PSYCHROTROPHIC BACTERIA OF RAW MILK

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By

Takeo NAKANISHI, Toshitaka NAKAE and Bihing S. RASAN

Laboratory of Chemistry and Technology of Animal Products, Faculty of Agriculture, Tohoku University, Senai, Japan

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Introduction

There are many terms which had been introduced and reviewed for bacteria able to grow at low temperatures (5°C or less), but recently it seems that the term psychrotrophic proposed by Eddy (1) is the most commonly accepted one.

In modern development in processing, keeping and shipping of foods in general where the refrigeration system is widely used, the importance of psychrotrophic microorganisms needs more attention because their growth in foods, in this case dairy products, can cause fruity, putrid and rancid flavors and odors, thus degrade the quality of the products and limit their uses.

This study was conducted, to gain information on the distribution, temperature relations of growth and proteolytic activity of these bacteria.

Materials and Methods

A. Bacterial distribution.

1. Raw milk samples.

The raw milk samples used in this study were received from a milk plant in Sendai, Miyagi Prefecture.

2. Bacterial counts.

Standard Plate Counts and Psychrotrophic Counts were conducted during 1966 in peptone-meat extract-glucose-agar (PMG) medium at 35°C, for 48 hours and 5°C for 10 days respectively (2).

The PMG medium was composed of 10 g peptone,5 g meat extract, 1 g glucose, 10 ml reconstituted milk, 15–25 g, agar, 1000 ml distilled water which gave a final pH of 6.8–7.0.

3. Bacterial isolation and identification.

One hundred eighty strains were isolated in PMG medium from raw milk samples which had been storaged in sterile skimmilk (1 ml of raw milk smaples in 100 ml sterile skimmilk) for 10 days at 5°C. Of these strains, 101 strains were selected and identified by common methods (3, 4, 5, 6, 7) by reference to Bergey's Manual (8).

Among the common techniques which had been used in identification tests of these isolates were: Gram stain, Leifson's flagella stain, motility, fermentation of carbohydrates, fluorescence in King, Ward & Raney's medium under ultraviolet light, hydrolysis of arginine in Thornley's semi-solid arginine medium, reaction in litmus milk, Kovacs' oxidase tests, catalase test, reduction of nitrates, indole production, gelatin liquefaction, proteolytic and lipolytic actions.

B. Temperature relations of growth.

1. Bacteria used.

Fifty one of the strains which had been identified in Table 2 were selected and studied for their temperature relations of growth by means of a temperature gradient incubator.

2. Temperature Gradient Incubator (TGI)

The temperature gradient incubator used in this study was designed by one of the authors (9).

The TGI was set 24 hours before the experiments were started. Except for coliform bacteria the temperature gradient which ranges from 5–43°C were used. For coliform bacteria the range of 5–53°C was used.

The medium used in the incubation tubes of the TGI was the PMG medium which contained 0.01% bromothymolblue (BTB) or bromocresolpurple (BCP) as indicator.

In this experiment the optimum temperature was determined by detecing and measuring the point where the growth zone of the bacteria in the incubation tubes of the TGI, first becomes visible.

The minimum and maximum temperatures were determined after 24 hours incubation in the TGI.

The lethal incubation temperature (10) was determined by 48 hours additional incubation at 22°C after the 24 hours incubation in the TGI.

Results

A. Bacterial distribution.

The result of bacterial counts conducted during 1966 is presented in Table 1. From this table it can be seen that the number of psychrotrophiles of raw milk samples is low in Spring, increases in Summer, decreases in Autumn and again slightly increases in Winter.

The bacterial species of raw milk samples are presented in Table 2. This table reveals that of the isolates 46.54% are considered to be Pseudomonas, 37.62% Achromobacter, 6.93% Coliform and 8.91% unidentified gram-negative rods.

In Table 3 the isolates are grouped according to their morphology, gram stain characteristics, motility, action on litmus milk, proteolysis and lipolysis. Among 180 strains isolated, 7.8% (14 strains) reduced the litmus milk into bluish

Table 1. The bacterial number of raw milk samples during 1966

Bacterial counts conducted during the indicated time	S.P.C. (35° C, 48 hr) (Bacterial number/ml of sample)	P.C. (4° C, 10 days) (Bacterial number/ml of sample)
March-May, 1966	1,100,000	73,000
June-August, 1966	997,000	151,000
Sept.-Nov., 1966	1,200,000	38,000
Nov.-Dec., 1966	190,000	96,000

S.P.C.: Standard Plate Count. P.C.: Psychrotrophic Count.

