PSYCHROPHILIC BACTERIA—A SANITATION PROBLEM[1,2]

R. B. PARKER, A. L. COLDWELL,* AND P. R. ELLIKER

Agricultural Experiment Station, Oregon State College, Corvallis, Oregon

The Weber and Black method for evaluating germicides was used to compare the efficiency of hypochlorite and quaternary ammonium compounds in the destruction of representative psychrophilic bacteria isolated from defective milk products.

Under conditions of the test, hypochlorites displayed a more rapid germicide action than quaternary ammonium compounds against suspensions of the psychrophilic bacteria studied.

The germicidal action of a quaternary ammonium compound was markedly stimulated by the addition of certain complex phosphate salts.

Centralization of dairy processing plants, with the attendant long storage periods of product, has posed a number of definite sanitation problems, especially those with respect to psychrophilic bacteria. Elimination of organisms capable of extensive growth at common refrigeration temperatures is essential if the product is to withstand storage. This fact must be considered in the design of any sanitation practice where these organisms are likely to be encountered.

Reports from various sources have indicated that pasteurized milk and cream, if consumed within a reasonable period of time, are not adversely influenced by psychrophilic bacteria, provided post-pasteurization contamination has been minimized. Burgwald and Josephson[1] concluded that milk of good quality could be expected to retain its bacteriological and flavor qualities for at least four days during summer months and six to seven days during winter months. The findings of Rojeck and Burgwald[9] indicate that most psychrophilic types are eliminated by pasteurization, and their presence in pasteurized milk is associated with improperly sanitized surfaces. Canadian workers[4,5] noted that, while objectional flavors were produced in milk by all of 722 bacterial strains capable of growth at 12.5°C, only four of the total survived pasteurization. Other studies have indicated similar heat sensitivities of various psychrophilic species.

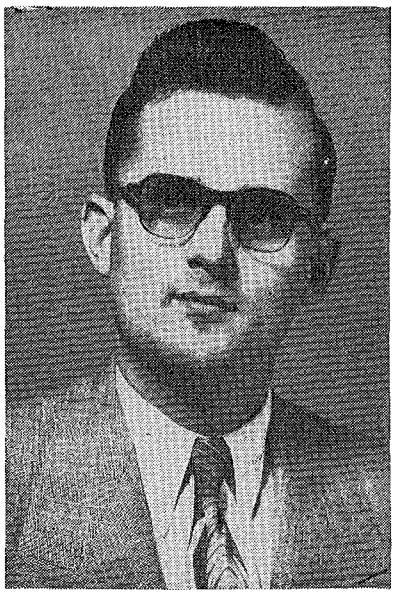
It is significant that not one of the 722 isolations mentioned previously were capable of growth at 35.5°C. Jezaski and Macy[6] incubated platings of creamery water and butter at 8, 20, and 37°C, respectively. Invariably, the plates incubated at 20°C yielded the highest counts. In some instances the plates incubated at 8°C yielded higher counts than those incubated at 37°C. Apparently the so-called "Total Plate Count" may in some instances represent a gross underestimation of the viable bacteria present. Even more important is the consideration that bacteria involved in product deterioration may remain undetected by Standard Methods.

Various species of *Flavobacterium, Achromobacter,* and *Alcaligenes* are encountered in refrigeration spoilages, but of all psychrophilic types, those representing the genus *Pseudomonas* are the most troublesome. Erdman and Thornton[5] found an intense bitter defect in milk held at 4.5°C for 15 days. All of the nine cultures found to produce the defect were identified as belonging to the tribe *Pseudomonadeae.* Three were not further classified, but three of the remaining were identified as variants of *Pseudomonas fluorescens,* and three closely resembled *Pseudomonas viscosa.*

The production of "surface taint" on butter by *Pseudomonas putrefaciens* is another example of one of the more offensive spoilages produced by this genus. The typical, cheesy, putrid, or rabbito flavor may appear within a few days in butter held at 50°C. Ripening of the cream with a lactic culture after pasteurization may allow production of sufficient acid to limit growth of the casual organism. In some localities, the defect has been so common as to constitute a serious problem. A New Zealand worker[10] was able to demonstrate the presence of *P. putrefaciens* in 91 out of 1,563 samples of butter. There appeared to be a rather close relationship between the presence of the organism and poor sanitation in the processing plant.

