ANALYSIS OF THE GROWTH AND CHARACTERISTICS OF MICROORGANISMS IN AN UNREFRIGERATED YOGHURT

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1-Introduction

- Some microorganisms are used in the production of dairy products.
- Others have the ability to spoil food by altering the organoleptic characteristics. Only a small number of microorganisms, pathogenic microorganisms, have the ability to cause disease.
- We tried to produce a culture medium for: didactic use, easy to produce and inexpensive, which would give some indications regarding the characteristics of the microorganisms cultivated.

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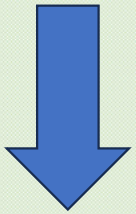
- Two media were produced based on almost all the constituents of the nutrient agar medium and some constituents of the MacConkey agar médium. The only difference between the two media is the concentration of lactose used, allowing you to select a least expensive one that produces good results.

Sample→ Yogurt made from cow's milk and enriched with bifidobacteria.



1. The fact that the yogurt is very sensitive during it's exposure time on supermarket shelves and at home;
2. Wide use in human food due to health benefits

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2- Research Objectives

- To produce a culture medium for: didactic use, easy to produce and inexpensive, which provides some indications regarding the characteristics of microorganisms cultured using solid yogurt rich in bifidobacteria;
- To study under the Optical Microscope the morphological characteristics of the CFU of the microorganisms present in yogurt;
- Apply the Gram stain and the catalase test to the different CFU present in the samples;
- Show the importance of refrigeration in the preservation of yogurt.

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3- Theoretical Framework

Characteristics of culture media

- Nutrient agar is a general medium, characterized by its basic nutrient composition, being suitable for a multitude of microbiological applications. The simple constitution of the medium, composed of peptone, beef extract, sodium chloride and agar, ensures the supply of the nutrients necessary for the replication of microorganisms.

- The function of sodium chloride is to maintain osmotic balance by ensuring that the pH of the medium remains stable during bacterial growth.

- Agar functions as a solidifying agent. Its inclusion ensures a stable surface for bacterial growth, facilitating the observation of colony morphology and allowing accurate enumeration of organisms.

- MacConkey Agar medium is a selective and differential culture medium. It is widely used in microbiology for the isolation and differentiation of enterobacteria, especially *Escherichia coli* and *Salmonella spp.*

- This medium is prepared with peptone, lactose, bile salts, crystal violet and phenol red. The bile salts and violet crystal inhibit the growth of gram-positive bacteria, allowing only the growth of gram-negative bacteria.

- In addition, the presence of lactose as a carbon source allows the identification of bacteria capable of fermenting this sugar.

- The lactose-fermenting bacteria will produce lactic acid during the fermentation process, which results in a decrease in the pH of the medium. This leads to the change of color of the pH indicator present in the MacConkey medium, from red to yellow, indicating lactose fermentation.

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Characteristics of bacteria

- Bacteria of the genus *Lactobacillus* are lactic acid, Gram positive bacilli, non-sporulated, and catalase-negative.
- *Streptococcus thermophilus* form large, unraised CFUs with wavy borders, cocci-shaped, Gram positive and catalase negative.
- *Staphylococcus aureus* forms a bumpy CFU, they are Gram positive cocci and catalase positive.
- Bifidobacteria form bumpy CFUs, are Gram-positive bacilli and catalase-negative.
- Total coliforms are Gram-negative and catalase-positive bacilli.

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- The family Enterobacteriaceae consists of bacilli, aerobic, or facultative anaerobic Gram-negative bacteria. They are glucose fermenters, producers of catalase and negative cytochrome oxidase

- Enterobacteriaceae include total coliforms, whose morphological and physiological characteristics can be evaluated by cultivation in selective culture media. They ferment lactose in addition to glucose, producing gas and acid.

