RELATION OF MAXIMUM GROWTH TEMPERATURE TO RESISTANCE TO HEAT[1]

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An old and incompletely worked-out problem in bacteriology is the nature of heat injury in bacteria, and the elucidation of the factors involved in the thermal resistance of endospores. The greatest advances have been made in determining those conditions in the external environment of the spore which influence the ability to resist heat. Much less progress is evident in isolating the factors within the cell. The present paper presents some data which suggest a relation between the maximum growth temperature of a bacterial species and the capacity of that species and its spores to survive heat injury. Physiologically considered, the relationship is an entirely logical one as the mechanism for thermal resistance should be expected to be more efficient in the case of an organism able to grow at high temperatures.

The validity of such a generalization would be substantiated if the rule were found to function for vegetative cells. A body of data already exists which permits analysis. Sherman (1937) has summarized information concerning streptococci and gives data on the ability to grow at 45°C. and 10°C. and to tolerate heating at 60°C. for 30 minutes. His data reveal that the streptococci which resist heating at 60°C. for 30 minutes are those which can grow at 45°C. The pyogenic streptococci are interesting in this connection as all except some strains of the "H" group are not able to grow at 45°C. And it is only among the "H" members of the pyogenic streptococci that strains are found within the group which resist heating for 30 minutes at 60°C. The enterococcus species are the most heat-resistant and also those most able to grow at 45°C. Of all the streptococci, *Streptococcus thermophilus* is most successful in tolerating pasteurization temperatures. It is significant that this same species, growing readily at 50°C., has the highest maximum. The lactic group constitutes an exception. They do not grow at 45°C. and yet withstand heating fairly well. But the rule seems to hold within the group as *Streptococcus lactis*, which grows at 40°C., is more heat resistant than *Streptococcus cremoris*, not capable of growth at the same temperature.

Psychrophilic bacteria are the least resistant of all bacterial forms and lend weight to the knowledge that maximum growth temperature correlates with heat resistance. Zobell and Conn (1940) have shown that marine bacteria possess low thermal death times, so low in fact, that plating temperatures (42–45°C.) too long prolonged destroy them in great numbers and decrease

[1] The experimental data presented were collected at Cornell University, College of Agriculture, Ithaca, New York where the author was under the direction and stimulating guidance of Dr. Georges Knaysi.

opportunities of isolation. It is in the oceans of the world, and in large bodies of water which tend to keep a fairly constant low temperature, that psychrophiles occur in the greatest abundance.

Sherman, *et al.* (1941) have found that spoilage of raw milk at 0°C. is much more rapid than of pasteurized milk. Evidently the lowest-temperature-growing types of bacteria are destroyed by pasteurization.

Does the relationship carry over to bacterial endospores? Is the heat resistance of spores from strains with high maxima greater than for spores from strains with low maxima? The author is aware only of the work of Meyer (1906) and his student, Blau (1905) who have specifically considered the problem. They concluded that there was no evident relationship. Their data may well lead one to challenge the conclusion, for, though species with similar growth maxima show different thermal death times for spores, the *tendency* does exist for the thermal death time to increase with rise in the maximum for growth. They did comment that the thermophiles showed the highest spore resistance. Moreover, the conditions under which the data were collected were not sufficiently controlled. The spore crops were in all cases obtained from glucose agar at 28°C. Theophilus (1938) has proven that maximum spore resistance is to be obtained only by growth of the culture at the optimum temperature. Not all of Blau's strains had an optimum at 28°C. For comparable results, spore crops grown under conditions to yield maximum resistance is needed. Schmidt in 1906 was the first to demonstrate the influence of number of spores in suspension on heat tolerance, a factor not controlled in the earlier work of Meyer and Blau. Limitations of knowledge in their time did not permit the collection of strictly comparable data and justifies reinterpretation of their investigation and the gathering of more information.

Bergey (1919) has suggested that the extraordinarily great thermal resistance of spores from thermophiles might be related to the high optimum growth temperature. In addition, his data show the tendency for the highest spore resistance to occur for the thermophiles with the highest maximum growth temperature. Esty and Williams (1920) and others have noted that the true thermophiles are those yielding the most heat-tolerant spores.

For the genus *Clostridium* abundant data exist on thermal death times. But analysis is difficult, as no correlating studies on maximum growth temperature have been made by the same authors. Table 1 brings together Greer's (1926) study on maximum temperature of growth and Esty and Meyer's (1922) data on thermal death times of typical anaerobic spore-formers. Unfortunately, the latter grew all of their cultures at 35°C. and did not use similar numbers of spores in all the heating trials. The latter criticism also applies to Bergey's work with the thermophiles. From the material in table 1, the existence of a tendency for thermal death time to rise with increasing maximum temperature of growth is evident. It is probably of some significance that *C. botulinum* which produces spores of maximum resistance is able to grow at 55°C. *C. oedematiens* and the one strain of *C. welchii* behave contrary to the rule.

