

Reserches regarding viability of probiotic level of some lactic bacterium and bifidobacterium colonies in the feta cheese

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Abstract

The research conducted on the probe of Feta Cheese with lactic bacteria and bifidobacterias was rated the degree of survival of a lactic bacteria in bifidobacterias and Feta Cheese with added probiotic. Assessing the suitability of Feta cheese as a food carrier of probiotic cultures, in different combinations, as probiotic adjuncts. Probiotic, lactic starter (Bifidobacterium Longum, Bifidobacterium Infantis), and (Lactobacillus Acidophilus și Lactobacillus Casei) were counted at 0, 15, 30, 45 and 60 day of refrigerated storage. The results showed that nine different combinations of bifidobacteria and lactobacillus had a satisfactory viability in the Feta cheese. Both combinations of bifidobacteria and Lactobacillus Casei cultures assayed also showed a satisfactory survival.

Keywords: Bifidobacterium, Lactobacillus acidophilus, Lactobacillus casei, probiotics bacteria, Feta cheese, survival level

1. Introduction

Probiotics were defined by a group of experts convened by the Food and Agriculture Organization of the United Nations (FAO) as "live microorganisms administered in adequate amounts which confer a beneficial health effect on the host". Most probiotics are bacteria, which are small, single-celled organisms. Bacteria are categorized by scientists with genus, species and strain names

Many of acid dairy products include probiotics cultures between *Bifidobacterium*, *Lactobacillus Acidophilus* and *Lactobacillus Case* being used through their headlands for health, being used for many years. Among the health and adopted nutrition benefits from this probiotics bacteria includes the modification for sugar of milk intolerance some pathogenes microorganisms and virus

inhibition vitamins production on the intestinal level, the reduction of cholesterol level, the prevention of bowels, cancer and immunity improvement. For this performances, acid dairy products should contain about 10^6 /cfu/ml probiotics bacteria.

In the future the development of dairy products will include another probiotics bacteria such bifido bacteria and lactobacill. The Bifidobacteria incorporation in cheese is not difficult because is necessary anaerobic conditions and a pH between 6,5-7. More than that, in present is trying on to product industrially more cheese using lactic and probiotics cultures. but also traditional yogurt, buttermilk, icecream, butter and heifer.

Recently, the dairy products factories already started to produce probiotics bacteria cheese adding some adjuvants, but probably, in the future the probiotics cheese

production will be made only with Bifidobacteria or adding *Lactobacillus Acidophilus*, *Lactobacillus casei*. More recently, a small number of researchers and industries have started to produce cheeses with probiotic bacteria as adjuncts. Nowadays there are a lot of producers who used the probiotics like adjuvants on Cheddar cheese and Dalia pressed cheese. The survival of rate of this bacteria on cheese is moderate; appearing almost 20 cultures with 7 species of *Bifidobacteria*. In Cheddar cheese the *Infantis Bifidobacterium* and *Bifidobacterium Bifidum* remains viables between 14-28 weeks. The adapting for *Bifidobacterium Bifidum* and *Bifidobacterium Longum* in Dalia pressed cheese is better comparing the addition of *Bifidobacterium Infantis* in Cheddar cheese. On the other hand, *Bifidobacterium Bifidum* have a low survival rate in salty medium, like Telmea cheese. The using of bifidobacteria and *Lactobacillus Acidophilus* cultures like probiotic mixture in curd had a better viability and maintained around 14 weeks. Although the results obtained of probiotics bacteria success depends on the used species and culture, on the interactions between as lactic acid and starter culture and the fermentation condition, on the product pH, oxygen and temperature conditions.

Some of considered characteristics when the probiotics culture were added in fermented dairy products were their survival capacity in the stomach acid conditions. In Romania 48% of milk production is used in fabrication of cheese product and a lot of this products are commercialized in U.E space.

The aim of this study was to evaluate the viability of bifidobacteria, *Bifidobacteria Lactobacillus Acidophilus* and *Lactobacillus Casei* in different combination in different combinations, during refrigerated storage for a long period of time of Feta cheese, and their ability to survive in hydrochloric solutions.

2. Materials and method

In order to make this experiment were used four combinations of probiotic colonies, each combination consisting of at least two types of microorganisms.