Table 2. Bacterial species of raw milk samples

Name of specieces	Number	Percentage
Pseudomonas fluorescent group		
1. Ps.fluorescence	21	20.79
2. Ps.mildenbergii	9	8.91
3. P·s.incognita	7	6.93
4. Ps.convexa	3	2.97
5. Ps.striata	1	0.99
Pseudomonas achromogenic group		
6. Ps.fragi	5	4.95
7. Ps.synxnantha	1	0.99
Achromobacter		
8. Ach. liquefaciens	20	19.80
9. Ach. guttatus	18	17.82
Coliform		
10. Aerobacter aerogens	2	1.98
11. Aerobacter cloacae	1	0.99
12. Paraco.obactrum aerogenoides (Hafnia)	1	0.99
13. Escherichia freundii	2	1.98
14. Escherichia intermedia	1	0.99
Unidentified gram-negative rods	9	8.91

Table 3. Bacterial morphology, gram stain, action on litmus milk, proteolysis and lipolysis

Sample collected during indicated time	No, of strains isolated	Morphology		Gram stain		Motility		Litmus milk				Proteolysis		Lipolysis	
		Rod	Coc	+	−	+	−	Ac	Al	Dig	R	+	−	+	−
March-May, 1966	22	20	2	2	20	21	1	2	2	17	1	—*	—*	18	4
June-August, 1966	100	98	2	2	98	94	6	12	24	53	11	58	42	65	35
Sept.-Dec., 1966	58	57	1	1	57	56	2	11	29	16	2	39	19	47	11
	180	175	5	5	175	171	9	25	55	86	141	97	61	130	50

Coc: Coccus: Ac: Acid; Al: Alkaline; Dig: Digested; R: Reduced.
* The test was not conducted.

white, 13.9% (25 strains) changed it into acid reaction, 30.6% (55 strains) digested the protein of the milk.

97.2% of the isolates where gram-negative rods and the others were gram positive cocci.

Table 4. Temperature relations of the strains isolated.

Name of species	No.	Temperature relations			
		Optimum temp. (°C)	Maximum temp. (°C)	Minimum temp. (°C)	Lethal inc. temp. (°C)
Pseudomonas fluorescent group					
1. Ps fluorescence	1	31.0	36.0	11.0	41.2
	2	32.0	36.0	13.0	41.8
	3	31.5	35.0	13.0	41.5
	4	32.0	36.0	12.0	40.9
	5	32.0	36.0	10.0	42.0
	6	30.5	36.0	11.0	41.0
	7	32.0	36.0	11.0	41.0
	8	31.0	35.0	11.0	41.0
	9	31.0	35.5	11.0	41.0
	10	29.0	35.6	12.0	42.0
	11	28.5	34.0	15.0	37.0
	12	28.5	34.0	14.0	37.0
	13	31.0	34.5	12.0	38.0
2· Ps. convexa	14	31.0	33.5	12.0	38.0
	15	30.0	33.8	10.0	
	16	29.0	33.0	10.0	39.5
3. Ps mildenbergii	17	30.0	33.8	14.0	37.0
4. Ps incognita	18	27.0	31.0	12.0	42.0
5. Ps. striata	19	31.5	36.0	10.0	39.0
Pseudomonas achromogenic group					
6. Ps. fragi	20	29.0	31.5	13.0	41.0
	21	27.0	31.0	12.0	41.0
	22	28.0	31.0	10.0	
	23	28.0	31.8	10.0	
	24	28.0	32.0	9.0	
7, Ps. synxantha	25	28.0	32.0	11.0	42.0
Achromobacter					
8. Ach. liquefactions	26	28.0	31.0	10.0	41.0
	27	27.0	30.5	10.0	41.0
	28	28.5	32.0	8.0	41.0
	29	27.5	31.0	11.0	41.5
	30	29.0	32.0	11.0	41.5
	31	27.5	32.0	11.0	41.5
	32	28.0	32.0	11.0	41.5
	33	27.0	32.0	11.0	41.5
	34	28.0	31.9	10.0	42.0
	35	28.0	31.5	10.0	
	36	27.0	31.5	12.0	41.3
	37	27.5	31.9	10.0	42.0
	38	28.0	31.9	12.0	41.0
	39	28.0	30.8	10.5	42.0
	40	27.5	31.0	9.0	
	41	29.0	32.0	12.0	40.7
	42	27.6	33.0	10.0	
	43	29.0	33.0	9.0	41.5
9. Ach. guttatus	44	27.0	31.0	11.0	41.5
	45	27.5	31.5		
	46	28.0	30.8	10.0	42.0
	47	28.0	31.8	10.0	
	48	28.0	31.0	11.0	
	49	28.0	32.0	11.0	41.5
Coliform					
10. Escherichia freuindii	50	33.0	38.0	14.5	43.8
11. Aerobacter aerogenes	51	35.5	42.8	13.0	45.0

158 strains had been tested for their proteolytic action and among them 97 strains (61.4%) were proteolytic and the other 61 strains (38.6%) were non-proteolytic.