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4- Materials and Methods

- Petri dishes
- Hot plate
- Erlenmeyr flask
- Bunsen burners
- Glass stirring rod
- Vertical Autoclave
- Inoculation loop
- Agar
- Lactose
- Ethanol
- Peptone
- Yeast extract
- Neutral red

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1- Sample preparation (10^{-1}) and dilutions with peptone water (10^{-2}, 10^{-3} e 10^{-4});

2- Sterilization and identification of petri dishes;

3- Preparation of culture media (medium I and medium II) and filling of petri dishes;

4- Inoculation using a loop with 0.1 ml of the sample in 3 replicates by sample dilution ;

5- Incubation of the petri dishes at 30°C for 40 hours ;

6- Catalase test at the end of 20h and Gram stain at the end of 40h;

5- Results and discussion

Petri dishes	Dilution	Medium I				Medium II			
		20H	**20H - Média**	**40H**	**40H - Média**	**20H**	**20H – Média**	**40H**	**40H - Média**
1	10^{-1}	387	336,7	511	500,3	826	859,7	NC	NC
2		421		573		842		NC	
3		202		417		911		NC	
4	**10^{-2}**	**30**	**36,3**	**38**	**48,3**	**73**	**78,7**	**82**	**93,0**
5		**28**		**29**		**106**		**128**	
6		**51**		**78**		**57**		**69**	
7	10^{-3}	12	23,3	28	42,3	63	57	74	65
8		20		44		51		52	
9		38		55		57		69	
10	10^{-4}	0	10,3	6	15	29	20,3	55	32
11		21		22		20		25	
12		10		17		12		16	

Figure 1 - Table with the counts of the number of CFU in all Petri dishes and averages at each dilution and a half at the end of 20H and 40H of growth.

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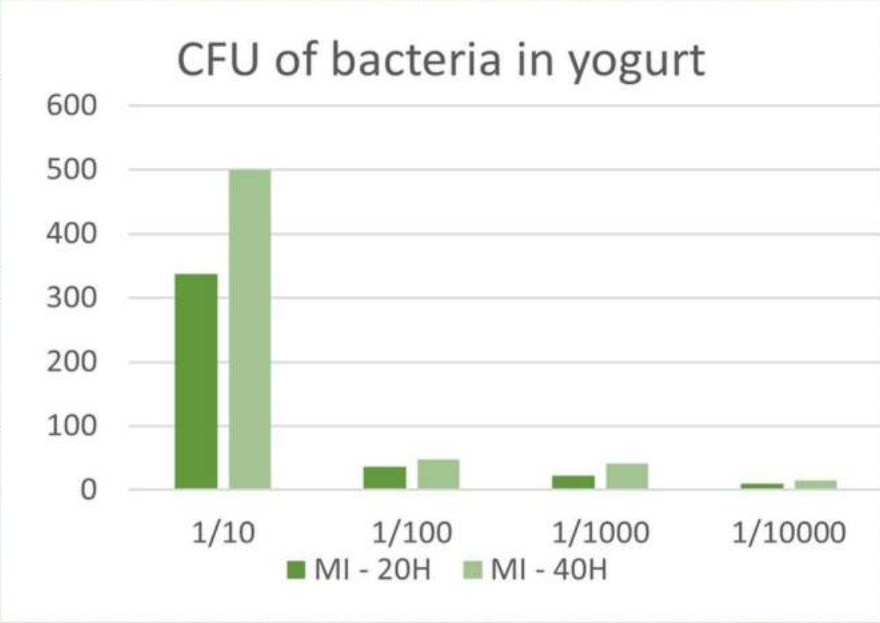

Figure 2 - Comparative graph of the number of CFU between the same medium with different sample dilutions and two different counting times (20H, 40H).