Determination of bacterial viability as illustrated by the numerous scientific papers published on the topic^{6,12,14}. *Cheese samples*. A total of 20 Feta cheese samples was obtained in a milk processing industrial unit from Maramures, using standardized cow's milk. The technological process of this cheese is the same with the one used in order to obtain classic cheese, described by Costin G.M⁷ what differences them is the type of starter colonies that were used. The cheese packing consisted of plastic boxes of 100 g. The cheese was stored at a temperature of 5°C for two months. The analyses that were made are according to the current standards which are:

- Sensorial analyze;
- Physico-chemical analyze: pH, humidity, fat, proteins, salt, ash, calcium, acidity;
- Microbiological analyze consisting of :
The counting of bacteria from the cheese was made after packing from 15 to 15 days for two months, the cheese being stored at a temperature of 5°C.

Microbiological Analysis.

For microbiological analysis, cheese samples (20 g) were diluted separately in 180 ml of 2% sodium citrate solution and homogenized (5 min) in a Lab Blender - Stomacher - Seward 80 Biomaster, and traditionally, plate counting has been the method of choice for viability assays, the plate counting described by Paola Camaschella and colleagues¹⁶. *Bacterial strains and growth conditions.* The strains used in this study are shown in **Table no.1**, strains were grown in GAM broth (Nissui Pharmaceutical Co, Tokyo, Japan) and incubated at 37°C in a Bactron X-2 SHEL LAB Anaerobic. *Statistical Analysis.* Data from viable counts were analyzed by the oneway MathCAD and Excel software.

Table no. 1.

Stain	Collection
<i>Lactobacillus acidophilus</i> La-5	-
<i>Lactobacillus casei</i> DSM 20011 ^T	German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany
<i>Bifidobacterium longum</i> JCM 1217 ^T	Japan Collection of Microorganisms, Saitama, Japan
<i>Bifidobacterium bifidum</i> JCM 1254 ^T	Japan Collection of Microorganisms, Saitama, Japan
<i>Bifidobacterium infantis</i> DSM 20088 ^T	German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

^T , type strain

3. Results and discussion

Sensorial and physio-chemical analyzes contents of moisture, pH, calcium,salt,

protein, ash, fat, and acidity, are presented in the following **Table no.1** and **Table no. 2.**

Table no. 2.

Senzorial characteristics

Aspect	The homogeneous without whey trinckling
Consistency	Godson, creamy, nonbrittle
Color	White-yellow, uniform
Smel and taste	Characterics of lactic fermentation

Table no.3.

Physico-chemical characterizes

pH	5,45
SU _T , [%]	45
Fat, [%]	14,23
Protein, [%]	23,48
Salt [%]	2
Calcium [%]	3,389
Ash, [%]	5,23
Acidity [°T]	108

In the following tables will be highlighted changes the probiotic microflora during storage from the Feta cheese long as 2 months , as seen on the ordinate and abscise will be represented logarithm number of cells / ml

The changing's of the probiotic microflora from the cheese that was stored for two months are studied according to the four combinations of bacteria colonies.

a). Combination: *Bifidobacterium Longum* si *Lactobacillus Acidophilus* during storage, the results that were obtained are presented in **Figure no.1** and **Table no.4**

Table no.4

Frequency of sampling days for counting (day)	The number of <i>Bifidobacterium Longum</i> (10^6 /cfu/ml)	The number of <i>Lactobacillus Acidophilus</i> (10^6 /cfu/ml)
0	6,29	6,33
15	6,89	7,09
30	6,56	7,81
45	6,32	6,59
60	5,95	6,11

