Psychrotrophic Micro-organisms in Animal Products

By NOBUHIRO YANO

Animal Products Division, National Institute of Animal Industry

Animal products are generally perishable due to microbial actions, and to prevent or lessen these actions low temperature has been widely applied for storage and distribution of animal products. No micro-organisms can grow in frozen states but freezing is not applicable to many animal products, because undesirable influences caused by freezing damage the qualities of the products. Therefore, such perishable products must be kept in unfrozen states. This means that the keeping qualities or shelf-lives of these products depend greatly on the contaminated psychrotrophic micro-organisms as they can grow relatively rapidly at low temperature ranges around 0°C. Thus interests have been paid more and more to the psychrotrophs with the scale up of the production and distribution of animal products. In Japan, low temperature storage and distribution of raw milk have become a common practice, and to improve the qualities of raw milk and to keep them at high levels, it is necessary to obtain information on the psychrotrophic micro-organisms. Therefore, the growth of psychrotrophic organisms in refrigerated raw milk samples[1] and the characteristics of the psychrotrophic bacterial strains isolated from these samples[2] were studied. Besides, microbiological study on refrigerated sliced pork has been carried out as a part of the research project on the irradiation of pork. The results are described here in brief.

Growth of bacteria in refrigerated raw milk

Herd milk produced in the dairy farm of National Institute of Animal Industry was used as the experimental material. Samples of milk were taken from can-milk at the time of reception in the dairy plant of the Institute. Evening milk was immersion-cooled overnight in the can at the farm before receiving. Viable and psychrotrophic counts of the milk samples were determined

Table 1. Initial bacterial counts per ml of can milk samples

Trial No.	Evening milk			Morning milk		
	Viable count (VC)	Psychrotrophic count (PC)	$\frac{PC}{VC}\times 100$	Viable count (VC)	Psychrotrophic count (PC)	$\frac{PC}{VC}\times 100$
1	70×10^4	58×10^3	8.3%	24×10^4	40×10^2	1.7%
2	90×10^3	40×10^2	4.4	20×10^4	67×10^2	3.4
3	16×10^5	18×10^4	11.2	11×10^5	24×10^3	2.2
4	15×10^5	86×10^3	5.7	20×10^4	12×10^3	6.0
5	55×10^4	23×10^3	4.2	85×10^3	10×10^2	1.2
6	11×10^5	12×10^3	1.1	25×10^4	10×10^2	0.4
7	23×10^5	21×10^4	9.1	59×10^4	52×10^2	0.9

with the plating method. Plates for viable counts and for psychrotrophic counts were incubated at 30°C for two days and at 5°C for 10 to 14 days, respectively. The result is shown in Table 1. With the exception of trial No. 2, viable counts of evening milk were 1.5 to 7.5 times higher than those of morning milk, and psychrotrophic counts of evening milk were 7 to 40 times higher than those of morning milk. This indicates that the storage conditions of the evening milk at the farm were inadequate. As the psychrotrophic counts of morning milk were generally in the range of 10^4 per ml, the quality of raw milk may be kept in a good state for a considerable length of time when milk is adequately cooled immediately after milking.

These milk samples were stored at 5°C and 0°C and changes of viable and psychrotrophic counts during the storage were determined. A representative result is shown in Fig. 1. Most of the bacteria present in the milk will survive under this experimental condition but only psychrotrophs can grow, so that the majority of the microflora of the stored samples is occupied by psychrotrophs. Initial flora of psychrotrophs in milk samples may be composed of various kinds of bacteria, but their growth rates may differ each other, so that the flora will become dominated by those having faster growth rate. Therefore, the apparent growth rate of psychrotrophs as a whole may vary along with the change of the microflora. Then, the apparent mean generation time of psychrotrophs in each sample milk was calculated in the range of the count of 10^5 to 10^6 per ml from the growth curve of psychrotrophs such as shown in Fig. 1. The calculated mean generation time is shown in Table 2. The mean generation times of psychrotrophs in raw milk held at 5°C and 0°C were 6.6 to 12.7 hr and 12.3 to 26.1 hr, respectively. At both temperatures, the shortest mean generation time was about a half of the longest. This means that the keeping quality of milk may vary considerably depending on the kinds of psychrotrophic organisms present. The ranges of the mean generation times observed in this experiment are similar to those of single strains of psychrotrophic bacteria in milk at low temperatures reported by Greene and Jezeski[3], and Lawton and Nelson[4]. The

