Observation and Direct Count of Bacterial Growth in Pasteurized and Unpasteurized Milk

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Introduction

The purpose of these experiments was to observe the level of bacterial growth on various milk samples using raw milk, fresh pasteurized milk, and past-date pasteurized milk. The process of pasteurization was first discovered by scientist Louis Pasteur in the 1800s involving a process where the item is heat treated with a temperature ranging from 57-72 degrees Celsius depending on how long it is kept at that temperature in order to kill the bacteria involved in food spoilage to prolong the storage and shelf life of the product (Suozzo, Andrea M, 2015). Bacteria such as *Mycobacterium tuberculosis*, is a non-spore forming pathogen that can be found in unpasteurized of an infected cow and can be fatal if consumed (G. B. Patel and G. Blankenagel, 1972). The process of pasteurization is used to eliminate these pathogens, however heat resistant forms of these pathogens, known as thermoduric, are capable of survival after pasteurization; some of these strains include spore forming bacteria such as *Clostridium, Bacillus*, and *Paenibacillus* as well as other pathogens of the Microbacterium and Streptococcus species (G. B. Patel and G. Blankenagel, 1972).

Advantages of using the standard plate count as seen in experiment 2 over the direct microscopic count in experiment 1 is that milk has a low bacteria content and SPC shows only viable organisms, as it can detect the organisms that use the nutrients within the milk for survival (Wessner. et al, 2017).

It is expected that the raw milk would have the largest number of colonies in the 10^{-2} dilution as the bacteria present in the raw unpasteurized milk as well as the lowest dilution theoretically has the greatest number of bacteria present before incubation and after incubation. Similarly, the fresh pasteurized milk is expected to have the lowest colony count in the 10^{-3}

dilution as the pasteurization process kills many bacteria and the highest dilution factor should theoretically yield the lowest number of bacteria present before and after incubation.

Materials and Methods

Please refer to Department of Biology, Winter 2019, Biology 241L Introduction to the Microbial World Laboratory Manual, Experiment 2: Standard Plate Count of Milk, pp. 37-41 for the full detailed procedure and material list for this lab. There were no deviations from the original guideline (Duxbury, 2019).

Results

Table 1 Cell count of various milk samples under a microscope in experiment 1

Milk Sample	Cells per field		Average # of cells per field	Cells per ml of milk
A. Raw milk	**10**	**4**	**6.7**	**$4.3x10^6$**
	7	**6**		
	8	**5**		
	9	**7**		
	5	**7**		
B. Fresh Pasteurized	**7**	**4**	**5.7**	**$3.6x10^6$**
	5	**5**		
	4	**6**		
	5	**7**		
	6	**8**		
C. Past-date Pasteurized	**10**	**12**	**12.9**	**$8.2x10^6$**
	11	**15**		
	12	**12**		
	14	**14**		
	13	**16**		

Table 1 displays data obtained from observing milk samples using a microscope to observe the number of cells visible per microscopic field of view. Common trends in the data is the decreased number of cells per field under the fresh pasteurized milk noted with the lowest cell per ml of milk and the past date pasteurized with the highest cells per field and cell per ml of milk.

Sample calculation of microscope factor

20 spaces across microscopic field

1. Diameter of microscopic field = 0.2 mm
    a. Radius of microscope field = 0.2 / 2 mm = 0.1 mm
2. Area of microscope field $= \pi r^2$
    a. = 3.14 x $(0.1\ mm)^2$
    = 3.14 x 0.01 mm^2
    = .0314 mm^2 → 0.000314 cm^2
3. Number of fields in 1 cm^2 = 1.0 cm^2 / 0.000314 cm^2 per field
    a. = 3184.71

4. Microscope factor = number of fields in 1 millimetre
    a. = number of fields in the 1 cm^2 area / volume of milk over 1 cm^2
    = 3184.71 fields/cm^2 / 0.005 ml/cm^2
    = 633, 6942.68 fields/ml → $6.3x10^6$ fields/ml

Sample calculation of cells per ml of milk

1. Average number of cells/field x Microscopic Factor
    a. = 6.7 x 6336942.68
    = 4267515.92 → $4.3x10^6$

Table 2: Observation of colony count on varying milk samples in experiment 2

Milk Sample	Colony count	Calculation of Standard plate count per milliliter (CFU/ml)
Sample A: raw milk unpasteurized	101 – 10^{-2} dilution	1.01 $x10^4$
Sample B: fresh pasteurized milk	11 – 10^0 dilution	1.1 x 10^1

Table 2 displays the data obtained from observing the milk samples using the Quebec colony counter to observe the number of colonies on each plate with respect to its dilution factor. The data obtained for the colony count was taken using the most accurate dilution with respect to the class' data trends; number of colony forming units per milliliter increases as dilution factor increases. One can expect to find more bacteria in the raw milk.