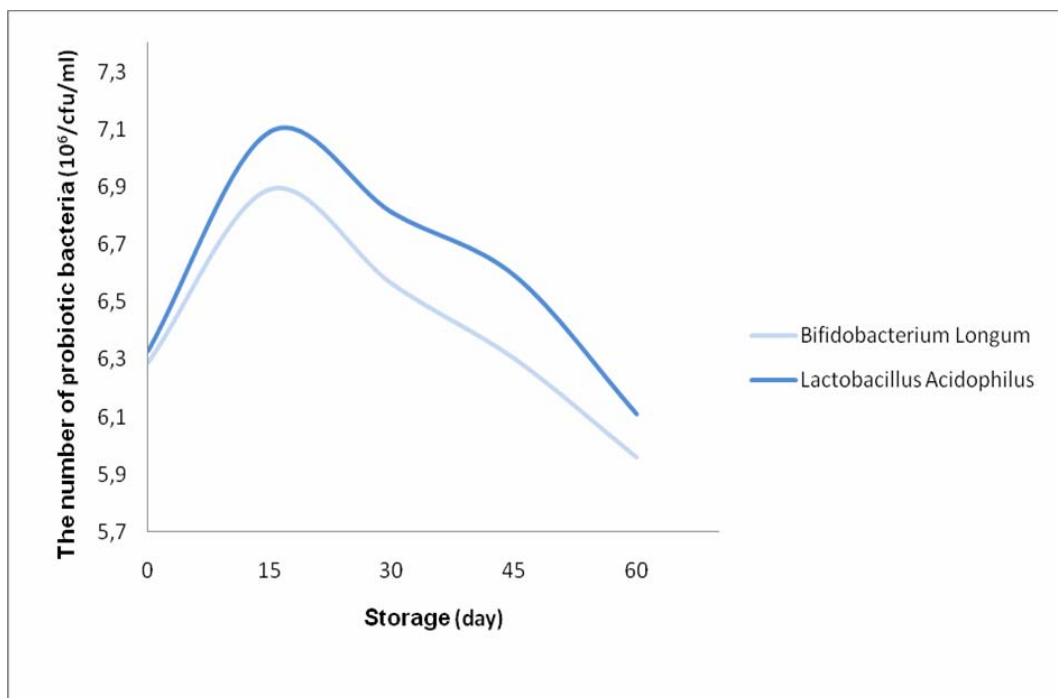


Figure no. 1 The survival level of the *Bifidobacterium Longum* si *Lactobacillus Acidophilus* colonies in cheese during storing

After counting the colonies of the *Bifidobacterium Longum* and *Lactobacillus Acidophilus* it was noticed that *Lactobacillus Acidophilus* had higher values for the whole maturation.

b). Combination: *Bifidobacterium Bifidum* and *Lactobacillus Casei* during storage , the results that were obtained are presented in **Figure no.1 and Table no.5**

Table no.5

Frequency of sampling days for counting (day)	The number of <i>Bifidobacterium Bifidum</i> (10^6 /cfu/ml)	The number of <i>Lactobacillus Casei</i> (10^6 /cfu/ml)
0	6.41	6.67
15	7.12	7.58
30	6.93	7.15
45	6.68	6.89
60	6.45	6.73

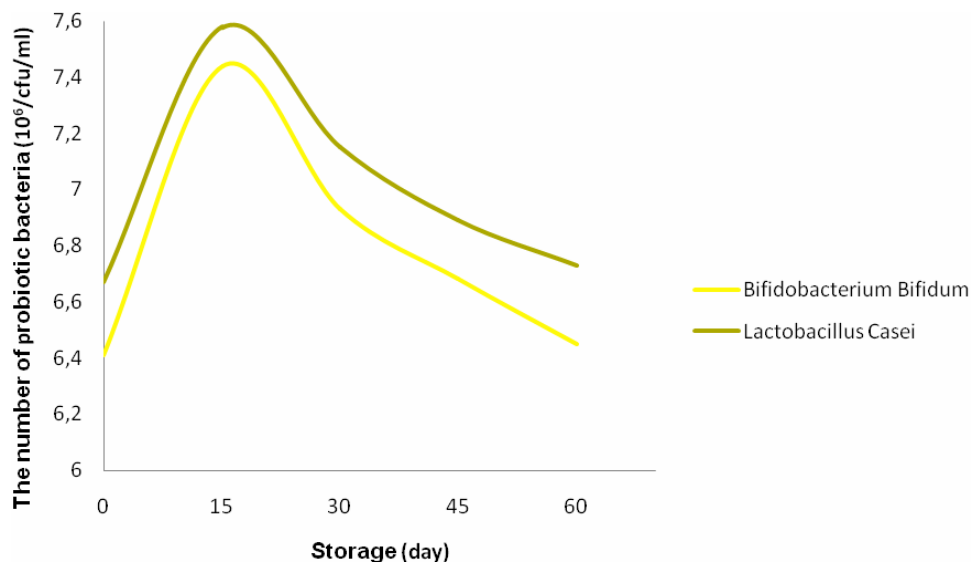


Figure no.2 The survival level of the *Bifidobacterium bifidum* and *Lactobacillus Casei* colonies in Feta cheese during storing

In this case survival lever in Feta cheese is satisfactory for the combination of *Bifidobacterium Bifidum* and *Lactobacillus Casei* cheese, being the most favourable case of *Bifidobacterium Bifidum* because it presents adaptability at some environment conditions such as: acid pH, and the adding of probiotic and lactic bacteria cultures

does not affect the Feta cheese composition, texture, flavour and they present resistency to alow pH.