Table 2. Apparent mean generation time of psychrotrophic micro-organisms in refrigerated raw milk

Sample No.*	Initial psychrotrophic count per ml	Apparent mean generation time (hr) 5°C	0°C
1—e	58×10^3	12.7	26.1
1—m	40×10^2	8.9	19.8
2—e	40×10^2	8.4	18.5
2—m	67×10^2	9.6	23.4
3—m	24×10^3	11.4	21.3
4—m	12×10^3	12.3	17.6
5—m	10×10^2	8.9	18.3
6—e	12×10^3	7.3	12.3
6—m	10×10^2	8.6	15.5
7—e	21×10^4	10.5	16.8
7—m	52×10^2	8.0	15.9
8—m	12×10^2	7.5	13.6
9—m	14×10^3	6.6	19.7
10—m	25×10^3	8.7	17.3

* e: evening milk, m: morning milk.

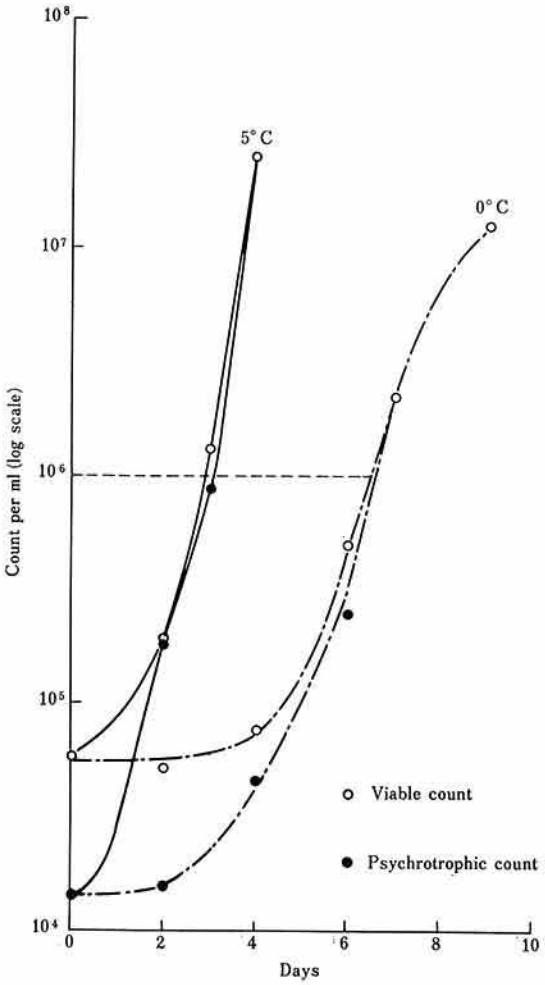

Fig. 1. Growth of micro-organisms in refrigerated raw milk

apparent mean generation time of psychrotrophs in each sample milk at 0°C was two times that at 5°C. From this fact it is expected that the keeping quality of raw milk will be doubled when the storage temperature is lowered from 5°C to 0°C. When the permissible limit of the bacteriological quality of raw milk is tentatively set at the plate count of 10^6 per ml, the keeping quality of these milk samples was calculated 2.0 to 4.9 days at 5°C and 3.9 to 12.7 days at 0°C.

It seems that the keeping quality of refrigerated raw milk has some correlation to the initial viable count rather than the initial numbers of psychrotrophic micro-organisms. This may be explained that the poorer the hygienic conditions of milk production, the more the chance to be contaminated by psychrotrophs having higher growth rate at low temperatures.

From these results it is concluded that the alternate day collection of raw milk can be introduced without any serious problems on the quality of milk, if milk is produced under good hygienic conditions and preserved at the temperature lower than 5°C.