Elliker and Horrall[3] reported that butter became distinctly flat prior to production of the definite "surface taint." They were able to show that the flatness corresponded to destruction of biacetyl. In a later study, Elliker[2] found that a number of other related psychrophilic types affected rapid destruction of the aroma compound.

Pasteurization studies indicate *P. putrefaciens* to be very heat sensitive. Long and Hammer[7] found that even the more resistant forms of this species were destroyed by



Mr. Parker served in the U. S. Army Air Corps, 1943-46. He attended Boise Jr. College, 1947, received his B. S., Oregon State College, June, 1950, and his M.S. in 1951. He now is Research Assistant, Oregon Agricultural Experiment Station, 1950. Recipient 1950 Phi Sigma award. Sigma Xi research honorary.

[1]Paper presented at the 16th Annual Klenzade Educational Seminar, Excelsior Springs, Missouri, March 20-22, 1952.

[2]Published as Technical Paper No. 747 with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution of the Department of Bacteriology.

*Present address: Langlois Cheese Makers, Langlois, Oregon.

an exposure to 61.7°C for 2 minutes.

Various other *Pseudomonadeae* are responsible for rancid, fruity, fishy, and a variety of other defects in butter. *Pseudomonas fluorescens* and *Pseudomonas fragi* are particularly noted for production of rancidity.

Recently two species of the genus *Pseudomonas* and one of the genus *Alcaligenes* have been shown to be responsible for a rapid and often intense surface spoilage of cottage cheese[8]. The nature of the defect may vary from a brownish- or yellowish-tinged slime to a white, translucent film. The physical manifestations may be accompanied by rotten, putrid, or fruity odors. Descriptive terms such as "tapioca," "glassy," and "slick" have been used by the trade. Here, as in the surface spoilage of butter, an extreme flatness or lack of aromatic flavor often precedes the physical spoilage. Three different species of bacteria are associated with the defect in its various forms. The darker spoilage is accompanied by rotten or putrid odors and is caused by an organism identified as *Pseudomonas viscosa.* A lighter-colored defect accompanied by fruity odors is attributed to *Pseudomonas fragi.* The organism causing an almost clear whitish spoilage is classified as *Alcaligenes metalcaligenes.* Pasteurization studies indicate that all three are killed by heating at 143°F for 30 minutes.

Studies on the effect of pH on extent of the gelatinous or slimy defect in uncreamed, unsalted curd indicate that *P. fragi* and *A. metalcaligenes* are effectively retarded at pH 5.0, but *P. viscosa* continues to develop until pH 4.8 is reached. Although exceptions may follow variation in composition of milk, experience has indicated that milk ripened to a titratable acidity of at least 0.55 percent before cutting usually will have a pH of 4.8 or lower. Increase in pH due to creaming will usually vary within a range of 0.2 to 0.35 of one pH unit.

Studies on effect of pH and salt in creamed curd indicate that *P. viscosa* again appears the most difficult to control. This organism is not limited at salt concentrations of less than 2.5 percent until pH is lowered to 4.8. Both *P. fragi* and *A. metalcaligenes* are retarded at pH 5.0 regardless of salt concentration. *P. fragi* is the least salt tolerant, being limited at 2.5 percent salt at pH 5.2. All three species are capable of growth in cheese at pH 5.2 with 2 percent salt content.

Growth of *P. viscosa* produces a greenish yellow pigment which fluoresces strikingly under long-wave ultra violet light (Mineralite or Black Light). Early detection of the defect through keeping quality tests at 15°C is greatly facilitated by the use of this device. Specks of beginning spoilage caused by *P. viscosa* can be seen in this manner when they are still invisible in ordinary light.

The long-wave ultraviolet light also has been of great help in rapid detection of milk stone deposits, especially those embedded in nicks and scratches on cottage cheese manufacturing and packaging equipment. These deposits have been shown to harbor the cottage cheese spoilage bacteria.

Recommendations for control of the above type of spoilage include ripening the milk during setting to a titratable acidity of at least 0.55 percent and maintaining a pH of not over 5.0 in the finished creamed curd. A salt concentration of at least 1.5 percent is also a helpful control measure. Unfortunately acidities and salt concentrations high enough to completely eliminate *P. viscosa* are objectionable to many consumers. Thus, the only satisfactory method of control involves strict plant sanitation to eliminate completely any spoilage bacteria.

