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Abstract

A yellow film of milkstone developed on a laboratory system that simulated unclean stainless steel surfaces. Cursory washing or presoiling with phospholipids and subsequent growth of *Pseudomonas* sp. in a milk film produced the yellow color in 1 to 2 days. Sequence of treatments, microenvironmental conditions, and microbial growth contributing to the formation of the yellow film were determined. *Pseudomonas* sp. was isolated from laboratory developed yellow films and from milk soil deposits on farm equipment. The overall requirements for the production of yellow films were: a) inadequate cleaning or a presoil of phospholipids, b) growth of *Pseudomonas* sp., c) high bacterial population, and d) available water.

Introduction

Cleanliness of food processing equipment surfaces commonly is visually evaluated. Soil (visible residue) on equipment surfaces may include food residues, cleaning agent residues, and residues from rinse water. According to Bourne and Jennings (4) and Hucker (7), soil is held to equipment surfaces by strong adhesive bonds between soil and surface and other cohesive bonds within the soil residue.

Both adhesion and cohesive bonding depend on the chemical nature of the soil. Fat-containing foods form a monomolecular layer on the surface of equipment which is difficult to clean (4). Fatty materials are most difficult to remove in circulation cleaning (1). In cleaning there is selective removal of the constituents. Least tenaciously absorbed constituents are removed first. Continued interaction of detergent and soil depletes detergent with increased challenge to remove the final residue. Variability in cleaning contributes to buildup of heterogeneous soil. Residues are indeed variable when cursory cleaning allows buildup to a visible film.

With repeated cursory washing, a visible yellow film (commonly termed "milkstone") may develop on dairy farm equipment where heat processing is not involved. This condition is associated with high bacterial counts from the equipment, but little is known of the nature of the soil. There is also belief that milkstone is associated with heating equipment and that it harbors mainly thermoduric organisms (6). The relationship between these two types of milkstone is not known.

The purpose of this study is to explore the role of the major constituents of milk, the interaction of bacteria, and the microenvironmental conditions that permit formation of a yellow film on dairy equipment.

Materials and Methods

Source of raw materials. Samples of whole Grade A mixed raw milk were collected from the dairy plant of the department of food science and technology, University of Nebraska. The standard plate count (12) was always less than 200,000 per ml. Homogenized, packaged, pasteurized milk was obtained from the above source. Butter was obtained from a large commercial dairy. Grade A eggs were obtained from the department of poultry science, University of Nebraska. All samples were stored at 5 C until used.

Skimmilk was prepared by reconstituting low heat nonfat dry milk solids to 10% w/v and then sterilized by autoclaving at 121 C for 12 to 13 min.

Sources of fat. Various sources of fat and methods of preparation of samples were used to see if the fat globule membrane material had a role in yellow film formation. The materials were prepared as follows:

a. *Butteroil.* Butter was melted at 45 C and filtered through cotton to separate oil from serum and phospholipids.

b. *Acid separated fat.* Babcock fat test, as described by Newlander et al. (11), was performed on whole Grade A mixed raw milk thereby separating milk fat from phospholipids. Milk fat as needed was then removed from the neck of the test bottle.

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c. *Solvent extraction and fractionation of milk fat.* Milk fat was extracted from fresh raw milk by reagents recommended in the Roese-Gottlieb procedure, later adopted for the Mojonnier fat test (11). After extraction, the solvent fraction was treated with anhydrous granular sodium sulfate, allowed to stand 30 min, and decanted into a rotary evaporator (Rinco Instruments Co., Inc.) for solvent removal.

The solvent extracted fat was dissolved in ether and phospholipids were precipitated by acetone (8). This process was repeated 4 to 5 times. The washings were collected and concentrated on a rotary evaporator.

Another fractionation was by the method of Acosta et al. (2), whereby a small amount of solvent extracted fat was dissolved in a chloroform-methanol 2:1 (v/v) mixture. Approximately 2 ml of the chloroform-methanol fat mixture (no quantification intended) was added to a silicic acid column. The column was eluted with anhydrous chloroform to collect neutral fats and with chloroform-methanol 2:1 (v/v) mixture to collect phospholipids. The fractions were concentrated using a rotary evaporator, flushed with nitrogen, sealed, and stored in a freezer.

d. *Lecithin from egg.* Lecithin was extracted from eggs according to the method of MacFarlane et al. (9). Three egg yolks were washed with distilled water, emulsified in 50 ml of .9% sodium chloride, and extracted 5 times with 50 ml volumes of ether. The lecithin was recovered through repeated washing with ether and precipitation with acetone. To prevent oxidation, the flask containing lecithin was flushed with nitrogen, sealed, and stored in a freezer.

Control of humidity. Relative humidities of 80 and 100% were obtained using a modification of the method of Winston et al. (13) as described in a previous paper (10).

Evaluation of growth. While the details are given in a previous paper (10), an inoculum of .01 ml of raw milk on stainless steel squares was incubated at 25 C and removed with sterile phosphate buffer (12) for enumeration of the microorganisms. The final count was expressed as .01 ml of initial sample.

