THE MINIMUM TEMPERATURES OF GROWTH OF SOME BACTERIA

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(With One Figure in the Text)

In a previous publication (Haines, 1933*a*) it was shown that the bacteria mainly responsible for the spoilage of lean meat during storage as practised in this country were members of the *Achromobacter* and *Pseudomonas* groups, Achromobacteria being the more abundant. Examination of chilled beef brought over from Australia in an atmosphere containing carbon dioxide revealed in some cases a limited flora composed chiefly of yeasts and Achromobacteria, in others a certain amount of mould growth has been reported. It is evident that the type of flora predominating on any stored foodstuff will depend on the following factors:

(*a*) the initial infection during preparation and any subsequent contamination during handling;

(*b*) the temperature of storage;

(*c*) modification of the environment, such as lowering the humidity, the use of carbon dioxide, etc.

Most foodstuffs are exposed to a fairly heavy infection with the common saprophytic bacteria abounding in air, soil and water. Details of the nature of this infection with regard to animal tissues, both in Australia and here, have been given elsewhere (Empey and Vickery, 1933; Haines, 1933*b*). It is therefore necessary, whenever cold storage is to be used, to be able to predict the behaviour of such bacteria over a given range of temperature. While there are in the literature numerous isolated examples of the growth of particular bacteria at low temperatures, there seem to be few systematic studies of the common saprophytes over the whole range of their viability.

Among the interesting cases of bacterial growth at low temperatures may be mentioned the luminescent bacteria from sea fish growing well at 0° C. (Forster, 1887), Rubentschik's uro-bacteria multiplying at −2·5 to −1·25° C. (Rubentschik, 1925), and Bedford's strains of Achromobacteria from the North Pacific growing down to −7·5° C. (Bedford, 1933). Müller (1903) gave an extensive survey of the earlier literature, and himself carried out a large number of counts, showing that several species of *fluorescens* and other groups could proliferate at 0° C. Barber's work on *B. coli* (Barber, 1908) suggested that this organism did not grow below about 10° C., but observations were

https://doi.org/10.1017/S0022172400034604 Published online by Cambridge University Press

confined to one strain. Graham-Smith (1920) investigated in detail the behaviour of several bacteria, including *Staphylococcus aureus*, *B. coli* and *B. pyocyaneus*, and he found that only slight multiplication occurred at 8–10° C., rapid decline in numbers taking place at lower temperatures. An extensive summary of much of the earlier work in relation to particular foods has recently been published by Tanner and Wallace (1933).

Experimental

In the present work an attempt was made to estimate the rate of growth of a number of common bacteria, at intervals of a few degrees, throughout the range 37 to −5° C. The method was to observe the time for a standard amount of growth at each temperature. It was carried out as follows. A thick saline suspension of the organism was made by vigorously shaking two loops of a young culture with 2 c.c. of sterile saline in a sterile tube. One loopful of the suspension was then streaked four times across nutrient agar *p*H 7·0 in a 10 cm. Petri dish, thus giving four parallel inoculations of decreasing density. Duplicate or triplicate plates were made, packed in cotton-wool and incubated at carefully controlled temperatures. The plates were examined daily, and a plus sign recorded for the appearance of visible growth along each streak. The point at which the first two of the four streaks appeared is regarded as indicating definite growth, and is the point recorded. In many cases duplicate experiments, made from different subcultures of the same organism, were carried out. In general good agreement was obtained, but sometimes a difference of a few days in the longer periods of incubation at the lower temperatures was noted. In such cases the two extremes were recorded. The organisms were obtained from several sources: some from the Lister Institute and the Pathological Laboratory, Cambridge, others isolated by the author from meat, abattoirs, etc. Cultures marked E were supplied by Mr W. A. Empey of the Australian Council for Scientific and Industrial Research, being isolated by him from similar sources in Australia.

Discussion of results

The experimental figures are displayed in detail in Table I, and shown graphically in Fig. 1, in which the time required for growth is plotted against temperature. For the sake of clearness not all the points are marked on the graph. It will be seen that the organisms studied may be divided into four groups as follows:

(1) The *Staphylococci*, optima at 37° C., growing very slowly if at all below 10° C.

(2) Most strains of *B. coli*, *B. proteus*, *B. subtilis* etc. (optima 37° C.) and *Micrococci* (optima 20–30° C.) growing very slowly in the range 5–0° C.

(3) Some strains of *B. proteus*, etc., capable of growth at 0° C.

Table I

The numerals represent the time in days for visible growth, as explained in the text, 1 day = 24 hours. 0 signifies no growth, cultures at temperatures at and below zero being kept for 3 months before being discarded. – signifies no measurement made.