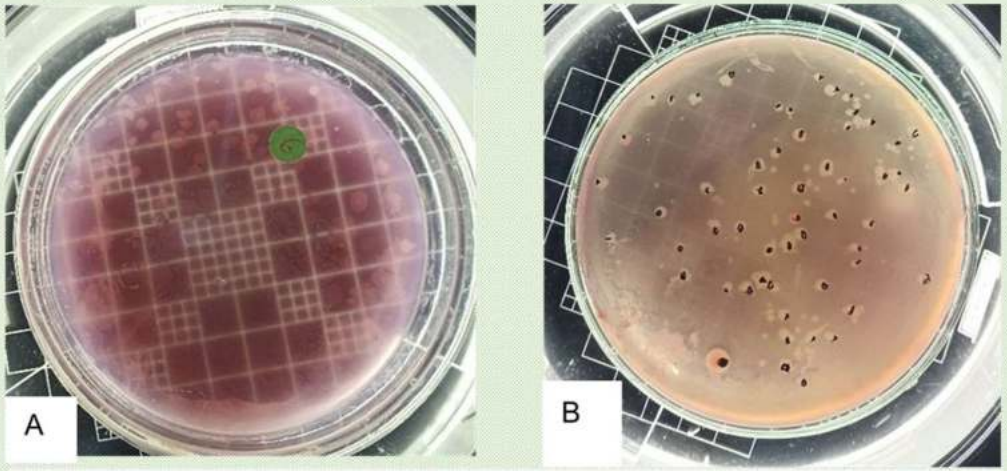

Figure 3 – CFU in the I6-20H Petri dish (A) and UFC in the II4-40H Petri dish (B).

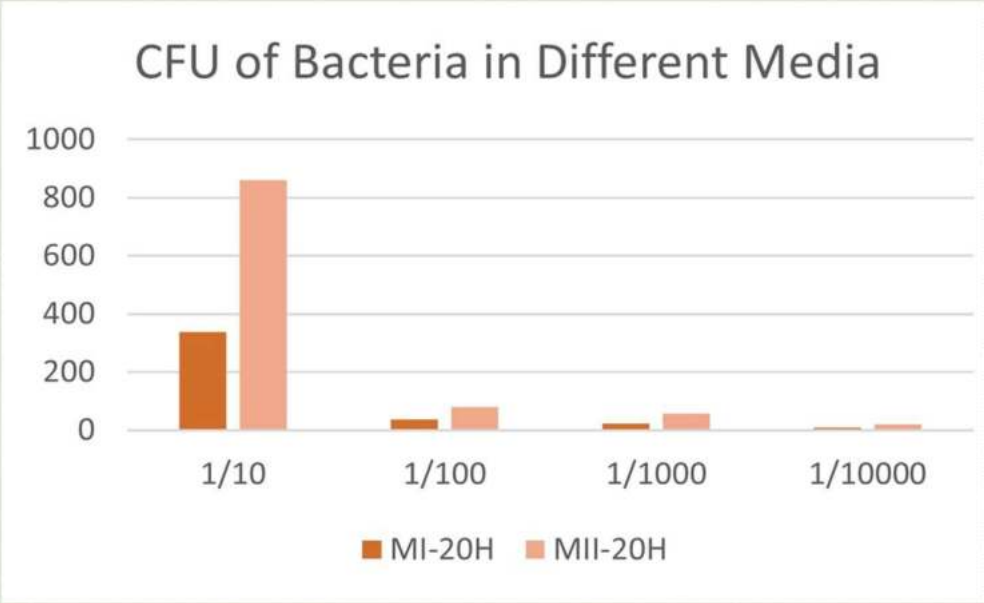

Figure 4 – Comparative graph of the CFU number between different lactose concentrations (medium I and medium II) in the different dilutions of the yogurt sample.

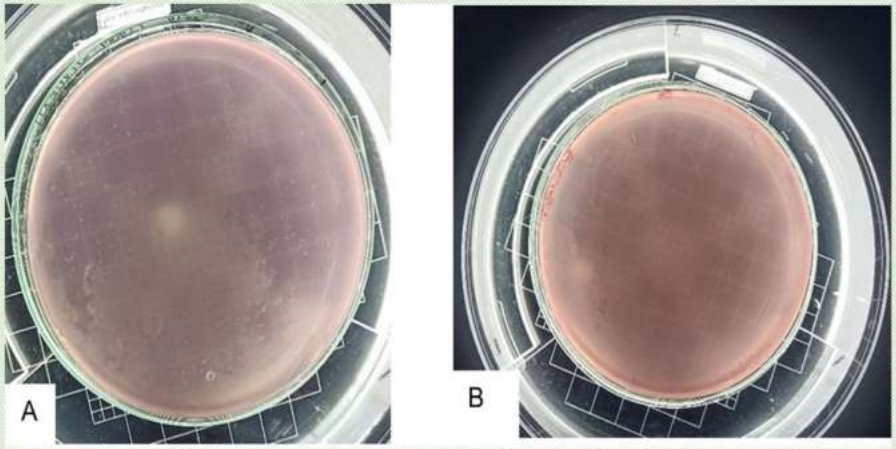

Figure 5- CFU in the I3-20H Petri Dish (A) and CFU in the I10-20H Petri Dish (B).