Results

Developing a yellow film. Numerous experiments with highly varied conditions were carried out to simulate cursory washing and storage in order to produce a yellow film of milkstone similar to the color of a grapefruit. This film was judged by sanitarians to look and smell like the yellowish film on improperly cleaned dairy farm utensils. The simplest reliable procedure involved the following sequence of treatments. A film of raw milk on a stainless steel square was incubated in 100% relative humidity for 24 hr, followed by rinsing in cold sterile distilled water. After cold water rinsing, the test square retained a barely visible film which constituted a presoiling without color for subsequent yellow film development. Another layer of raw milk was added and allowed to incubate in 100% relative humidity. With only one presoiling and subsequent raw milk layer, a yellow film developed within 2 to 3 days. Presoiling was necessary to produce a yellow film.

Fatty substance as a presoil in the production of a yellow film. Stainless steel squares (in triplicate) were presoiled with acid separated fat, solvent extracted fat, or butteroil, then followed by a raw milk layer. The test squares were then incubated in 100% relative humidity and observed visually for up to 5 days (Table 1). Test squares with the prelayer of solvent extracted fat commonly developed the yellow film in 1 to 2 days, while the other test squares produced no yellow film in 5 days.

One apparent difference in types of fat was the phospholipid content. Thus, solvent extracted milk fat was fractionated to obtain phospholipids. A very thin film of phospholipid (approximately .1 mg) was placed on test squares to simulate a presoil. A similar treatment was given test squares using the non-phospholipid fraction of solvent extracted milk fat. A subsequent layer of raw milk was then added to each of the squares. The test squares were incubated in 100% relative humidity and observed daily (Table 2). Within 1 to 3 days, all samples with phospholipid presoiling produced a yellow film. Those without phospholipid in the presoil

TABLE 1. Effect of various kinds of fat presoils and a subsequent layer of raw milk on development of yellow film in 100% relative humidity.

Type of fat	Appearance of film
Control (no presoil)	No change
Solvent extracted	Yellow in 1-2 days
Acid separated	No change
Butteroil	No change

TABLE 3. Relation between bacterial population and production of yellow film.

Time	Presoil fat + whole raw milk		Presoil fat + skimmilk	
	Appearance of film	Count/ml[a]	Appearance of film	Count/ml
(hr)				
24	No yellow	2.7×10^{10}	No yellow	2.8×10^{8}
48	Yellow	2.4×10^{10}	No yellow	9.7×10^{9}
72	Yellow	7.2×10^{10}	Yellow	8.5×10^{10}

[a] Results expressed as the original .01 ml of initial sample.

showed no apparent change. Lecithin from egg yolk produced a yellow color whereas the non-lecithin fraction did not.

Bacterial contribution in producing a yellow film. Stainless steel squares were presoiled with solvent extracted fat and then treated with sterile, homogenized milk and with raw milk. During incubation in 100% relative humidity, a yellow film developed within 1 to 2 days with raw milk while none appeared in 5 days with sterile milk.

Another approach to the evaluation of microbial contribution to a yellow film was by comparing raw and pasteurized milk. Samples were taken directly from the vacuum tank of a pasteurizing system, attempting to avoid post-pasteurization contamination. Using solvent extracted milk fat as a presoil and incubation in 100% relative humidity, the raw milk developed a yellow film in 1 to 2 days while the pasteurized milk did not in 5 days.

A yellow film was detected sooner on test squares with homogenized milk than with skimmilk. The final color was also more intense with homogenized milk. Since neither pasteurized milk nor sterile milk produced a yellow film, natural color pigments were apparently without effect.

To determine the causative organisms, plate count agar was used to recover the microorganisms from yellow films. A great variety of colonies were subcultured and inoculated into sterile skimmilk and sterile homogenized milk for study on test squares. Organisms producing a yellow color within 5 days belonged to the genus *Pseudomonas* (5).

Population density associated with a yellow film. Test squares presoiled with solvent extracted milk fat were inoculated with raw milk and incubated at 100% relative humidity to maximize growth and at 80% relative humidity to provide moisture, yet below the water activity required for growth of gram-negative organisms (10). Within 5 days a yellow film developed at the relative humidity of 100% but none at 80%.

To determine the relation between bacterial population and yellow film, test squares presoiled with solvent extracted milk fat were inoculated with raw milk or reconstituted skimmilk and incubated in 100% relative humidity. Soil was recovered from representative test squares after 24, 48, and 72 hr for evaluation by standard plate counts. An average of three trials (Table 3) indicated that a high bacterial population and time were required to produce a yellow film. In whole raw milk film, essentially the maximum population was reached in 24 hr, which was before the yellow color was apparent.