COMPARATIVE EFFECT OF QAC AND NaOCl ON VARIOUS PSYCHROPHILIC SPECIES

The extensive use of quaternary ammonium germicides (QAC) and hypochlorites in dairy sanitation suggested an investigation into the relative merits of these compounds against several important psychrophilic species. All organisms used in the study were originally isolated from some form of defective milk product. Organisms used were *P. viscosa, P. fragi, P. fluorescens, P. putrefaciens,* and *A. metalcaligenes.* The Weber and Black technique for evaluating practical performance of germicides was used in all trials[11]. Alkyl dimethyl benzyl ammonium chloride was selected as the QAC, along with one of the representative commercial sodium hypochlorites. Activity of hypochlorite was determined at pH 9.5 and activity of QAC at pH 7.2.

To evaluate the effectiveness of the compounds under conditions simulating those of water sanitation, low concentrations of germicide were used against an approximate concentration of 20,000 bacteria per ml. Germicide concentrations of 5, 10, 20, and 30 ppm, respectively, were used, and the results of trials with 10 ppm are presented in table 1.

The various psychrophilic species employed displayed a wide range of resistance to 10 ppm QAC. Destruction after 30 seconds exposure at this concentration ranged from about three-fourths destruction of *P. viscosa,* to complete destruction of *P. fragi* and *A. metalcaligenes.* It is notable that the latter two organisms were the only species completely eliminated after the full 300 seconds exposure to 10 ppm QAC. In decreasing order of resistance to QAC, the respective organisms are: *P. viscosa, P. fluorescens, P. putrefaciens, A. metalcaligenes,* and *P. fragi.* All species were effectively eliminated by hypochlorite within the initial 15-second exposure period.

Although not tabulated in this paper, similar gradations of resistance were exhibited by the various species of the other QAC germicide concentrations used. A more outstanding example of hypochlorite activity is presented in data which are also untabulated for the sake of brevity. The data show that all species used in the study were destroyed by a 15-second exposure to 5 ppm hypochlorite.

Table 2 indicates results of a study on the effects of high concentrations of germicide against high concentrations of bacterial cells. It was felt that these conditions more closely approximate those found in equipment sanitation. The data indicate complete inactivation of all strains by a 15-second exposure to 100 ppm hypochlorite germicide. However, when QAC was used as

TABLE 1—EFFECT OF 10 PPM QAC OR NaOCl ON LOW NUMBERS OF VARIOUS PSYCHROPHILIC SPECIES

Germicide	Exposure time	Percent destruction of the following species: *P. viscosa*	*P. fluorescens*	*P. putrefaciens*	*P. fragi*	*A. metalcaligenes*
	seconds	*% kill*	*% kill*	*% kill*	*% kill*	*% kill*
QAC	15	74.1	85.3	97.2	99.8	99.7
	30	76.3	96.5	99.0	100	100
	60	79.6	97.5	99.6	100	100
	120	85.5	98.5	99.8	100	100
	300	88.8	99.3	99.9	100	100
NaOCl	15	100	100	100	100	100
	30	100	100	100	100	100
	60	100	100	100	100	100
	120	100	100	100	100	100
	300	100	100	100	100	100
No. trials		3	2	3	2	2
No. organisms exposed to germicide		13×10^3	12×10^3	12×10^3	12.5×10^3	10.5×10^3

QAC alkyl dimethyl benzyl ammonium chloride

TABLE 2—EFFECT OF 100 PPM QAC OR NaOCl ON HIGH NUMBERS OF VARIOUS PSYCHROPHILIC SPECIES

Germicide	Exposure time	Percent destruction of the following species: *P. viscosa*	*P. fluorescens*	*P. putrefaciens*	*P. fragi*	*A. metalcaligenes*
	seconds	*% kill*	*% kill*	*% kill*	*% kill*	*% kill*
QAC	15	99.9	99.9	99.9	100	99.9
	30	99.9	100	99.9	100	99.9
	60	100	100	99.9	100	100
	120	100	100	99.9	100	100
	300	100	100	100	100	100
NaOCl	15	100	100	100	100	100
	30	100	100	100	100	100
	60	100	100	100	100	100
	120	100	100	100	100	100
	300	100	100	100	100	100
No. trials		3	2	3	2	2
No. organisms exposed to germicide		8×10^7	8.6×10^7	10.5×10^7	9×10^7	8×10^7

QAC alkyl dimethyl benzyl ammonium chloride

the germicide, a full 300-second exposure period to the same concentration was required to eliminate *P. putrefaciens*. For other species a shorter period sufficed, but only *P. fragi* was destroyed by a 15-second exposure to 100 ppm QAC. At this concentration, *A. metalcaligenes* showed slightly greater resistance to QAC action than at lower concentrations, and it is believed that the slime coat of this organism may serve as a protective mechanism.

