Cell Viability of *Lactobacillus plantarum* CM114 and *Lactobacillus rhamnosus* CW48 in skim milk during refrigeration

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Abstract

There is an increasing commercial interest to add probiotic bacteria to fermented dairy products. Besides basic nutrition, probiotic foods provide health benefits to the consumers. In order to maintain the claimed health benefit, the probiotic bacteria must ensure a greater survival and viability. Therefore, the microbiological enumeration of probiotic bacteria in fermented foods is essential.

This work aimed to evaluate the viable count of Lactobacillus plantarum CM114 and Lactobacillus rhamnosus CW48 in skim milk during refrigeration. Both the lactobacilli isolates were grown in skim milk at 37°C for 24h. Fermented skim milk was stored at two different refrigeration temperatures 4°C and -20°C. Viable cell count was determined in skim milk before storage and at an interval of 5 days during the total storage period of 15 days using standard pour plate method and the viability loss in percentage was calculated. Viable cell count of L. plantarum CM114 and L. rhamnosus CW48 was 48×10^9 and 62×10^9 CFU/ml respectively in skim milk before storage. The desired viability ($\geq10^6$ CFU /ml) was maintained by both the cultures at 4° and -20°C upto 15 and 10 days of storage period respectively. These cultures may provide good prospects for the development of new probiotic foods. They can be explored further for the actual capacity to exert functional effects.

Keywords: *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, skimmed milk, viability.

Introduction

Fermented milk products are created when milk ferments with lactic acid bacteria such as lactobacilli or bifidobacteria species. The fermentation process makes the fermented milk product easier to digest, especially for people who have milk allergies or are lactose-intolerant. Fermentation also increases the shelf-life of the milk product. Addition of probiotic bacteria in fermented milk also seems to help lower blood pressure, lower cholesterol and prevent cancer from growing.[3] These improvements might be due to the antioxidant or immune-stimulating effects of fermented milk products. In the modern life the intake of probiotic foods that improve health is increasingly sought by consumers.[4]

Monitoring the viability of probiotic microorganism is an important parameter in assessing probiotic product quality. Reliable and novel methods are essential for enumeration of bacteria and to monitor the possible physiological or biochemical changes in the probiotic bacterial population during the storage of commercial products.[5] The viability of probiotic bacteria in fermented foods depends on many factors including the strains used, temperature pH and storage period, amount of lactic acid and acetic acids produced during fermentation etc.[7,8]

The aim of the present study is to determine the viable count of lactobacilli in skimmed milk during refrigeration. The data from this study will be useful to verify the benefits of lactobacilli cultures in food products.

Material and Methods

Source and maintenance of cultures: *Lactobacillus plantarum* CM114 (LS992102) and *Lactobacillus rhamnosus* CW48 (LT79532) used in the present investigation were previously identified using morphological, biochemical and molecular characterization. *Lactobacillus plantarum* CM114 was isolated from raw camel milk and *Lactobacillus rhamnosus* CW48 was isolated from raw cow milk. Both the isolates showed probiotic properties such as antibacterial activity, bile salt tolerance and antibiotic resistance. Both the isolates were maintained using MRS broth and MRS agar at 37°C for 24 h.

Determination of viable cell count in skim milk during storage: Each culture was grown in MRS broth and inoculated at 1% concentration in skim milk. Skim milk tubes were then incubated at 37°C for 24 h. After incubation, samples were stored in refrigerator for 15 days. Viable count was determined initially after 24 h which was kept as control. After that, the tubes were stored at two different temperatures i.e. 4°C and -20°C in refrigerator and deep freeze respectively.

Viable cell count was determined by standard plate count method using MRS agar. Plates were incubated at 37°C for 48 h. The experiment was performed twice. The viable cell count was expressed as CFU/ml and calculated using the given formula:

$$\text{Numberofcolony forming units/ ml} = \frac{\text{Number ofcolonies}}{\text{Amount plated} \times \text{dilution}}$$

Results and Discussion

Viable cell count of *Lactobacillus plantarum* CM114 and *Lactobacillus rhamnosus* CW48 was determined in skim milk before storage and during the storage of 15 days at two different temperatures i.e. 4°C and -20°C. The viability was determined in CFU/ml and expressed in the form of percentage viability loss during storage period. The initial viable cell count of *L. plantarum* CM114 and *L. rhamnosus* CW48 in skim milk after 24 h of incubation before any storage was 48×10^9 and 62×10^9 CFU/ml respectively. During the storage period of 15 days gradual decrease in viable cell count at both the refrigeration temperatures (4°C and -20°C) was observed. The data for the same has been presented in figure 1 and 2 respectively.

