THE RELATIVE THERMAL DEATH RATES OF YOUNG AND MATURE BACTERIAL CELLS

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The conception of a definite period of youth in the life of bacteria now appears to be well established.

From the standpoint of the initiation of reproduction in cultures it has long been known that transplants from cultures only a few hours old start reproduction at once, whereas inoculations from old laboratory cultures require a period of dormancy before reproduction begins. This phenomenon was observed microscopically by Barber (1908), and was later studied more in detail with the aid of growth curves by Penfold (1914) and Chesney (1916).

From the work of Clark and Ruehl (1919) and Henrici (1921) it is known that the morphology of bacterial cells varies greatly with the age of the culture. Henrici has carefully studied this subject and the biological significance of these morphologic changes has been interpreted by him in a series of subsequent papers (1928).

Sherman and Albus (1923) studied young and old bacterial cells with the idea of detecting physiological differences. A number of tests were found which revealed definite physiological distinctions between young and mature cells. Differences were observed with respect to acid agglutinability, and resistance to harmful environmental factors, such as extremes of temperature, germicidal substances and osmotic pressure. These workers concluded that a period of physiological youth is characteristic of bacterial cells.

Among the many problems with which the bacteriologist is confronted, none is of more importance than the factors which

influence the death rate of organisms at high temperatures. The purpose of this investigation was to obtain more definite information concerning the relative heat resistance of young and mature cells of a few important types of bacteria.

EXPERIMENTAL

The cultures used in this study were six strains of *Streptococcus fecalis*, a relatively heat resistant species of streptococcus, and three strains each of two capsulated organisms which were isolated from "ropy milk."

Prior to the heat tests the cultures were grown in sterile skimmed milk for different periods of time in order to obtain cells of varying ages. The *Streptococcus fecalis* cultures were incubated at 37°C. before the heat treatment, while the slime producing organisms were incubated at laboratory temperature. A 1-cc. amount of the culture under test was transferred from the milk in which it was grown to a tube containing 9 cc. of sterile skimmed milk previously heated to the temperature (62.8°C.) used for these tests. The tubes in which the heat tests were made were closed with sterile rubber stoppers in order to prevent undue surface cooling by evaporation, and also to allow thorough mixing, by shaking, at the beginning of the heat treatment. At the end of the heat treatment, samples were obtained from near the bottom of the tube without agitation and without touching the inside surface of the tube above the heating medium. These precautions are essential in order to obviate experimental error due to the adhesion of the bacteria to the walls of the tube above the liquid.

The ordinary plate culture method was used for determining the bacterial counts before and after the heat treatment. The *Streptococcus fecalis* counts were made using lactose nutrient agar and the plates were incubated at 37°C. for two days. The capsulated organisms were plated on plain nutrient agar and the plates were incubated at room temperature and counted at the end of two days. As these capsule forming organisms grow rapidly at comparatively low temperatures and produce large colonies the two-day incubation period at laboratory temperature

was shown to be adequate. All plates were made in duplicate and each culture was tested a number of times, always obtaining similar results.

From the data reported in tables 1, 2 and 3 it may be seen that the thermal death rate of the young cells is distinctly greater

TABLE 1

The relative resistance to heat of bacterial cells of different ages (Streptococcus fecalis)

CULTURE NUMBER	AGE	BACTERIAL PLATE COUNT		RATIO BEFORE : AFTER	PER CENT SURVIVAL
		Before pasteurization	After pasteurization		
	hours				
18	24	109,000,000	2,870,000	37:1	2.63
	48	135,500,000	2,150,000	63:1	1.58
	4	7,000,000	18,000	388:1	0.25
20	24	102,500,000	1,310,000	78:1	1.27
	48	106,500,000	845,000	126:1	0.79
	4	8,100,000	14,000	578:1	0.17
21	24	135,000,000	1,200,000	112:1	0.88
	48	110,500,000	1,445,000	76:1	1.37
	4	9,150,000	21,700	421:1	0.23
22	24	102,500,000	3,190,000	32:1	3.11
	48	113,000,000	1,905,000	58:1	1.68
	4	13,450,000	32,600	412:1	0.24
23	24	149,500,000	3,250,000	46:1	2.17
	48	121,500,000	445,000	273:1	0.36
	4	11,050,000	10,900	1013:1	0.09
24	24	87,500,000	470,000	186:1	0.53
	48	144,000,000	530,000	271:1	0.36
	4	12,000,000	2,400	5000:1	0.02

than that of the older cells. In addition some other points, perhaps, are worthy of being noted. In the case of *Streptococcus fecalis* incubated at 37°C., a temperature at which the growth rate is rapid and the whole growth cycle therefore of brief duration, the cells from the twenty-four-hour culture were apparently

more resistant than those from a culture forty-eight hours old. This is entirely logical, as it might be expected that the cells

TABLE 2

The relative resistance to heat of bacterial cells of different ages

(Ropy milk organisms—Type I)

CULTURE NUMBER	AGE	BACTERIAL PLATE COUNT		RATIO BEFORE:AFTER	PER CENT SURVIVAL
		Before pasteurization	After pasteurization		
	hours				
2	54	84,000,000	23,350	3,597:1	0.026
	24	33,000,000	5,900	5,593:1	0.018
	6	17,850	<1	>17,850:1	<0.005
4	54	91,000,000	45,000	2,022:1	0.0494
	24	61,000,000	5,100	11,960:1	0.0083
	6	98,000	<1	>98,000:1	<0.0010
6	54	136,000,000	93,500	1,454:1	0.068000
	24	23,600,000	8,400	2,809:1	0.035000
	6	3,850,000	<1	>3,850,000:1	<0.000002

TABLE 3

The relative resistance to heat of bacterial cells of different ages

(Ropy milk organisms—Type II)

CULTURE NUMBER	AGE	BACTERIAL PLATE COUNT		RATIO BEFORE:AFTER	PER CENT SURVIVAL
		Before pasteurization	After pasteurization		
	hours				
12	54	17,650,000	1,895	9,314:1	0.010700
	24	1,190,000	<1	>1,190,000:1	<0.000084
	6	5,350,000	<1	>5,350,000:1	<0.000019
14	54	21,900,000	6,750	3,244:1	0.030000
	24	183,000	<1	>183,000:1	<0.000540
	6	485,000	<1	>485,000:1	<0.000206
16	54	12,700,000	160	79,375:1	0.001200
	24	1,680,000	<1	>1,680,000:1	<0.000060
	6	6,600,000	<1	>6,600,000:1	<0.000015

would lose some of their resistance before the population of the culture begins its numerical decline. Of practical interest is

the fact that the thermal death rates of the young and old cells are so different that such commercially important organisms as the "ropy milk" bacteria may survive pasteurization or be entirely eliminated, depending upon the physiological age of the cells.

SUMMARY

Thermal death rates of young and mature cells of *Streptococcus fecalis* and of two species of capsulated bacteria were studied.

In each case the young cells were markedly less resistant than were the mature cells of the same organism.

It is thought that more emphasis should be placed on this physiological difference in practical work.

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