Characteristics of psychrotrophic bacteria isolated from raw milk

Two hundreds and thirty strains of psychrotrophic bacteria were isolated from the above mentioned fresh and cold-stored raw milk samples and some other farm milk samples. Almost all of the isolates, namely, 228 strains were catalase-positive Gram-negative rods while two strains were psychrotrophic Gram-positive cocci. The Gram-

Table 3. Grouping of psychrotrophic Gram-negative rods isolated from raw milk

Group	I—1	I—2	I—3	II	III	IV	V	VI	VII	VIII
Numbers of isolates	160	42	2	2	2	1	1	3	13	2
Cytochrome oxidase	+	+	+	+	+	+	+	—	—	—
Reaction of Hugh-Leifson's medium*	0	0	0	—	0	0	F	—	0	F
Fluorescens or pigment	Fluorescent			—	Yellow	Violet	—	—	Yellow	Yellow
Change of litmus milk	Peptonized	Acid	Reduced	—	—	—	Acid, clot	— or reduced	— or reduced	Acid, clot, slimy

*: 0; oxidative, F; fermentative, —; inactive

negative strains were tentatively divided into eight groups according to cytochrome oxidase test, reaction in Hugh-Leifson's medium, production of pigment or fluorescence, as shown in Table 3. Most of the isolated strains were the members of the group I, and this group is considered to belong to Genus *Pseudomonas*. Group I was further divided into three sub-groups by the change of litmus milk culture. About 80% of the strains in group I showed alkaline reaction and peptonization in litmus milk culture.

From taxonomic standpoint, group I–1 is considered to be *Pseudomonas fluorescens* type, and group III and VII seem to be *Flavobacterium*. Strains of group IV can be identified as *Chromobacterium* according to its deep violet colored colonies. For the other groups it is difficult to identify definitely their generic names. Because of the lack of information on these types of bacteria, the opinions of taxonomists are diverse, and it seems unnecessary at present to classify the groups more precisely.

Growth response to temperature and decomposition of milk constituents were examined on the representative strains of each group. The results are summarized in Table 4. Among these characteristics, actions on milk constituents and growth at low temperature are most important from the standpoint of deterioration of milk and milk products. Organisms of group I and V should be noted that they grow relatively rapid at low temperature and are active against milk constituents. Most of the strains examined failed to grow at 37°C, and their optimum growth temperatures were in the range of 20°C to 30°C, that is, these psychrotrophs are all mesophiles concerning with their optimum growth temperatures. Their mean generation times in pasteurized reconstituted skimmilk were; 6 to 22 hr at 5°C and majority were less than 10 hr, and 13 to 34 hr at 0°C, most were around 20 hr, but some could not multiply at this temperature. Many strains exhibited strong proteolysis and lipolysis against milk casein and milkfat. Some of these strains did not show the lytic actions when they were incubated at 30°C in spite of exhibiting strong actions at lower temperatures.

Two strains of Gram-positive cocci clotted litmus milk after reduction of litmus, did not grow in carbohydrate-free medium, did not produce gas from carbohydrates, and were catalase-negative. They were identified as lactic streptococcus. Their acid production in milk at 20°C or 30°C was rather slow, and the amounts of the acid produced were not so much. Their mean generation times

Table 4. Characteristics of psychrotrophic Gram-negative rods isolated from raw milk

Group	I — 1	I — 2	I — 3	II	III	IV	V	VI	VII	VIII
Numbers of strains examined	6	3	2	1	2	1	1	3	4	2
Optimum growth temperature (°C)	20–30	20–30	25–30	25	20–30	20	20–30	20–30	20–25	30
Mean generation time in skimmilk (hr)										
at 5°C	6–9	6. 5–10	6. 5–10	7	10–17	8	7–8	8–20	6–16	14–22
at 0°C	14–24	15–19	13–22	21	21–34	20	17–22	22–∞	15–23	33–∞
Growth at 37°C	—	—	—	—	—	—	+	—	—	+
Proteolysis of milk casein	+*	+*	—	—	—	+	+	—	1/4**	—
Lipolysis of milkfat	+*	2/3*	—	+	—	+	+	+	—	1/2

Note: + growth or positive, — no growth or negative.
* some strains were negative at 30°C.
** numbers of positive strains/numbers of strains examined.