For *L. plantarum* CM114, viability loss at 4°C during 15 days of storage ranged between 41.67 to 79.17%. Residual percentage viability after the end of storage period of 15 days was 20.83%. At -20°C, viability loss ranged from 70.83 to 77.08% up to 10 days of storage and 100% loss of viability loss was observed at the end of storage period of 15 days. The data for the same has been presented in table 1.

For *L. rhamnosus* CW48, viability loss at 4°C upto 15 days storage ranged from 53.22 to 82.25%. Residual percentage viability after the end of storage period of 15 days was 17.75%. At -20°C, percentage viability loss ranged from 64.51to 79.03% up to 10 days of storage and 100% viability loss was observed at the end of 15 days of storage. The data for the same has been presented in table 1.

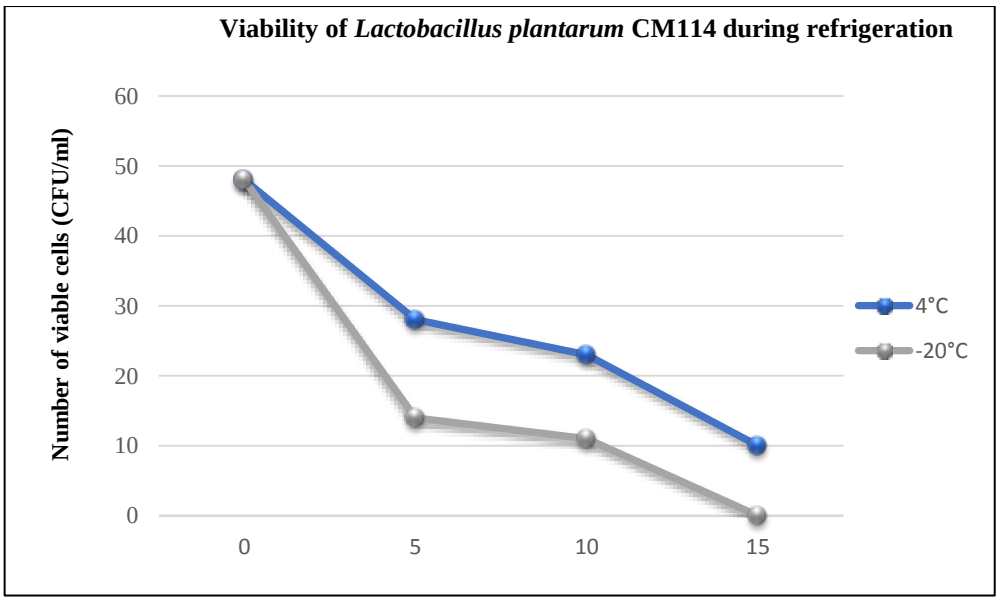

Figure 1: Number of colonies of *Lactobacillus plantarum* CM114 in skim milk during storage at 4°C and -20°C during 15 days of storage