c). **Combination:** *Bifidobacterium Bifidum* si *Lactobacillus Acidophilus* during storage, the results that were obtained are presented in **Figure no.3 and Table no.6**

Table no.6

Frequency of sampling days for counting (day)	The number of <i>Bifidobacterium Bifidum</i> (10^6 /cfu/ml)	The number of <i>Lactobacillus Acidophilus</i> (10^6 /cfu/ml)
0	6.23	6,35
15	7,78	7,01
30	7,48	6,87
45	6,18	6,63
60	5,96	6,16

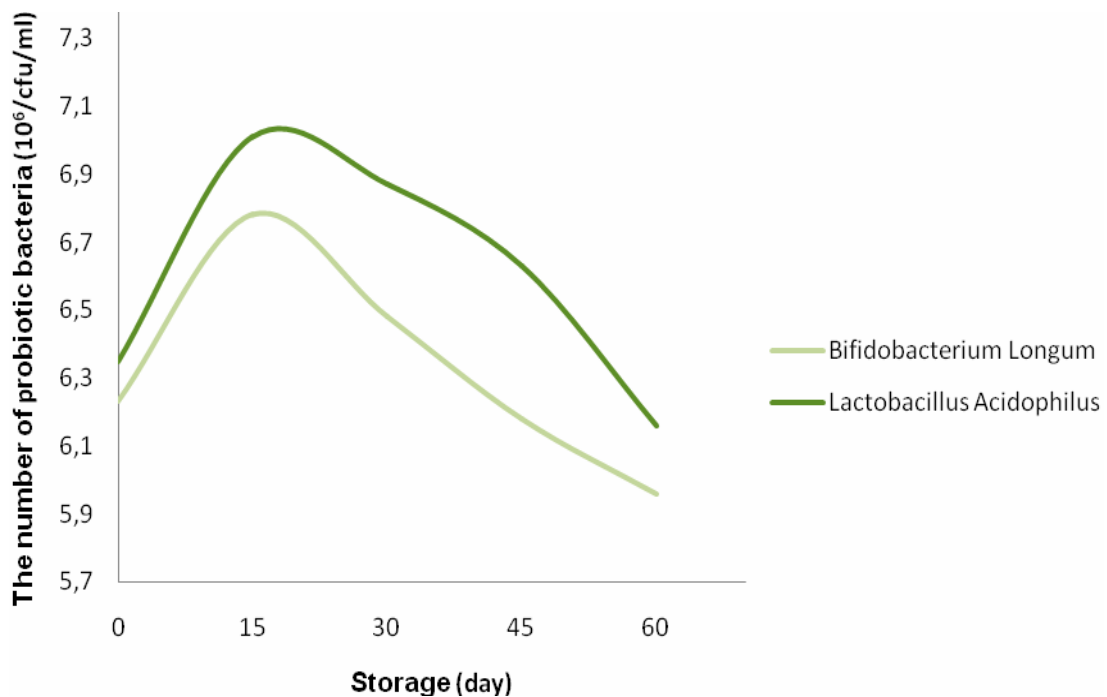


Figure no.3 The survival level of the *Bifidobacterium bifidum* and *Lactobacillus Acidophilus* colonies in cheese during storing

The survival lever of *Lactobacillus Acidophilus* a in this combination is bigger at the beginning and lower at the end, at this is due to an inhibition action caused by the excess lactic acid the was formed. The *Bifidobacterium Bifidum* cultures presented adatability to a lactic acid environment

because in this case the diminution number of cells is not as low as in other cases,

d). Combination: *Bifidobacterium Infantis* si *Lactobacillus Casei* during storage , the results that were obtained are presented in **Figure no. 4 and Table no.7.**

Table no.7

Frequency of sampling days for counting (day)	The number of <i>Bifidobacterium Infantis</i> (10^6 /cfu/ml)	The number of <i>Lactobacillus Casei</i> (10^6 /cfu/ml)
0	6.31	6,56
15	7,10	7,15
30	7,89	7,05
45	6,75	6,89
60	6,11	6,65