All isolates were tested for their lipolytic action. 130 strains (72.2%) were lipolytic and the others were non-lipolytic.

B. Temperature relations of growth.

The results of the determination of the temperature relations of growth of 51 isolates are compiled in Table 4, summarized in Table 5, and arranged diagrammatically in Fig. 1a, b, c, d. In Fig. 2, 5 of incubation tubes which reveal the optimum, maximum, minimum and lethal incubation temperatures of the isolates is presented. The dark zones which can be visualized in these incubation tubes are the growth growth zones of the isolates.

Table 5. The distribution of psychrotrophiles according to their temperature relations

Optimum temp. (°C)	Number of isolates	Percentage	Minimum temp. (°C)	Number of isolates	Pecentage
27	6	11.8	8	1	2.0
28	20	39.2	9	3	6.0
29	9	17.6	10	14	28.0
30	2	3.9	11	14	28.0
31	6	11.8	12	10	20.0
32	6	11.8	13	4	8.0
33	1*	2.0	14	2	4.0
Above 34	1*	2.0	15	2	4.0
Maximum temp.			*Lethal incubation temp.*		
31	11	21.5	37	3	7.3
32	18	35.3	38	1	2.4
33	3	5.9	39	2	4.9
34	5	9.8	40	2	4.9
35	3	5.9	41	13	31.7
36	9	17.6	42	18	43.9
37	0	0.0	43	0	0.0
38	1*	2.0	44	1*	2.4
43	1*	2.0	45	1*	2.4

* Escherichia intermedia or Aerobacter aerogenes.

These results reveal that 96.1% of the isolates studied have the optimum temperature ranges from 27–32°C. The growth of 92.2% of the isolates could be detected at 8–13°C after 24 hours incubation in the TGI. 72.5% of the isolates have the maximum temperature below 35°C, but 96.1% of them have the maximum temperature below 37°C. 41 of the isolates had been determined for their lethal incubation temperature, and 95.1% 0f them have the lethal incubation temperature below 43°C.