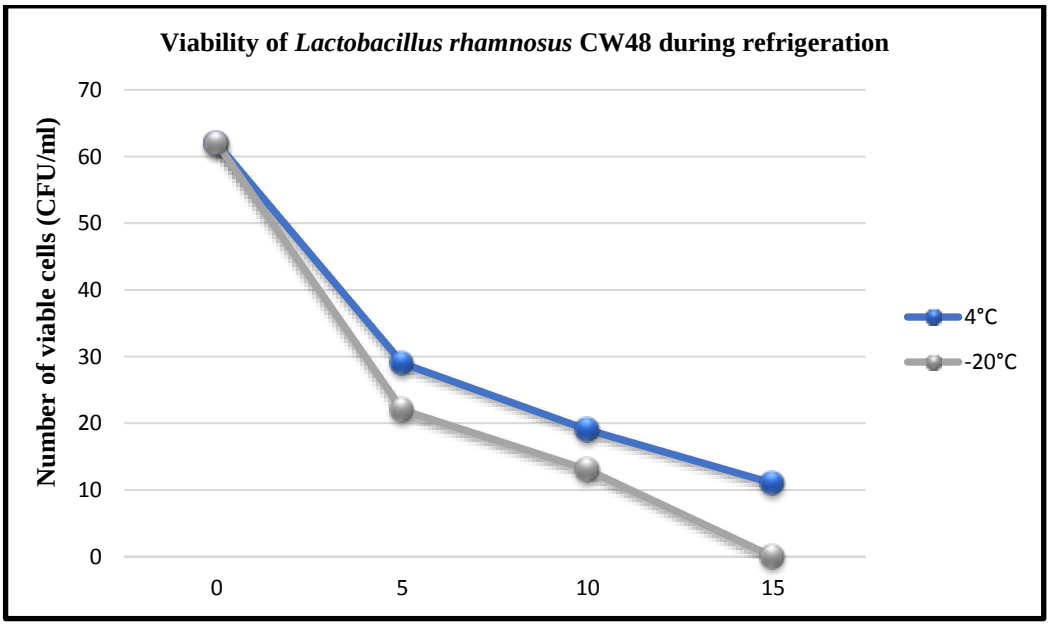

Figure 2: Number of colonies of *Lactobacillus rhamnosus* CW48 in skim milk during storage at 4°C and -20°C during 15 days of storage

Table 1
Percentage viability loss of *Lactobacillus plantarum* CM114 and *Lactobacillus rhamnosus* CW48 in skim milk during 15 days of storage period at 4°C and -20°C.

S.N.	Lactobacilli isolates	Storage temperature	Storage period (days)		
			5	10	15
1	*Lactobacillus plantarum* CM114	4°C	41.67%	52.08%	79.17%
		-20°C	70.83%	77.08%	100%
2	*Lactobacillus rhamnosus* CW48	4°C	53.22%	69.35%	82.25%
		-20°C	64.51%	79.03%	100%

Both the isolates *L. plantarum* CM114 and *L. rhamnosus* CW48 showed considerable viable cell count in skim milk at refrigeration temperature 4°C up to 15 days and at -20°C up to 10 days of storage period. Viable cells show inverse relationship with duration of storage, as the storage period increases loss of viable cells. Both the isolates *L. plantarum* CM114 and *L. rhamnosus* CW48 showed viable cell count in the range of 10^9 CFU/ml in fermented skimmed milk during storage in refrigerator. For both the isolates, viable cells were present at 4°C for 15 days and at -20°C for 10 days of storage period. The rate of survival of both *L. plantarum* CM114 and *L. rhamnosus* CW48 in skimmed milk was significantly greater at 4°C than -20°C.

Similar findings suggesting better survival rate of lactobacilli at 4°C were given by Canganella et al.[1] Present investigation is in agreement with above mentioned study. Lower viable counts (11×10^9 to 22×10^9 CFU/ml) in both the cultures at -20°C were linked to the low temperature of the product being a frozen preparation. Frozen environment is not optimum for the survival of bacteria.[2] The freezing process of the mix may cause a loss in the viable counts. The fluctuation in temperature causing large ice crystal formation during re-freezing may rupture bacterial cells and reduce viability.

In this study, both the isolates *L. plantarum* CM114 and *L. rhamnosus* CW48 showed the lowest viable counts (10×10^9 and 11×10^9 CFU/ml respectively at 4°C after 15 days of storage period) which were still more than the prescribed minimum viable count. A minimum viable number of 10^6 CFU/ml or gram was suggested while a viable count of 10^8 CFU/g was recommended to compensate for reduction during passage through the gut.[6] It is generally accepted that at the point of consumption of probiotic products, the probiotic bacterial count should be >1 x 10^6 CFU/ ml or gram and that a total of 10^8 to 10^9 probiotic microorganisms should be consumed daily if therapeutic effects are to be realized.

Conclusion

The traditional concept for control of cell viability acts as an important tool for determining the probiotic value of the food. *L. plantarum* CM114 and *L. rhamnosus* CW48 show probiotic properties including bile tolerance and antibacterial activity. These cultures maintain considerable viability when stored at low temperatures for more than a week. They can prove to be promising isolates and can be incorporated in fermented foods. Further investigations are also required to study the effect of probiotication on the sensory scores.

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