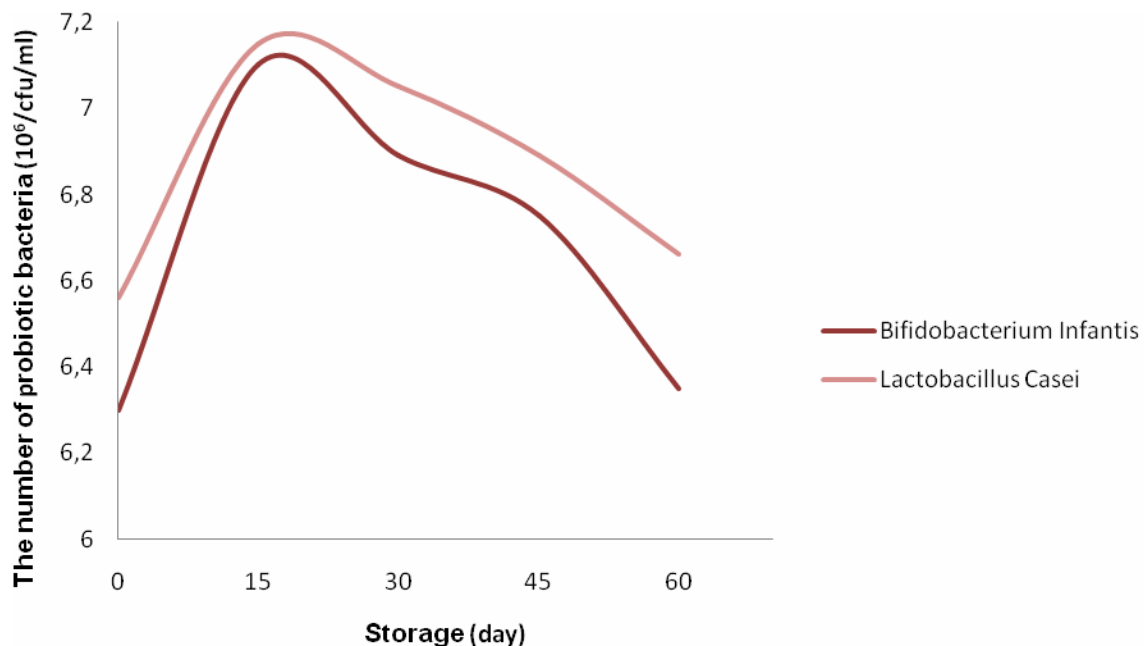


Figure no. 4 The survival level of the *Bifidobacterium* : *Bifidobacterium Infantis* si *Lactobacillus Casei* colonies in cheese during storing

The *Lactobacillus Casei* in this case has registered excellent survival conditions at the end of the storing period but the *Bifidobacterium Infantis* presented a diminution at the end of this period because of the sensibility of the stem.

4. Conclusions

In this study we can observe that making probiotic cheese were used different probiotics cultures and it was make an evaluation of this survival rate and adaptability at medium conditions from

curd. *Bifidobacterium Bifidum* and *Lactobacillus Casei* cultures used in combinations demonstrate a satisfactory survival among 2 months. In all cases the number of viable cells was over the level suggested by the producers.

Bifidobacterium Bifidum and *Lactobacillus Casei* culture as an adjunct probiotic culture instead, because *Lactobacillus Casei* is an acid-resistant microorganism^{11,15} and it survives in the cheese mass during ripening^{1,4,17}

The conclusions of this experiment was that (*Lactobacillus Casei* ,*Bifidobacterium*

Bifidus, *Bifidobacterium Infantis* and *Lactobacillus Acidophilus*) in the Feta cheese keeping maximum 2 months at 5 temperature. accrue a satisfactory survival rate and Bifidobacteria sensibility was due to a lower pH and also lower oxygen concentration.

The production of probiotic cheese in which the probiotic culture would survive and develop during manufacture and throughout its shelf life could lead to a major economic advantage.

These results demonstrated that *Bifidobacterium Bifidum* and *Lactobacillus Casei* cultures were more adaptable to the Feta cheese environment. In all cases, final numbers of viable cells were still above the levels suggested to produce their claimed health benefits.

The use of bifidobacteria as a starter adjunct to produce probiotic cheese has recently been applied in Gouda, Cheddar, and cottage cheeses^{2, 3, 8, 10}

A recent review updated the concept of prebiotics and suggested that other components suitable for inclusion in the diet, may exert specific effects upon gut bacteria². These were germinated barley foodstuffs, oligodextrans, gluconic acid, gentiooligosaccharides, pectic-oligosaccharides, mannan oligosaccharides, lactose, glutamine and hemicellulose rich substrates, resistant starch and its derivatives, oligosaccharides from melibiose, lactoferrin-derived peptides and N-acetylchito-oligosaccharides. As the use of more refined and reliable technologies are applied to prebiotic research then the list of candidate materials for food use is likely to grow.

A further important initiative for this area has been the development of an international society dedicated to prebiotic and probiotic research⁵

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