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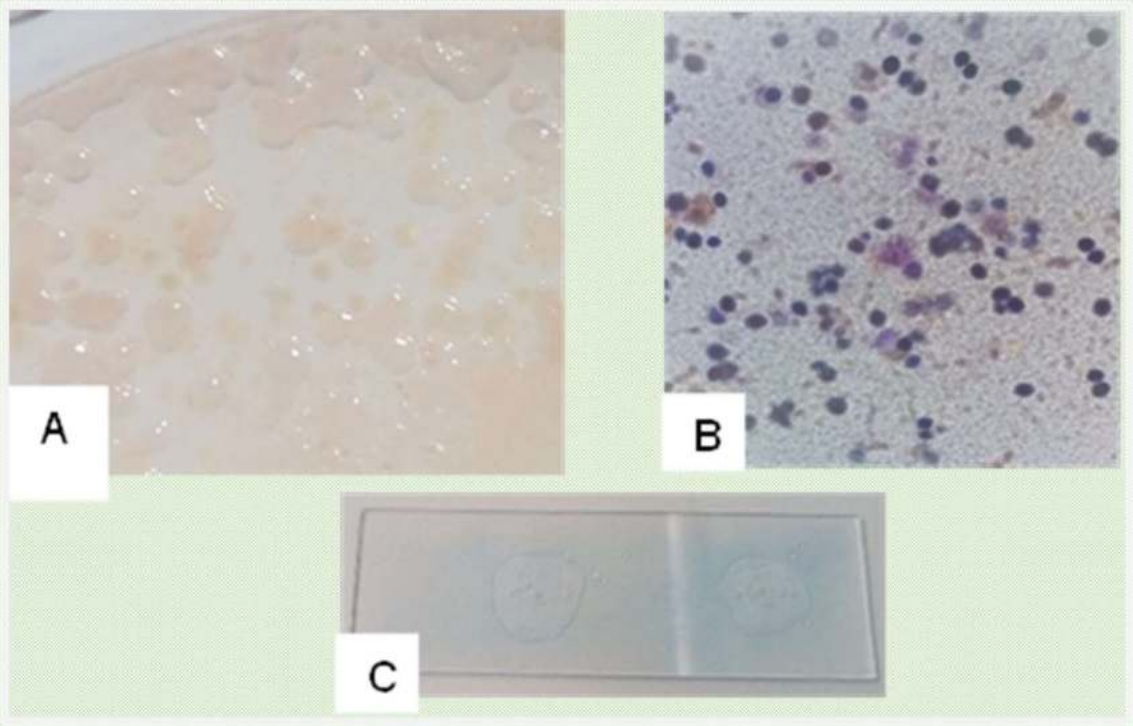

Figure 6 - Typical appearance of CFU (A) in the form of cocci, Gram positive, magnified 1000x (B) and catalase negative (C).