Rinsing as a factor in production of a yellow film. To determine the effect of rinsing, approximately .01 ml of raw milk was applied to each square, followed by incubation for 24 hr in 100% relative humidity. The test squares were then rinsed with distilled or tap water at either 5 or 82 C. Visible water was removed

TABLE 2. Comparative effect of fractions of fat as a presoil in production of yellow film in 100% relative humidity.

Type of presoiling	Visual observation
Column fractionated phospholipids	Yellow film in 1-3 days
Non-phospholipid fraction	No yellow film produced
Ether-acetone fractionated phospholipids	Yellow film in 1-3 days
Non-phospholipid fraction	No yellow film produced
Lecithin (from egg)	Yellow film in 1-3 days
Non-phospholipid fraction	No yellow film produced

by blotting with a paper towel and another film of raw milk was added. After incubation, all of the samples turned yellow in 2 to 3 days. Thus, neither water hardness nor rinse water temperature appeared to be a factor in the production of a yellow film.

Yellow film in field conditions. Samples of soil with a yellowish cast were collected from raw milk handling equipment (milker inflations, bulk tank valves, and vacuum hoses). Plate counts were made and isolates were taken for subsequent pure culture observations. Pure cultures were added to homogenized milk and placed on test squares presoiled with solvent extracted fat. Many of these isolates contributed to yellow film formation. These organisms appeared identical to those previously isolated from laboratory produced yellow film.

Discussion

Laboratory conditions of presoiling to produce a yellow film were similar to dairy farm conditions of cursory washing and subsequent accumulation of yellowish milkstone. Phospholipids remained after the cursory washing and contributed to accumulation of additional soil, which nurtured subsequent growth of bacteria. The requirement of a presoil surface film agrees with the results of Barnhart et al. (3) who reported a residual film that interacted with subsequent milk (soil) and retarded the loss of moisture, thereby permitting growth of microorganisms. The function of phospholipid was perhaps related to their dipolar nature, influenced adsorption to the stainless steel, and subsequent interaction with other soil constituents.

The major factors involved in producing a yellow film are a) inadequate cleaning to yield a presoil containing phospholipid, b) growth of *Pseudomonas* sp., c) high population density, and d) available water. Thus, it would appear there is an interaction between the microorganisms and the presoiling film. Natural color compounds apparently do not contribute to a yellow film.

It is recognized that the work reported here deals with only one type of milkstone, which is commonly related to cursory cleaned dairy utensils. The relationship, if any, between this problem and milkstone associated with heat processing equipment and harborages of thermoduric contaminants (6) is not known.

These observations deal with conditions of visibly detectable soil and gross contamination. Perhaps a more important consideration for future work is the chemical and microbiological conditions prior to the appearance of a visible film. Further study on the interaction of *Pseudomonas* sp. and the presoil film should also be rewarding.

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References

(1) Arnold, R. G., and R. B. Maxcy. 1970. Evaluating circulation cleaning by analysis of soil depletion from surfaces. J. Dairy Sci., 53:1540.

(2) Acosta, S. O., W. W. Marion, and R. H. Forsythe. 1966. Total lipids and phospholipids in turkey tissue. Poultry Sci., 45:169.

(3) Barnhart, H. M., Jr., R. B. Maxcy, and C. E. Georgi. 1970. Effect of solute concentration and pre-soil interaction on the microflora of dairy origin on stainless steel surfaces. J. Milk Food Technol., 33:541.

(4) Bourne, M. C., and W. G. Jennings. 1961. Some physicochemical relationships in cleaning hard surfaces. Food Technol., 15: 495.

(5) Breed, R. S., E. G. D. Murray, and N. R. Smith. 1957. Bergy's Manual of Determinative Bacteriology. 7th ed. The Williams and Wilkins Company, Baltimore, Md.

(6) Elliker, P. R. 1949. Practical Dairy Bacteriology. 1st ed. McGraw-Hill Book Company, Inc., New York, New York.

(7) Hucker, G. J. 1954. The adherence of organisms and soil to surfaces of eating utensils. J. Milk Food Technol., 17:48.

(8) Jenness, J., and S. Patton. 1959. Principles of Dairy Chemistry. John Wiley and Sons, Inc., New York, New York.

(9) MacFarlane, Marjorie G., and B. C. J. G. Knight. 1941. The biochemistry of bacterial toxins. I. The lecithinase activity of *Clostridium welchii* toxins. Biochem. J., 35:884.

(10) Maxcy, R. B. Factors in the ecosystem of food processing equipment contributing to outgrowth of microorganisms on stainless steel surfaces. J. Milk Food Technol., 34: 569.

(11) Newlander, J. A., and H. V. Atherton. 1964. The Chemistry and Testing of Dairy Products. 3rd ed. Olsen Publishing Company, Milwaukee, Wisc.

(12) Walter, W. G. 1967. Standard Methods for the Examination of Dairy Products. 12th ed. American Public Health Association, Inc., New York, N.Y.

(13) Winston, P. W., and D. H. Bates. 1960. Saturated solutions for the control of humidity in biological research. Ecology 41: 232.