Sample calculation for CFU/mL

1. Number of colonies counter / dilution factor
    a. = 220 / 10^{-2}
    =22,000

Discussion

The purpose of the experiments is to identify the level of bacterial growth using 2 different quantifying methods of pasteurized, unpasteurized, and past-date pasteurized milk samples. Some noticeable trends found in experiment 1 for the direct microscopic field count of bacteria in the milk sample is the fresh pasteurized milk yielding the lowest value of colony forming units per ml of milk. The past-date pasteurized milk yielded the greatest number of colony forming units, while the raw milk was the middle of the two, as seen in *Table 1*. These values are expected as the pasteurization process kills the bacteria involved in spoilage in order to prolong shelf life and storage. Past-date pasteurized milk does have the benefit of having the bacteria to slow down the spoilage, however, there are still bacteria that are heat resistance that are left behind after pasteurization known as thermoduric (G. B. Patel and G. Blankenagel, 1972). Allowing the milk to be left out will spoil it sooner due to the refrigeration process slowing down the metabolic rate of the bacteria, thus yield an increased number of colonial growth. Class data follows a similar trend, most students' fresh pasteurized milk had the lowest number of microbial growth per ml of milk. However, data suggests a discrepancy between the past date pasteurized milk and the raw milk as both are expected to have a larger value of CFU/ml than the pasteurized milk as roughly half of the student's values were greatest for past date milk whilst the others had the greatest value in the raw milk.

Upon observing the bacteria under the microscope in experiment 1, the bacteria found in sample A resemble that of *E.coli*, this is indicative of the bacillus (rod-shaped) dark specs that seemed to have pick up the methylene blue stain. Further, *E.coli* is gram-negative and seems to only be seen in the raw milk sample as these type of bacteria are not heat resistant and only have a thin peptidoglycan layer making them more readily to be destroyed in the pasteurization

process (Mohammadi. et al, 2013). The pasteurized and the past date pasteurized milk had similar looking bacteria under the microscope. Both resemble the bacteria *Bacillus cereus* as a pathogen in the milk samples, this is indicative of the species being a thermoduric bacteria allowing it to survive the pasteurization process and the rod-shaped morphology as seen in *figures 2 and 3* leans more towards this assumption.

A study done at the University College Cork in Ireland was done to observe the microbial contents of raw and pasteurized milk and it was found that the raw milk had a significantly greater amount of Proteobacteria than the pasteurized milk (Quigley. et al, 2013). Proteobacteria is a phylum of gram-negative bacteria that are non-spore forming which indicate a bacteria that would likely be readily reduced after pasteurization (Quigley. et al, 2013). This helps support the data that there are a reduced number of bacteria in the pasteurized milk, in both experiments 1 and 2.

The Quebec colony count method differs from the direct microscopic count is that the Quebec colony count only displays viable organisms while the direct microscopic count method counts all bacteria visible within the microscopic field of view. These methods yielded different results as the Quebec colony count method yielded a lower value of colony forming units per ml of milk in both the pasteurized and raw milk samples. This difference can be noted in how the Quebec colony counting method counts only viable cells that grow in colonies that are visible on the agar medium while the direct microscopic field count shows all visible cells found within the field. However, both involve a direct count of the bacteria so both have a similar degree of inaccuracy when counting the bacteria.