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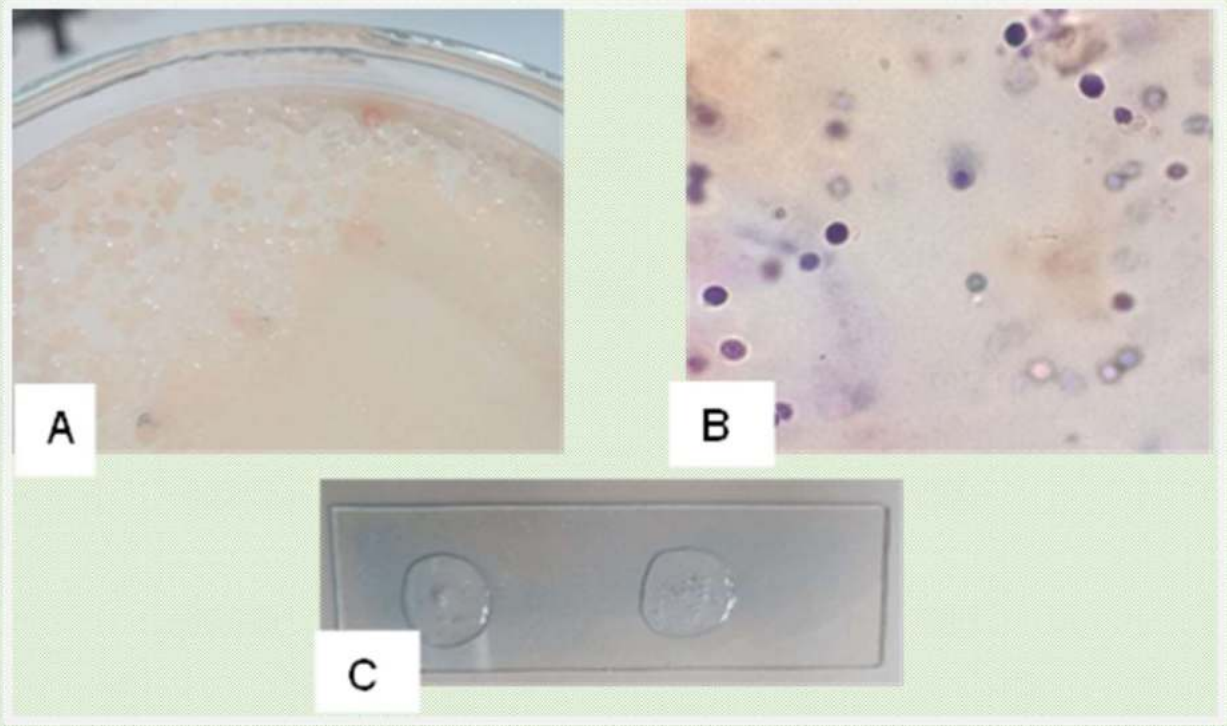

Figure 7 - Typical appearance of CFU (A) in the form of cocci, Gram positive, magnified 1000x (B) and catalase positive (C).

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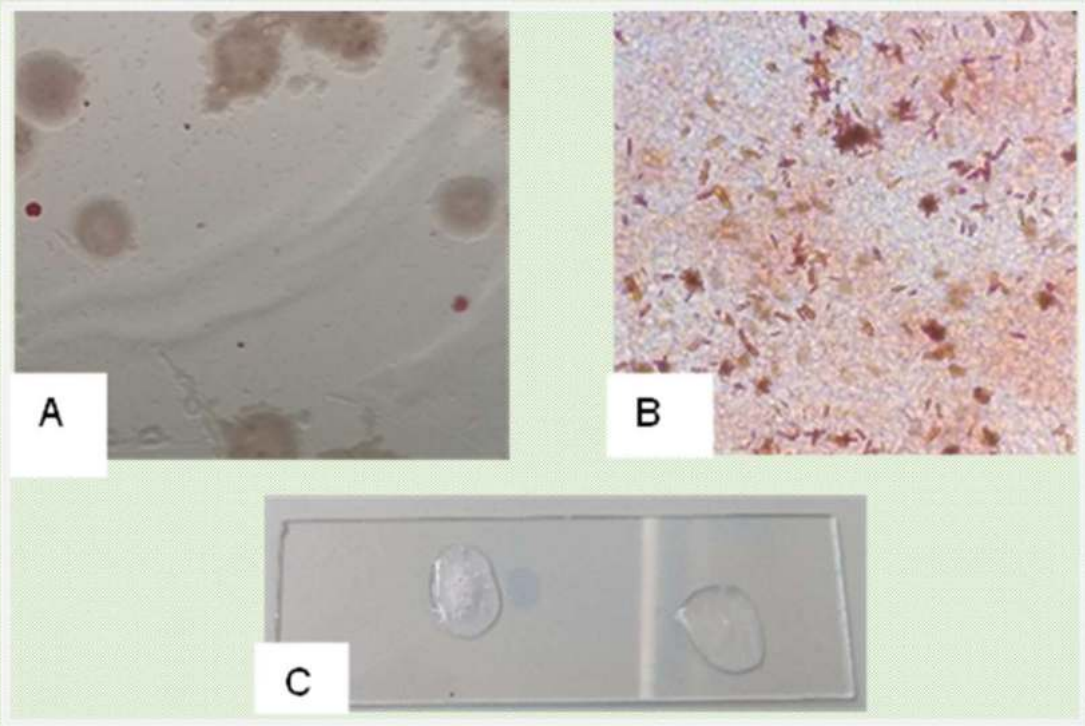