Nomenclature of organism			Temperature ° C.*								
Bergey's	Usual name	Source and strain	37	20	15	10	5	0	−1	−2	−3	−5
Achromobacter	?	Sq, slimy beef	0	2	2	3	4	10–12	13	13–16	26	–
,,	(*Achromobacter lique-*	A_1, ,,	0	2	2	3	4	6–11	14	20	30	>3 months
,,	*faciens* = *B. liquefaciens*	A_8, ,,	0	2	3	6	6	14	17	21	32	3 months
,,	of Frankland)	A_5, ,,	0	2	2	4–5	11	>30	–	0	0	–
,,	.	A_{17K}, beef	0	1	1–2	2	3	5	–	8	14	–
,,	.	S_{54}, ,,	0	1	2	2	3	5	–	13	17	–
,,	.	A_4, ,,	0	1	2	3	4	16	–	41	>71	–
,,	.	A_2, ,,	0	2	2–3	3	4	6	–	11	18	–
,,	.	A_3, ,,	0	2	2	3	3	5	–	11	–	–
Aerobacter	*B. lactis aerogenes*	Lister No. 418	<1	2	3	9	36	0	–	–	–	–
B. mesentericus	*B. mesentericus*	Path. Lab. Cambridge	<1	2	3	5	12	43	–	–	–	–
B. subtilis	*B. subtilis*	Lister No. 2586	<0·5	2	8–11	>30	–	–	–	–	–	–
Escherichia	*B. coli* Escherich	Lister No. 86	<0·5	2	3	5	0	0	–	–	–	–
,,	*B. coli communior*	Abattoir	<0·5	1–2	2	6	0	0	–	–	–	–
,,	*B. coli communis*	,,	<0·5	2	2	4	8–9	29	–	>30	–	–
Flavobacterium	*B. fulvus*?	Chilled beef	<1	<1	1	2	4–7	>30	–	–	>43	–
Micrococcus luteus	*Micrococcus luteus*	,,	1–2	2	6	23	–	>30	–	–	–	–
M. aurantiacus	*M. aurantiacus*	,,	1	2	4	5	15	24	–	>30	–	–
Proteus	*B. proteus*	Pr, putrid beef	<1	2	4	4–5	7	8	–	–	–	–
,,	*B. proteus vulgaris*	Lister	<0·5	2	3	6	–	0	–	0	0	–
,,	*B. proteus*	Cow dung, P_6	<0·5	1	2	3	5	8	–	−30	–	–
,,	*B. proteus*	N.Y.U. Lister No. 401	<0·5	2	3	5	24	0	–	–	–	–
Pseudomonas pyocyanea	*B. pyocyaneus*	Lister	<0·5	2	4	10	0	0	–	0	0	0
P. pyocyanea	*B. fluorescens*	$P_8$1, horse dung	0	1–2	2	3	6	13	18	23	33	>70<87
P. pyocyanea	*B. fluorescens*	$P_3$1 E, chilled beef	0	1	2	3	4	6	–	23	37	–
Rhodococcus cinnebareus	*Micrococcus cinnebareus*	Abattoir	0	2–3	6	–	25–28	0	–	–	–	–
Sarcina flava	*Sarcina flava*	,,	0	2	4	5	12	75	–	–	–	–
Staphylococcus albus	*Staphylococcus albus*	Lister	<0·5	2	3–4	20	0	–	–	–	–	–
S. albus	*S. albus*	C_2, chilled beef	<0·5	3	>20	–	0	0	–	–	–	–
S. aureus	*S. aureus*	Lister	<0·5	3	6	20	0	–	–	–	–	–
S. pyogenes	*S. pyogenes*	,,	<0·5	2	4	15	0	–	–	–	–	–
Yeast (*Torula*?)	.	Chilled beef	0	2	2	4	7	11	–	16	23	>40
,, ,,	.	,,	0	2	2	3	6	10	–	15	20	>40

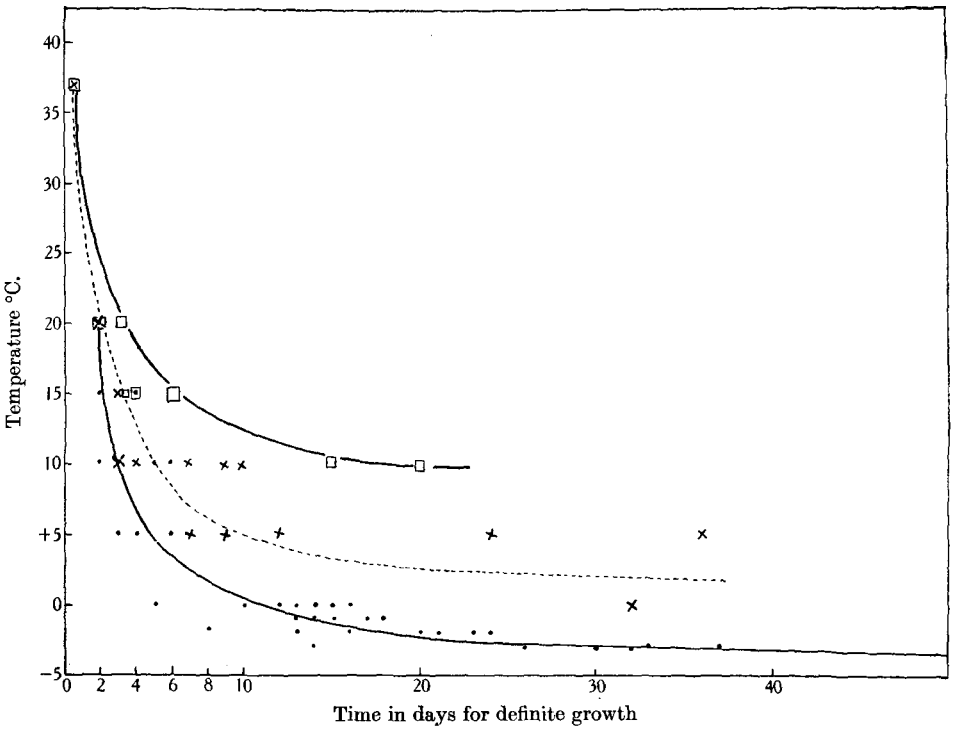
* To the nearest degree in the headings. The exact range is shown in the appended Note.