Some errors noted in the lab is that the amount bacteria within the milk cannot be uniformly dispersed within each sample for the conductors to use so the level of microbial

growth after incubation will vary from student to student. Also, both methods in experiment 1 and 2 involve counting the number of colonies using a microscope in experiment 1 and the Quebec colony counter in experiment 2. These methods utilize human ability to count each colony in experiment 2, so it is impossible for each colony to be counter with a high degree of accuracy, especially without access to a counter device in the lab. This leads to an inaccurate value for the colony counts and the number of colony forming units per millilitre of milk. Similarly, in experiment 1, experimenters use direct microscopic count by counting the amount of bacteria visible in a single microscopic field 10 times using a different field, however there's only so much bacteria present in the milk samples that counting the same bacteria on a separate field is inevitable, yielding a higher cells per ml of milk value.

In experiment 1 the average number of cells per field was 6.7, 5.7, and 12.9 for the raw milk, pasteurized milk, and past-date pasteurized milk respectively, similarly the cells per ml of milk for the raw milk, pasteurized milk, and past-date pasteurized milk are $4.3x10^6$, $3.6x10^6$, and $8.2x10^6$ respectively. In experiment 2, the colony count for the raw milk and pasteurized milk were 101 for the 10^{-2} dilution and 11 for the 10^0 dilution respectively. Overall, this experiment was done with a high degree of accuracy and effectiveness as the expected results of the pasteurized milk yielding the lowest number of colony forming units and the raw milk and past date pasteurized milk having a higher number.

Appendix

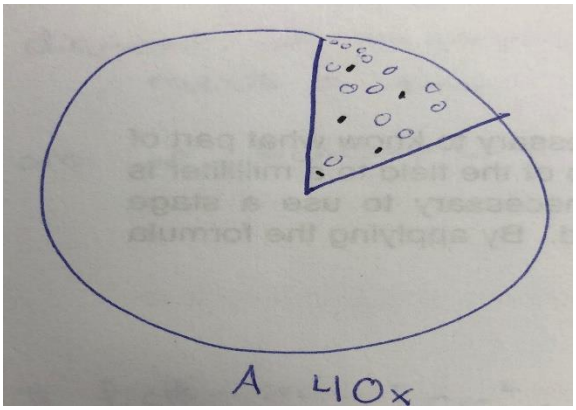

Figure 1: image of sample A, raw unpasteurized under 40x magnification under a microscope, noticeable black specs are representative of the desired bacteria to be counted that picked up the methylene stain and resemble a rod-shaped morphology

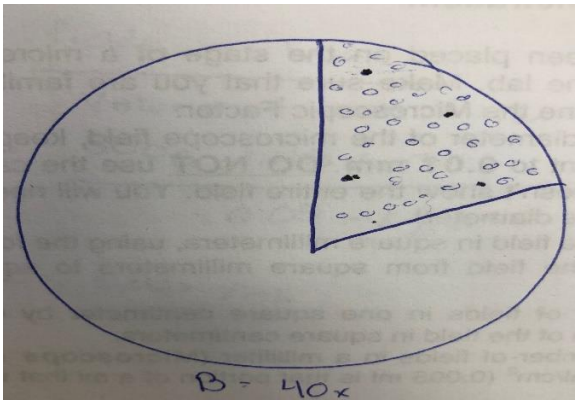

Figure 2: image of sample C, fresh pasteurized milk under 40x magnification under a microscope, noticeable black specs are representative of the desired bacteria to be counted that picked up the methylene stain and resemble a cocci morphology

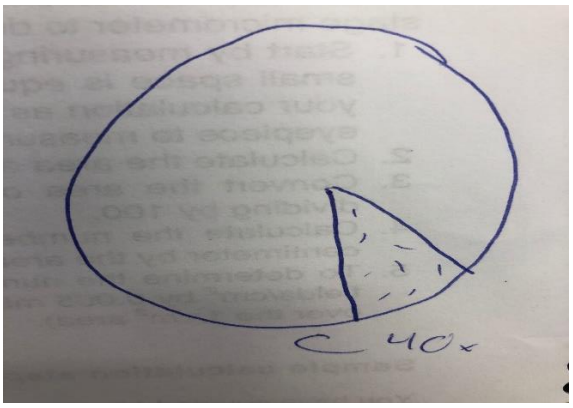

Figure 3: image of sample C, past-date pasteurized under 40x magnification under a microscope, noticeable black specs are representative of the desired bacteria to be counted that picked up the methylene stain and resemble a cocci morphology

Work Cited

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