Figure 8 - Typical appearance of CFU (A) in the form of bacilli, Gram negative, magnified 1000x (B) and catalase positive (C).

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6- Discussion

- This shows that CFU increased with the incubation time for all dilutions and with the decrease in the amount of lactose in the médium, and decreased with increasing dilutions. These results are in line with expectations, as the peptone and yeast extract available in the media provide the bacteria with nutrients to grow and multiply and the lactose allows the production of energy for bacterial metabolism (Tankeshwarin, 2024; Mazura-Reetz, 1979).

- Interestingly, in medium I consisting of: 1.5 g of yeast extract, 5 g of peptone, 5 g of NaCl, 1.5 ml of neutral red at 2% in ethanol, 15 g of agar and 10 g of lactose per liter we obtained lower growth than in medium II, whose composition is equal, but have 5 g of lactose per liter of solution. Thus, the medium with less lactose presents greater growth, probably this nutrient becomes limiting when present in higher values, as it happens in medium I.

- In figure 3 it was possible to know that the production of lactic acid after 40 hours is higher than after 20 hours. as the middle turned from red to yellowish.

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6- Discussion

- In the microbiological analysis, it was possible to distinguish three groups of bacteria. The group depicted in figure 6 has characteristics compatible with *Streptococcus thermophilus, Lactococcus lactis and Enterococcus*, as it was found that they form large, reliefless CFUs with wavy borders, have the shape of cocos, are Gram positive and catalase negative (Zirnstein, 1999) and are part of the bacteria used in the production of probiotic-rich yogurt (Song *et al* 2017; Saad, 2006; Stefe *et al*., 2008; Santos, 2010).

- The group depicted in figure 7 has typical characteristics of *Staphilococus aureus,* as it forms a fuse with relief, are Gram positive cocci and catalase positive (Silva, 2020).

- The group depicted in figure 8 are compatible with coliforms, as they are Gram-negative and catalase-positive bacilli (Silva, 2020).
- Since *Staphilococus aureus* and coliform bacteria are not part of the microorganisms present in a yogurt that follows food safety standards (Estevinho, 2014; Cunha, 2017), confirming that contamination occurred during the time the sample was not refrigerated.

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7- Conclusions

- The number of colony-forming units (CFU) increases with the incubation time for all dilutions and with the decrease of lactose present in the medium and decreased with the increase of dilutions;

- These results allowed us to achieve one of the objectives of the work , the production of a medium for didactic use, easy to produce , with good growth , which provides some indications regarding the characteristics of the microorganisms cultured and less expensive , as it uses less amount of lactose per experimental assay;

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- It was possible to distinguish three groups of bacteria (cocci, gram positive and catalase negative; cocci, gram positive and catalase positive and bacilli, gram negative and catalase positive). It was possible to accomplish two more objectives of the work, since it was possible to study the morphological characteristics of the CFU of the microorganisms present in the yogurt and to apply the Gram stain and the catalase test to the different CFU present in the samples, contributing to their classification.

- *We have three groups of bactéria compatible with: Streptococcus thermophilus, Lactococcus lactis, Enterococcus*, *Staphilococus aureus* and coliforms.

- The presence of *Staphilococus aureus* and coliform bacteria shows that the yogurt has been contaminated over the time it has not been refrigerated, showing the importance of this method of preservation.

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