk and on starch solution, the one did not change milk but fermented starch to maltose, the other produced an acid reaction in milk with coagulation and subsequent digestion of the casein but only very slight action on starch. Both cultures liquefied gelatin but neither reduced nitrate. The maximum temperature of growth was about 70°C., and both were killed at 100°C., in two hours.

Although the thermophilic bacteria are widely distributed in nature it is evident that their activities can be brought into play under exceptional conditions. These organisms have been found in thermal springs where temperature conditions

	TRUE THERMOPHILES										FACULTATIVE
	Type 1	Type 2	Type 3	Type 4	Type 5		Type 6	Type 7	Type 8		Type 9
					Var. a	Var. b			Var. a	Var. b	
Source of organism	Dust and contaminated milk medium	Dust and soil	Dust, soil horse manure	Dust, soil, horse manure	Dust, g. pig feces, horse manure	Dust, cheese, g.pig feces	Horse manure	Horse manure	Rabbit's stomach	Contamination on agar	Dust, soil, horse manure
Form	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Thread	Thread	Rod
Motility	+	+	+	+	+	+	+	+	−	−	+
Size in microns	0.6–0.8 x 3–4	0.5–0.7 x 2–3	0.6–0.8 x 4–5	0.6–0.8 x 2–4	0.2–0.4 x 2–3	0.2–0.4 x 2–3	0.5–0.6 x 2–2.5	0.3–0.4 x1.5–2			0.5–0.7 x2–3
Grouping	Chains	Chains	Singly	Singly	Singly	Singly	Singly	Singly			Short chains
Location of spores	Central	Central	Central	Polar	Polar	Polar	−	−	Conidia	Conidia	Central
Spores larger or smaller diameter than rods	Smaller	Greater	Greater	Greater	Greater	Greater	−	−			Smaller
Gelatin liquefied	+	−	+	+	+	−	+	−	+	+	+
Nitrates reduced	NH_3	N_2O_3	±	N_2O_3	N_2O_3		±	N_2O_3	±	±	NH_3
Starch reduced	+	±	+	+	±	±	−	−	+	−	+
Action in litmus milk	Aid coagulation	Slight acidity	Slight acidity	Slight acidity	Slight acidity	Slight acidity	Slight acidity	Slight acidity	Slight acidity	Acid coagulation and digestion	Acid coagulation and digestion

Minimum temperature......	50–60	37	37–50	50	37	37	37	37	60	60	20–37
Maximum temperature......	75–80	70	70–75	75–80	60–70	60	60	60	70	70	50–60
Thermal-death point at 100° C. in minutes.	400	300	200	180	120	60	5	5	120	120	15–60

favor their development. In the surface layers of the soil in the temperate and torrid zones the temperature frequently rises to a point where they can develop and it is probable that they find an important field for action under these conditions.

There are other conditions in nature where it is probable that these organisms find suitable environment for their activities. In the fermentation of silage temperatures have been recorded as high as 55°C., but more commonly the temperature ranges between 35 and 45°C. The production of heat is probably not due to chemical changes in the fodder, by the tissue cells, but primarily to the activities of the thermophilic bacteria. Under these conditions it seems likely that the facultative thermophiles begin the fermentation processes and when the temperature rises to a sufficient degree then the true thermophiles begin to develop. Their ability to act on carbohydrates and protein indicates that they probably find sufficient food for development in various fodder crops.

The spoiling of hay and other fodder crops and of grain, when insufficiently cured, is no doubt due largely to the action of the thermophilic bacteria. The charring effect noticed in piles of improperly cured hay and fodder and in manure is probably caused by the thermophilic bacteria.

The conditions of temperature under which the thermophilic bacteria thrive are paradoxical when we note that the optimum temperature of growth is at about the temperature at which ordinary egg and serum albumin begin to coagulate. The maximum temperature of growth is decidedly above the temperature at which egg and serum albumin coagulate. This phenomenal characteristic may be due to the reaction of the medium in which the bacteria are growing or to the mineral content of their own protoplasm.

The investigation was interrupted in 1917 on entering the government service and the cultures were discarded. This accounts for the lack of details that would be desirable but it was thought advisable to publish the results that had been noted.