The existence of the same tendency among the fungi is probable. The temperature relations of the genera *Aspergillus* and *Penicillium* (both belonging to the same family, the *Aspergillaceae*) would lead us to believe that *Aspergillus* with the higher temperature maximum (Thom, 1930; Thom and Church, 1926) should have the conidia of greater thermal resistance. The study of the effects of pasteurization temperature on conidia of species of these genera by Thom and Ayers (1916) justifies the conclusion.

MATERIAL AND METHODS

As part of a study of 72 strains of mesophilic aerobic spore-formers, information was collected on maximum growth temperature and spore thermal resistance. The method of running maximum growth temperature has been recorded elsewhere (Lamanna, 1940).

TABLE 1

Relation of maximum growth temperature to thermal resistance of spores in the genus Clostridium

ORGANISM	TEMPERATURE OF GROWTH (GREER, 1926)	RESISTANCE OF SPORES AT 105°C. (ESTY AND MEYER, 1922)	
		Maximum	Average
		minutes	*minutes*
C. botulinum (109 strains)	Growth at 55°C.	85	35.2
C. sporogenes (33 strains)	Growth at 55°C.	45	12
C. oedematiens (1 strain)	Growth at 55°C.	Less than 6	
C. bifermentans (1 strain)	Growth at 55°C.	Survived 18 min. not 21	
C. welchii (2 strains)	No growth at 55°C.; growth at 50°C.	1 strain survived 18 min. not 21 1 strain survived 5 min. not 10 at 100°C.	
C. tetani (24 strains)	No growth at 50°C.; growth at 37°C.	25	9.2
C. histolyticum (1 strain)	No growth at 50°C.; growth at 37°C.	Less than 6	

Spore crops were obtained by incubating the strains at either 30, 35, or 40°C. depending on which temperature gave maximum percentage of sporulation. The strains were grown on agar slants of a medium containing 0.1 per cent sodium chloride, 0.1 per cent beef extract and 0.2 per cent peptone for two or three transfers at 24- to 48-hour intervals before the final culture was allowed to age for 13 to 16 days. The spore crop was harvested by washing off the growth with sterile distilled water, centrifuging, and decanting off the water. The spores were resuspended in buffer solution, washed twice by centrifuging, shaken vigorously with chemically clean sterile sand in order to break up clumps, and filtered through sterile cotton. By means of the Petroff-Hauser counter, suspensions were diluted with sterile buffer to give a final concentration of five million spores per milliliter. The buffer solution used in all cases was made up by dissolving four grams of monobasic sodium phosphate and six grams of dibasic potassium phosphate in a liter of distilled water. Esty and Cathcart (1921) have shown that heating in soft or hard glass changes the pH of a solution. The buffer has been reported by Morrison and Rettger (1930) to resist change in pH at the temperatures and times employed.

For determining heat resistance, one ml. portions of the standardized suspension were removed to soft glass tubes six cm. high, eight mm. of internal diameter, and one mm. thick. These were heated in a small oxygen flame 1.5 to 2.5 cm. above the surface of the suspension and drawn out to fine capillaries about seven inches long. Tanner and McCrea (1923) report that spores heated in a tube with a capillary opening require a longer time to be killed than in the case of a closed tube. Although this was not tested in the present experiment, we are inclined to believe that the depth at which the tubes were suspended and small volume of material makes such differences insignificant. In any case our interest is in comparative results. A gas-heated water bath whose temperature did not fluctuate more than 0.2°C. was used. The surface was covered with an oil layer to reduce evaporation and a stirring mechanism insured even distribution of heat.

The spore suspensions were preheated in another bath for one minute at 1°C. higher than the test temperature. At the end of the given time interval three tubes were removed, cooled immediately in ice-water, dried, and opened by applying red hot glass to a scratch made with a file. The contents of each were poured into a separate petri dish and sterile agar containing 0.5 per cent peptone and 0.5 per cent yeast extract added. According to the study of Curran and Evans (1937) which appeared after this work was done, the medium used was not the ideal one. The plates were incubated at about the organisms' optimum growth temperature and examined for growth at the end of six days. Throughout the whole procedure every precaution was taken to prevent contamination. Plating out the heated spore suspensions enables one to determine by the growth of nontypical colonies when contamination has occurred. Also a fair idea is obtained of the number of spores surviving.

RESULTS AND DISCUSSION

In table 2 the results are brought together according to species and arranged in the approximate order of decreasing thermal resistance. The heavy horizontal line separates the two groups into which the organisms seem to fall according to the thermal resistance of the spores. *Bacillus subtilis* and *Bacillus agri* both growing at 55°C. produce the spores of maximum resistance. *Bacillus vulgatus* and *Bacillus mesentericus* with lower maxima produce spores less resistant than *B. subtilis* but more resistant than spores of *Bacillus cereus*, *Bacillus mycoides*, and *Bacillus megatherium*. The latter three species usually have a maximum lower than 50°C. The author (1940) has divided *B. cereus* into three physiological groups of which group II has the highest maximum growth temperature and generally the spores of greatest thermal resistance.