Results with QACs on psychrophilic bacteria led to attempts to accelerate or potentiate their germicidal activity. It was observed that distilled or soft water solutions of detergent-sanitizers produced more rapid germicidal action than the constituent QAC alone. This suggested one or more of the detergent compounds in the preparation to be the potentiating agent. Various common detergent agents were investigated for their potentiating effect. The polyphosphates were found to be the active ingredient and one illustration of the effect of simple and complex phosphate salts on quaternary activity is presented in table 3. Results with the simple phosphate are included to provide an example of effect of detergent ingredients other than complex phosphate. Some unpublished reports also have attributed potentiating activity to simple as well as complex phosphate. A pH of 9.5 was chosen, as previous studies had demonstrated increased activity as the alkalinity increased. Also the pH of several of the more representative detergent sanitizers is near this level. Distilled water was used in making up the buffer to eliminate interference by water minerals. Tests were made using the buffer alone, the buffer plus trisodium phosphate, and the buffer plus tetrasodium pyrophosphate.

As is indicated in table 3, the addition of 0.1 percent trisodium phosphate to the buffered solution of QAC did not noticeably increase activity over that obtained with the buffer alone. Addition of the pyrophosphate, however, produced a substantial increase in germicidal activity. For example: with either borate buffer, or borate buffer plus TSP, a period of 60 seconds was required for complete kill of

TABLE 3—EFFECT OF ADDITION OF PHOSPHATE SALT TO QAC ON THE RATE OF DESTRUCTION OF A 24-HR. CULTURE OF P. VISCOSA

Phosphate added to QAC	Time of exposure	Percent destruction with the following concentration of germicide	
		50 ppm	100 ppm
	seconds	*% kill*	*% kill*
None	15	99.6	99.9
	30	99.8	99.9
	60	100	100
0.1% tri sodium phosphate	15	99.6	99.9
	30	99.9	99.9
	60	100	100
0.1% tetra sodium pyrophosphate	15	99.9	100
	30	100	100
	60	100	100

QAC alkyl dimethyl benzyl ammonium chloride

P. viscosa with either 50 or 100 ppm of QAC. When 0.1 percent pyrophosphate was added, only 30 seconds were required for complete kill with 50 ppm QAC, and only 15 seconds when 100 ppm QAC was used. In spite of this marked potentiation, an active hypochlorite preparation appears superior to QACs for destruction of psychrophilic bacteria in water and on equipment. Further studies on potentiation of QACs under hard water conditions will be reported in another paper.

SUMMARY

It is generally agreed that the more important psychrophilic species are eliminated in pasteurization. Thus, for the most part the control of these types seems to resolve itself into a program of efficient sanitation of water and plant equipment. For pasteurized products handled in closed equipment (such as fluid milk and cream), elimination of spoilage bacteria is not difficult. Products such as butter and cottage cheese, however, constitute a more serious problem as they require washing, working, and some degree of manual manipulation with equipment which is often difficult to sanitize.

The results presented in this paper very definitely favor the use of hypochlorite compounds for destruction of psychrophilic bacteria in water and on equipment. It is doubtful, however, that either QAC or hypochlorite would prove effective if employed on a dirty surface. A long wave ultra violet light is recommended for the general detection of milkstone and especially for deposits left in nicks and scratches. The importance of removing these deposits cannot be over-emphasized. They constitute a potential source of all types of organisms and are almost impossible to sanitize. Even 200 ppm hypochlorite is ineffectual when bacteria are protected with a milkstone film.

Practically all psychrophilic species are common soil and water types, and evidence will frequently point to these as the original sources of contamination in instances where refrigeration spoilage is encountered. The chilled water used in washing butter and cottage cheese has proved particularly troublesome in this respect. These data indicate that a residual of 5 to 10 ppm available chlorine will effectively eliminate psychrophilic species unless they are imbedded in a slime layer. The presence of high levels of organic matter in water should also interfere with hypochlorite action, but in this event the

(Continued on page 152)

PUBLIC HEALTH ASPECTS

(Continued from page 148)

farms was colder than those in milk plants.

