

衛生福利部疾病管制署

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衛福部 疾病管制署
中區傳染病防治醫療網
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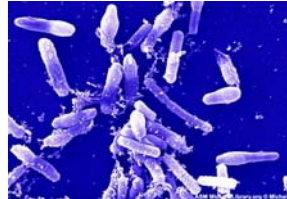
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How does pasteurization affect the activation of *Bacillus* spores in milk?

Bacillus is a family of bacterium that is characterized primarily for endospores, highly resistant, dormant forms of life. They also have the following characteristics:

- o Gram-positive
- o Rod shaped
- o α -hemolysis
- o Catalase positive
- o Extreme Thermophiles



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Background – Endospores

Endospores are extremely resistant to any environmental conditions and are the known source for species survival.


They are produced through sporulation usually triggered by poor growth conditions.

They remain dormant until conditions are right again for the live bacterium is able to survive.

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Background – Pasteurization



Defined as: "The heating of every particle of milk or milk product to a specific temperature for a specified period of time without allowing recontamination of that milk or milk product during the heat treatment process."

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Background – Pasteurization

- o Is used to improve the quality of dairy products
- o To decrease health risks associated with bacterium normally found in dairy products



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Experiment

- o The extreme heat associated with pasteurization could be responsible for the activation of bacterial endospores derived from *Bacillus*, and therefore an increase in reproduction and concentration of cells.
- o We plan on isolating *Bacillus* from both spoiled and non-spoiled milk and comparing the concentrations of *Bacillus* in each.
- o If our hypothesis is true, then there should be a higher concentration of *Bacillus* in the spoiled milk.

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Experiment – Protocol

1. Make Dextrose-Tryptone Agar :
 - minimal media, selective for *Bacillus*
 - Bromcresol Purple is a pH indicator that turn yellow when the pH becomes very low due to the fermentation of sugars such as dextrose
2. Plate both spoiled and non-spoiled milk (whole and regular) onto dextrose-tryptone agar and TSA plates in serial dilutions to obtain concentrations.
3. Perform a series of tests to be sure that we have obtained *Bacillus*

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Methods

Our protocol will test our hypothesis by finding the concentrations of *Bacillus* using serial dilutions and confirm the presence of *Bacillus* by performing tests that the results would be characteristic of *Bacillus*. These tests include:

- o Catalase
- o MacConkey's Media
- o Blood Agar
- o Endospore stain

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Results

| (TSA) | Regular Whole Milk | Sour Whole Milk | Regular Skim Milk | Sour Skim Milk |
|------------------------|----------------------|----------------------|----------------------|----------------------|
| Dilution | 1*10 ⁻⁴ | 1*10 ⁻⁵ | 1*10 ⁻¹ | 1*10 ⁻⁵ |
| # of Colonies | 191 | 205 | 363 | 198 |
| Concentration (CFU/ml) | 1.91*10 ⁷ | 2.05*10 ⁸ | 3.63*10 ⁴ | 1.98*10 ⁸ |

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Results

| (Dextrose-Tryptone) | Regular Whole Milk | Sour Whole Milk | Regular Skim Milk | Sour Skim Milk |
|------------------------|----------------------------|----------------------|----------------------|----------------------|
| Dilution | Unable to Count (Too tiny) | 1*10 ⁻⁵ | 1*10 ⁻¹ | 1*10 ⁻⁵ |
| # of Colonies | N/A | 227 | 207 | 169 |
| Concentration (CFU/ml) | N/A | 2.27*10 ⁸ | 2.07*10 ⁴ | 1.69*10 ⁸ |

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Result

| | Regular whole milk | Sour Whole Milk | Regular Skim Milk | Sour Skim Milk |
|------------|--------------------|-----------------|-------------------|----------------|
| Blood Agar | Alpha | Alpha | Alpha | Gamma |



Regular Skim Milk



Sour Skim Milk

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Result

| | Regular whole milk | Sour Whole Milk | Regular Skim Milk | Sour Skim Milk |
|-----------|--------------------|-----------------|-------------------|----------------|
| MacConkey | White Colonies | No Growth | No Growth | No Growth |



Whole Regular Milk,
the only Gram-negative species

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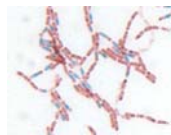
Result

| | Regular Whole Milk | Sour Whole Milk | Regular Skim Milk | Sour Skim Milk |
|----------|--------------------|-----------------|-------------------|----------------|
| Catalase | Positive | Negative | Negative | Negative |

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Results




Bacillus cereus, control

| | Regular Whole Milk | Sour Whole Milk | Regular Skim Milk | Sour Skim Milk |
|------------|--------------------|-----------------|-------------------|----------------|
| Endospores | Negative | Negative | Negative | Negative |


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Results




Whole Regular Milk



Whole Sour Milk



Skim Regular Milk



Skim Sour Milk


Rods

Cocci

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Results



Skim Regular 10⁻¹

Skim Regular 10⁻⁵

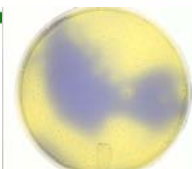
Skim Sour 10⁻⁵

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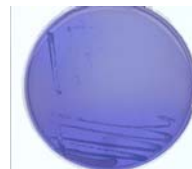
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Whole Regular Milk 10⁻⁵



Whole Sour Milk 10⁻⁵



Whole Regular Milk Streak Plate



Whole Sour Milk Streak Plate

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Expected Results?

- o Not Completely
 - By just looking at the Dextrose-Tryptone plates, it seems like our hypothesis was true because there was an increase in concentration of cells and they changed the color indicating a change in pH.
 - But....upon further testing to make sure we had *Bacillus*, we found that the bacteria on the plates was in fact not *Bacillus*.
 - Then what is it?

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Discussion

Streptococcus is a common cause of Mastitis in cows. It is a non-endospore forming, catalase positive cocci shaped bacterium. Mastitis is easily seen by an inflamed udder in the cow and is caused by a bacterial infection. This is what we think we recovered in the sour milk.



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Diseases

- o *B. cereus* causes food-poisoning syndromes (found in milk):
 1. Rapid-onset emetic syndrome
-nausea, vomiting
 2. Slower onset diarrheal syndrome
- o Drink water and eat garlic if extremely severe then antibiotics may be necessary such as: erythromycin, ciprofloxacin and chloramphenicol. These antibiotics break down the cell wall or prevent synthesis of proteins.
- o *B. anthracis*, causes anthrax under skin, in the lungs (pneumonia) and intestine.
- o These diseases are rare and can be treated by antibiotic therapy such as: penicillin, doxycycline and fluoroquinolones (especially in inhaled *Bacillus*).

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What if we found *Bacillus*?

- o Finding an increased concentration of *Bacillus* in the sour milk would mean that Pasteurization would in fact activate the spores and *Bacillus* would be present in sour milk. This would lead to possible food-poisoning caused by *B. cereus*.
- o Pasteurization is not as effective in preventing health risks associated with contaminated food as thought.

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Conclusions

- o We didn't actually find *Bacillus* in the sour milk, which disproves our hypothesis that the endospores were activated due to the extreme heat.
- o This experiment makes sense and it could only have been human errors that caused us to deem our hypothesis as false.

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