NOTE. The exact temperatures of incubation were as follows:

37 = 35 ± 2° C.	15 = 14·9 ± 0·2° C.	5 = 5·4 ± 0·2° C.	−1 = −0·9 ± 0·1° C.	−3 = −3·0 ± 0·2° C.
20 = 20 ± 1° C.	10 = 9·8 ± 0·2° C.	0 = 0·5 ± 0·3° C.	−2 = −1·9 ± 0·2° C.	−5 = −4·8 ± 0·1° C.

(4) Most strains of *Achromobacter*, *Pseudomonas*, and various yeasts showing comparatively rapid growth at 0° C. (sometimes in 5 days) and growing down to about −5° C. on supercooled media.

At all the temperatures above −5° C. the agar remained unfrozen unless special steps were taken to bring about separation of ice: at −5° C. some of the plates froze and others remained supercooled. Growth has not been obtained on frozen agar at −5° C. with bacteria. In a few cases it has been observed with *Pseudomonas* and *Achromobacter* on frozen media at −3° C.



□ = Staphylococci. × = *Coli*, *Proteus*, etc. • = *Achromobacter*, *Pseudomonas*, etc.

Fig. 1

Multiplication of yeasts and moulds on frozen tissues stored at −5° C. has previously been noted (Haines, 1931). Tomkins (see Griffiths, Vickery and Holmes, 1932, p. 159) found that several fungi could grow down to about −7° C., and Haines (1932) studying strains of *Actinomyces* concluded that these did not grow much below 0° C. The lower limit of growth of micro-organisms is thus determined by two factors; firstly, the temperature, and secondly the amount of water frozen out of the medium. As regards temperature, the lower limit of growth of the so-called psychrophilic micro-organisms, whether yeasts, moulds or bacteria, lies between −5 and −10° C., probably near −7° C., including the majority of the cases reported in the

https://doi.org/10.1017/S0022172400034604 Published online by Cambridge University Press

literature and those examined here. When, however, the medium freezes, yeasts and moulds tend to preponderate and bacteria to be largely inhibited, especially a few degrees below zero. It is known that yeasts and moulds are able to tolerate higher osmotic pressures of the medium than bacteria. Thus, Karaffa-Korbutt (1912) found that while 8–12 per cent. of sodium chloride, added to nutrient broth, inhibited the multiplication of the common bacteria, yeasts were able to proliferate in media containing 25 per cent. sodium chloride. Moran (1930) estimated that frozen mammalian muscle allowed to come to equilibrium at −3° C. had 70 per cent. of its water present as ice, and at −5° C. 82 per cent. These facts probably explain why the growth of bacteria is brought to a standstill on frozen media before that of yeasts and moulds, at temperatures at which some members of all three groups of organisms could otherwise grow. The explanation of the observation that, at temperatures above freezing, lowering the humidity also tends to favour yeast and mould growth, probably also lies here.

SUMMARY

1. The commonly occurring bacteria may be divided into four groups on the basis of their behaviour at various temperatures. These are (i) the Staphylococci, not growing below 10° C., (ii) most strains of *B. coli*, *B. proteus* and Micrococci ceasing growth in the range 5–0° C., (iii) some strains of *B. proteus* etc. capable of growth at 0° C., (iv) many strains of *Achromobacter*, *Pseudomonas*, and various yeasts growing rapidly at 0° C. (sometimes in 5 days), and down to about −5° C. on unfrozen media.

2. Bacterial growth on frozen media has not been observed below −3° C.

The author is indebted to Dr G. S. Graham-Smith, F.R.S., who read the manuscript, to Mr W. A. Empey for the supply of cultures, and to Mr J. Barlow for assistance in the experimental work.

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(*MS. received for publication* 25. IV. 1934.—Ed.)