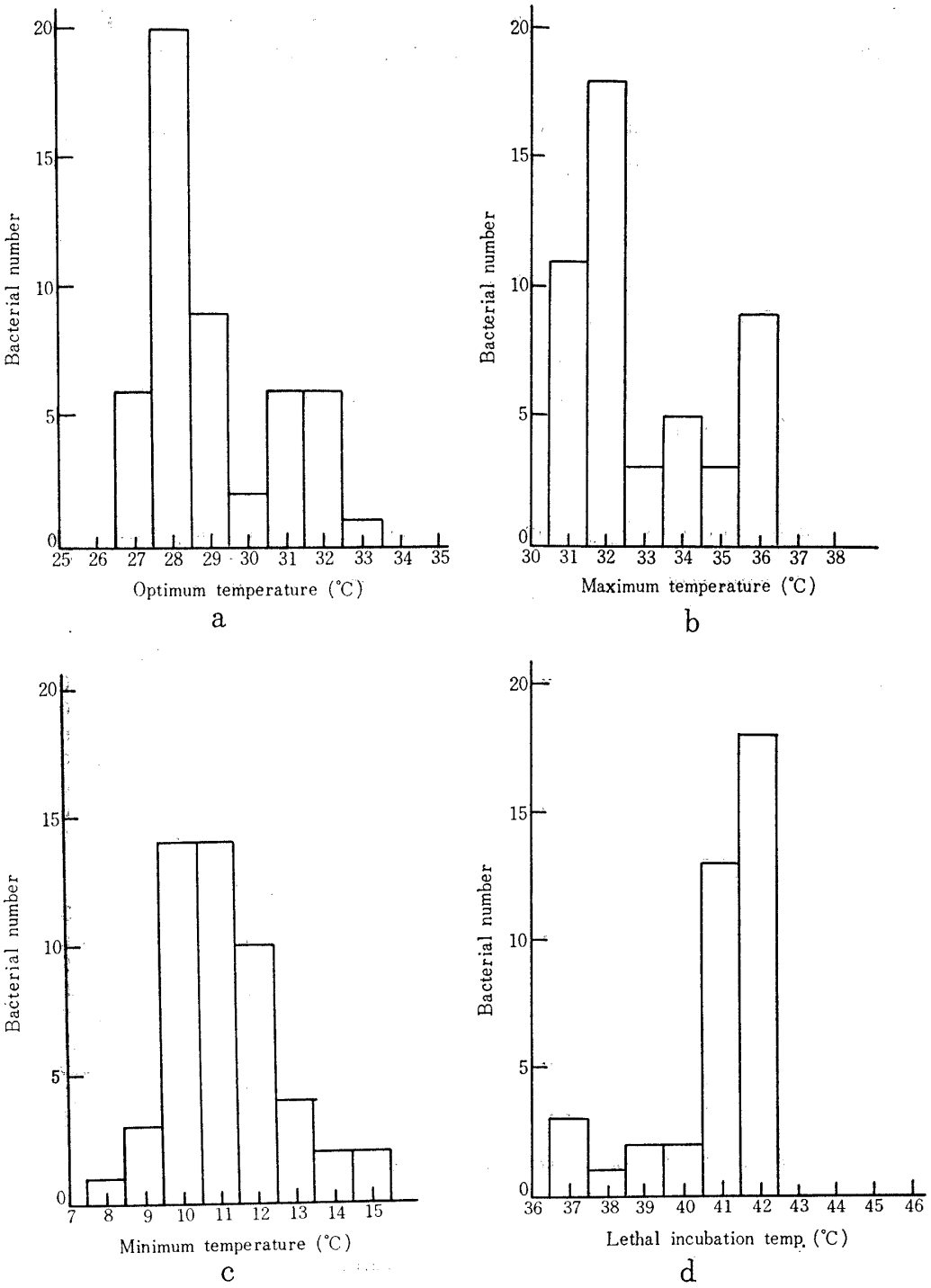
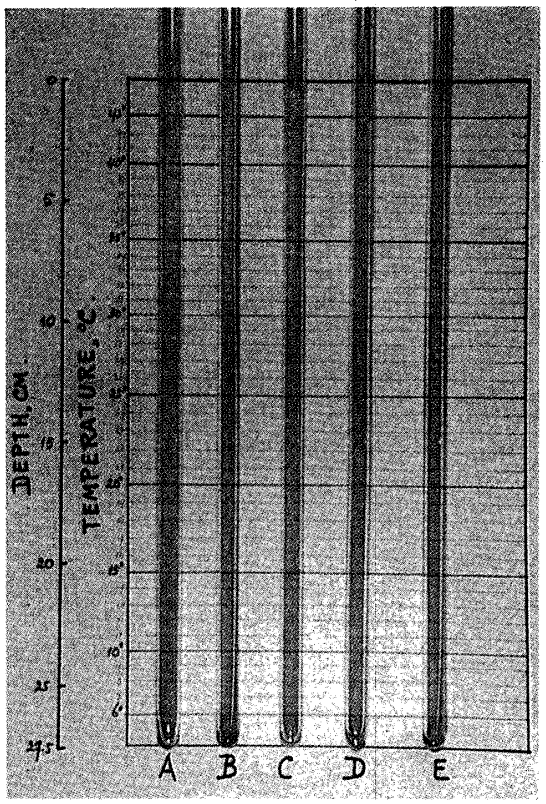


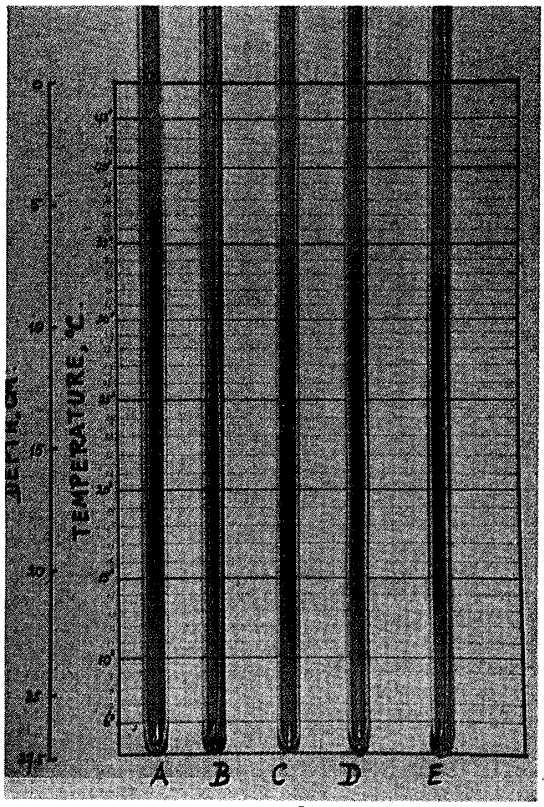
Fig. 1. The distribution of psychrotrophiles of raw milk according to their optimum (a), maximum (b), minimum (c), and lethal incubation (d) temperatures.