The bacterial counts for pasteurized milk ran:

Percentage of raw count:	2 percent
Below 30,000 per ml	91 percent
Below 20,000 per ml	86 percent
Logarithmic average on all counts	5,980 per ml
Coliforms in	less than 1* per ml
Psychrophils	1* per ml

(*recontamination after pasteurization)

The report recommends that health departments make laboratory tests of pasteurized milk at least once a week, and further states that testing should include temperature, sediment, bacterial plate count, coliform count, and the phosphatase test. Analyses for butterfat and specific gravity should also be made, and when the addition of water is suspected the total solids and freezing point should be determined.

Inspections of milk plants in each city revealed violations of what are considered to be essential sanitary requirements.

Tests in pasteurized milk showed that it kept well for four days when properly refrigerated—ample for every-other-day or three-times a-week delivery. However, when kept at 44°F for 7 days, off-flavors and bacteria developed. No relationship was observed between the sanitary conditions of milk production on the farm and the keeping quality of the pasteurized milk.

Sale of raw milk was prohibited in six out of the eight cities studied. Six cities limited sales to only one grade, and only one grade was sold in seven cities.

This study showed that those dairy farms subjected to the most detailed regulations and rigid enforcement procedures produced milk of the best sanitary quality, as measured by its bacterial content. However, the findings indicate that this was due to strict enforcement of a limited number of essential requirements. While many other detailed requirements might be useful, the study concluded that since they are not essential they should be recommended rather than required.

EFFECT OF SELECTED ANTIBIOTIC

Continued from Page 127

substitute for unsanitary practices. There is no particular advantage in adding these compounds to a good quality dairy product.

REFERENCES

1. Curran, H. R., and Evans, F. R., The Activity of Penicillin in Relation to Bacterial Spores and the Preservation of Milk. *J Bact.*, 52, 89-98, (1946).

2. Foley, E. J., and Byrne, J. V., Penicillin as an Adjunct to the Preservation of Quality of Raw and Pasteurized Milk *J. Milk and Food Technol,* 13, 170, (1950).

A SANITATION PROBLEM

Continued from Page 139

problem would be so serious as to justify suitable modifications in the water supply system. A thorough cleaning of water tanks and lines may be required in some instances.

LITERATURE CITED

1. Burgwald, L. H. and Josephson, D. V. The Effect of Refrigerator Storage on the Keeping Qualities of Pasteurized Milk. *J. Dairy Sci.* 30, 371-383 (1947).

2. Elliker, P. R. Effect of Various Bacteria on Diacetyl Content and Flavor of Butter. *Ibid* 28, 93-102 (1945).

3. Elliker, P. R. and Horrall, B. E. Effect of Growth of *Pseudomonas putrefaciens* on Diacetyl and Flavor of Butter. *Ibid* 26, 943-949 (1943).

4. Erdman, I. E. and Thornton, H. R. Psychrophilic Bacteria in Edmonton Milk and Cream. I. Numbers. *Can. J. of Technology* 29, 232-237 (1951).

5. *Ibid.* II. Kinds. *Loc. cit.* 29, 238-242.

6. Jezeski, J. J. and Macy, H. Cryophillic Organisms in Water and Butter. *J. Dairy Sci.* 29, 439-452 (1946).

7. Long, H. F. and Hammer, B. W. Classification of the Organisms Important in Dairy Products. III. *Pseudomonas putrefaciens. Iowa Agri. Res. Bul.* 285. (1941).

8. Parker, R. B., Smith, V. N., and Elliker, P. R. Bacteria Associated with a Gelatinous or Slimy Curd Defect of Cottage Cheese. *J. Dairy Sci.* 34, 887-893 (1950).

9. Rojick, F. A. and Burgwald, L. H. A Study on Psychrophilic Bacteria in Market Milk. *Loc. cet.* 33, 403 (1950). (Abstract).

10. Thomson, G. I. *Pseudomonas putrefaciens* in New Zealand Butter. *J. Dairy Res.* 17, 66-71 (1950).

11. Weber, G. R. and Black, L. A. Laboratory Procedure for Evaluating Practical Performance of Quaternary Ammonium and Other Germicides Proposed for Sanitizing Food Utensils. *Amer. J. Pub. Health,* 38, 1405-1417 (1948).

CONNECTICUT ASSOCIATION OF DAIRY AND FOOD SANITARIANS

Our Association now has 265 members, and all memberships must include affiliate membership in the International and obviously subscriptions to the Journal.

At our coming annual meeting in