TABLE 2

Record of thermal resistance and maximum growth temperature of 72 strains of aerobic spore-forming bacteria

NO. OF STRAINS	SPECIES	MAXIMUM GROWTH TEMPERATURE					SPORE RESISTANCE IN MINUTES AT 95°C.
		35	40	45	50	55	
		°C.	°C	°C	°C	°C	
14	*B. subtilis*	+	+	+	+	+	Greater than 45
1		+	+	+	+	+	30–45
4	*B. agri*	+	+	+	+	+	Greater than 45
1		+	+	+	+	0	Greater than 45
1		+	+	+	+	0	50% dead within 45
1	Unidentified	+	+	+	+	sl.	Greater than 45
1		+	+	+	+	sl.	20
1	*B. vulgatus*	+	+	+	+	+	45
1		+	+	+	+	sl.	Greater than 45
1		+	+	+	+	sl. +	45
1		+	+	+	+	v. sl. +	Greater than 45
1		+	+	+	+	0	45
1		+	+	+	+	0	20 min. at 98°C.
1	*B. mesentericus*	+	+	+	+	+	20–30
1		+	+	+	+	sl. +	30
1		+	+	+	+	0	Greater than 45
1		+	+	+	+	0	20
2		+	+	+	+	0	15
2	*B. cereus* Group II	+	+	+	+	0	45
1		+	+	+	0	0	20
1		+	+	0	0	0	20
1		+	+	+	0	0	15
1		+	+	+	0	0	10
1	*B. cereus* Group I	+	+	+	0	0	20–30
1		+	+	0	0	0	20
1		+	+	v. sl. +	0	0	10
1		+	+	0	0	0	10
2		+	+	0	0	0	5
2		+	+	0	0	0	Less than 5
1		+	+	0	0	0	Less than 5
1		+	+	0	0	0	20 min. at 90°C.
3		+	+	0	0	0	Less than 2
1	*B. cereus* Group III	+	±	0	0	0	10
1	Unidentified	+	+	+	0	0	30–45
1		+	+	+	0	0	30
1		+	+	+	v. sl. +	0	10–15

TABLE 2 (*Concluded*)

NO. OF STRAINS	SPECIES	MAXIMUM GROWTH TEMPERATURE					SPORE RESISTANCE IN MINUTES AT 95°C.
		35	40	45	50	55	
		°C.	°C	°C	°C	°C	
1	*B. mycoides*	+	+	±	0	0	30–45
1		+	+	+	+	0	10
1		+	+	0	0	0	Less than 5
1		+	+	+	±	0	Less than 2
1	*B. megatherium*	+	+	+	0	0	10
1		+	+	±	0	0	10
1		+	+	+	0	0	5
1		+	+	+	0	0	Less than 5
2		+	+	±	0	0	Less than 5
1		+	0	0	0	0	Less than 5
3		+	+·	+	+	0	Less than 2

v. sl. = very slight growth. sl. = slight growth.
None of the above strains grow at 60°C.

From the data presented it is evident that the members of the genus *Bacillus* may be separated into three groups on the basis of heat tolerance by spores. The thermophiles produce the spores of greatest resistance and have the highest growth temperature. The species with a maximum below 50°C. possess spores of least resistance while non-thermophilic types with maxima between 50–60°C. have spores of intermediate heat resistance. The lines of demarcation between these groups are not sharp.

The data also show individual exceptions especially within the *B. mycoides* and *B. megatherium* groups. This fact is of theoretical interest and does not necessarily negate the evidence for the existence of a relationship of thermal resistance to maximum growth temperatures. Establishing the influence of a variable upon a phenomenon demands the stabilization of all other known variables. When, in spite of the effort to control the variables, data show significant but inexact correspondence of the factor and phenomenon under investigation the conclusion may be drawn that deficiencies of experimental procedure exist and that unknown influences are at work. In the case of the relation of maximum temperature of growth to heat tolerance the correspondence is significant but not exact. Such data may be interpreted to mean that a complex of factors within the vegetative cell and spore are involved in thermal resistance. The characters that delimit the highest temperature of growth are among the total controlling heat tolerance. Apparently the explanation for limitation of growth at a maximum temperature will not provide the complete answer to thermal resistance. In the case of the spore, the spore coat is assumed to play a special part. Knaysi (1938) has indicated that the role of the spore coat may probably be established by studying spore germination.

CONCLUSIONS

The heat resistance of bacterial cells is related to the maximum temperature of growth.

In the case of bacterial spores a similar relationship exists.

Within the genus *Bacillus* three groups are separable with respect to spore resistance and relation to maximum growth temperature.

The inexact correspondence of maximum growth temperature to thermal resistance of spores is interpreted to mean that factors in addition to those which determine maximum temperature of growth are involved in thermal resistance.

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