Discussion

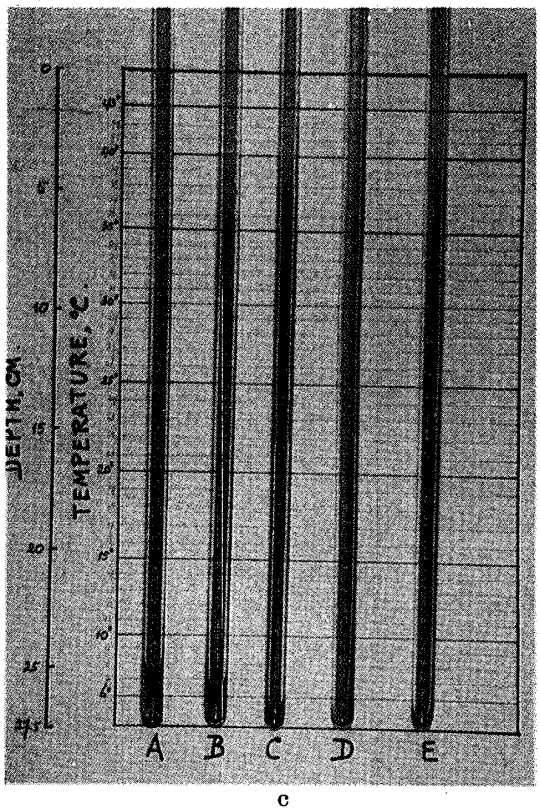
The bacterial distribution of raw milk depends on many factors such as the place where the sample is collected or taken, and the treatment of the sample. So, it is very hard to say that the result of this study can be applied to the other samples



a



b



c

Fig. 2a: Optimum temperature of A: Pseudomonas fluorescence. B: Pseudomonas fragi. C and D: Achromobacter liquefaciens. E: Achromobacter guttatus.

Fig. 2b: Maximum and minimum temperatures of the same bacteria as indicated in Fig. 2a.

Fig. 2c: Lethal incubation temperature of the bacteria indicated in Fig. 2a, b.

from other places. However, this result may be adduced as an instance when an extensive study which covers various samples from various places is conducted.

There are many publications concerning the cold growing bacteria as a whole or those of dairy products have been published. However there is little if any sublications which can reveal a general mode of temperature relations of these organisms, especially those of raw milk.

As mentioned previously, Eddy (1) proposed the use of term "psychrotrophic" for bacteria able to grow at 5°C and below, whatever their optimum temperature, and suggested that psychrophiles should be defined as bacteria with a maximum growth temperature below 35°C. If this suggestion is followed, of these isolates which had been studied here 72.5% were psychrophiles. However, if the result of this study can be regarded as a reflection of the general mode of the temperature relations of psychrotrophic bacteria of raw milk or dairy products as a whole, the authors would like to define the psychrotrophic bacteria of raw milk as bacteria which is able to grow at 5°C or less and have the optimum, maximum, and lethal incubation temperatures in the order of 27–32°C, below 37°C and below 43°C respectively.

Summary

1. The number of psychrotrophic bacteria of raw milk was low in Spring, increases in Summer, decreases in Autumn and again slightly increases in Winter.

2. Psychrotrophic bacteria of raw milk studied was composed of Pseudomonas (46.54%), Achromobacter (37.62%), Coliform (6.93%), and unidentified gram-negative rods (8.91%).

3. 61.4% of the strains studied were proteolytic, and 72.2% were lipolytic.

4. As determined by Temperature Gradient Incubator, 96.1% of the psychrotrophic bacteria of raw milk have the optimum temperature ranges from 27–32°C; 96.1% have the maximum temperature below 37°C. Among the isolates which were determined for their lethal incubation temperature, 95.1% have the lethal incubation temperature below 43°C.

The visible growth of 92.2% of the psychrotrophs of raw milk can be detected at 8–13°C after 24 hours incubation in the TGI.

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