Table 5. Change of viable counts of psychrotrophic Gram-negative rods in tap water

Group	I − 1	I − 2	I − 3	V	Control	
Strain No.	121	238	221	675	*E. coli*	*Str. faecalis*
Initial count/ml	94×10^2	23×10^2	64×10^2	27×10^2	68×10^2	19×10^2
after 24 hr.	14×10^5	(2)	25×10^4	51×10^4	140	17×10^2
after 48 hr.	22×10^5	<1	12×10^5	12×10^5	16	20×10^2
after 3 days	35×10^5		89×10^5	13×10^5	<1	
after 7 days	38×10^5		12×10^6	87×10^4		
after 10 days	34×10^5		10×10^6	13×10^5		
after 20 days	13×10^5		68×10^5	11×10^5		

in milk at 5°C was 12 to 20 hr, and 26 to 60 hr at 0°C.

Strains of psychrotrophs which showed strong actions on milk constituents were generally more resistant to various chemicals. Some strains could grow in the presence of; crystal violet 10 ppm, penicillin-G potasium salt 10 mg/ml, dihydrostreptomycin sulfate 10 mg/ml, chlortetracycline 20 ppm, and 0.4% of sorbic acid at pH 6.2. Some strains survived after exposure to hypochlorite solution containing 100 ppm available chlorine for 5 min or chloramine T solution containing 200 ppm available chlorine for 15 min.

These psychrotrophic strains were not heat resistant. Their decimal reduction times at 63°C were 1.0 to 3.8 min, and strains of group I were less heat resistant. Their D_{63} values were 1 to 2.3 min. From these D-values, none of them will survive for heating at 63°C for 30 min in so far as there is no heavy contaminations.

Behaviour of psychrotrophs in water

Four strains of psychrotrophic bacteria isolated from raw milk and one strain of each of *Escherichia coli* and *Streptococcus faecalis* as controls were used in the experiment. Broth cultures of these strains were diluted with sterilized tap water in the ratio of 1:100,000, and allowed to stand at room temperature (around 20°C). Changes of viable counts of the experimental strains are shown in Table 5. Only one strain among four psychrotrophs showed decrease in viable count in a manner similar to *E. coli* strain. The other three psychrotrophic strains showed definite increase in viable counts, which reached to the level of 1,000,000 per ml in less than 48 hr. This level of viable counts was kept for more than 20 days thereafter. It is well known that psychrotrophic bacteria have generally rather simple nutritional requirements, and it is no wonder that some of them can multiply to some extent under this condition. However, the fact that they can survive for long time in water should be noticed and such behaviors of psychrotrophs in water will give some important suggestions in preventing the contamination of animal products by psychrotrophic micro-organisms.

Psychrotrophic micro-organisms in pork

Sliced pork samples were irradiated by γ-ray and then stored under refrigeration together with unirradiated control samples. Micro-organisms developed on the surface of the sample meat were observed. When the meat samples were stored in closed containers with air, Gram-negative rods usually developed and slimes were observed in unirradiated control samples, while in irradiated meat, sometimes yeasts developed on the surface of meat, and pungent odor was observed in this case.

When the pork samples were stored under vacuum-packed state, most of the micro-organisms developed during cold-storage

were Gram-positive rods. These organisms seem to be lactobacilli of streptobacteria-type.

References

1) Yano, N., Morichi, T. & Kembo, H.: Growth of psychrotrophic bacteria in refrigerated raw milk. *Bull. Nat. Inst. Anim. Ind.*, 28, 41–46 (1974). [In Japanese, English summary].
2) Yano, N. & Morichi, T.: Psychrotrophic bacteria isolated from raw milk. *Bull. Nat. Inst. Anim. Ind.*, 28, 47–58 (1974). [In Japanese, English summary].
3) Greene, V. W. & Jezeski, J. J.: Influence of temperature on the development of several psychrophilic bacteria of dairy origin. *Appl. Microbiol.*, 2, 110–117 (1954).
4) Lawton, W. C. & Nelson, F. E.: The effect of storage temperatures on the growth of psychrophilic organisms in sterile and laboratory pasteurized skimmilk. *J. Dairy Sci.*, 37, 1164–1172 (1954).