DOI : 10.5958/2277-8934.2020.00011.9 *Vol 27 No 1, p 77-79*

VIABILITY OF *Lactobacillus fermentum* CM36 AND *Lactobacillus rhamnosus* CW40 IN SKIMMED MILK DURING REFRIGERATION

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ABSTRACT

Two lactobacilli isolates namely *Lactobacillus fermentum* CM36 and *Lactobacillus rhamnosus* CW40 were used in present study. Both the lactobacilli isolates were grown in skimmed milk at 37°C for 24h. Fermented skimmed milk was stored at two different refrigeration temperatures, i.e. 4°C and -20°C. Viable cell count was determined in skimmed milk before storage and at an interval of 5 days during the total storage period of 20 days using standard pour plate method and the viability loss in percentage was calculated. Viable cell count of *L. fermentum* CM36 and *L. rhamnosus* CW40 was 52×10^9 cfu/ml and 88×10^9 cfu/ml, respectively in skimmed milk before storage. Viable cell count observed throughout the storage was in 10^9 cfu/ml range, 4°C proved to be a better storage temperature than -20°C for both the *Lactobacillus* isolates.

Key words: *Lactobacillus fermentum*, *Lactobacillus rhamnosus*, skim milk, viability

Lactic acid bacteria, particularly those belonging to non-pathogenic and beneficial genera (*Lactobacillus*, *Pediococcus*, *Lactococcus, Streptococcus,* and *Leuconostoc*) are widely used in food industry as biopreservative agents (Arokiyamary and Sivakumal, 2011). A large number of *Lactobacillus* species are frequently used because of their antagonistic activity against pathogenic and food spoilage microorganisms (Uraz *et al,* 2001).

Health benefits of probiotic microorganisms should be documented with strains and dosage requirement (Guarner and Schaafsma, 1998). Viability of probiotic organisms is affected by low pH conditions during the process of fermentation, oxygen distribution during storage and high acidity conditions during storage or after consumption in human stomach (Shah, 2007; Kailaspathy and Chin, 2000). The present investigation was undertaken to study the viability of lactobacilli in fermented food product.

Materials and Methods

Source and Maintenance of Cultures: *Lactobacillus* used in the present investigation were previously identified using morphological, biochemical and molecular characterisation. *Lactobacillus fermentum* CM36 was isolated from raw camel milk and *Lactobacillus rhamnosus* CW40 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, MRS agar after incubation at 37°C for 24 h and skimmed milk was used for storage of isolates.

Determination of viability in skimmed milk during storage: Each culture was grown in MRS broth and inoculated at 1% concentration in skimmed milk. Skimmed milk tubes were then incubated at 37°C for 24 h. After incubation, samples were stored in a refrigerator for 3 weeks. Viability was determined initially after 24 h which was kept as control. After that, the tubes were stored at 2 different temperatures i.e. 4°C and -20°C in refrigerator and deep freeze, respectively. Viability 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:

$$\text{Number of cells/ ml} = \frac{\text{Number of colonies}}{\text{Amount plated} \times \text{dilution}}$$

Results and Discussion

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. fermentum* CM36 and *L. rhamnosus* CW40 after 24 h

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of incubation before any storage was 52×10^9 cfu/ml and 88×10^9 cfu/ml, respectively. During the storage period of 20 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 Figs 1 and 2, respectively.

Percentage viability loss observed in both the lactobacilli isolates is represented in Table 1. For *L. fermentum* CM36, viability loss at 4°C during 20 days of storage ranged between 11.53 to 80.76%. Residual percentage viability after the end of storage period of 20 days was 19.24%. At -20°C, viability loss ranged from 26.92 to 57.69% up to 15 days of storage and 100% loss of viability loss was observed at the end of storage period of 20 days. For *L. rhamnosus* CW40, viability loss at 4°C up to 15 days storage ranged from 77.27 to 97.72%, after 20 days of storage period 100% viable cells were lost. At -20°C, percentage viability loss ranged from 84.09 to 88.63%, 100% viability loss was observed at the end of 15 days of storage and onwards. In the case of *L. rhamnosus* CW40 viable cell count decreased sharply during storage. Problems with the stability of strains of lactobacilli in fermented milk products have been reported earlier (Gilliland and Speck, 1977 and Shah *et al,* 1995). The number of viable cells decline greatly with time indicating poor survival rate of *lactobacilli* as probiotic starter culture. Results of the present investigation were in agreement with the above mentioned studies in the case of *L. rhamnosus* CW40. The production of hydrogen peroxide by starter culture during storage may adversely affect the viability of probiotic culture (Hull *et al,* 1984), and this can be the probable reason of low viable cell count in the case of *L. rhamnosus* CW40.

Viable cells showed inverse relationship with duration of storage, as the storage period increases loss of viable cells was observed. Both the isolates *Lactobacillus fermentum* CM36 and *Lactobacillus rhamnosus* CW40 showed viable cell count at the concentration 10^9 cfu/ml in fermented skim milk during storage in refrigerator. For *L. fermentum* CM36

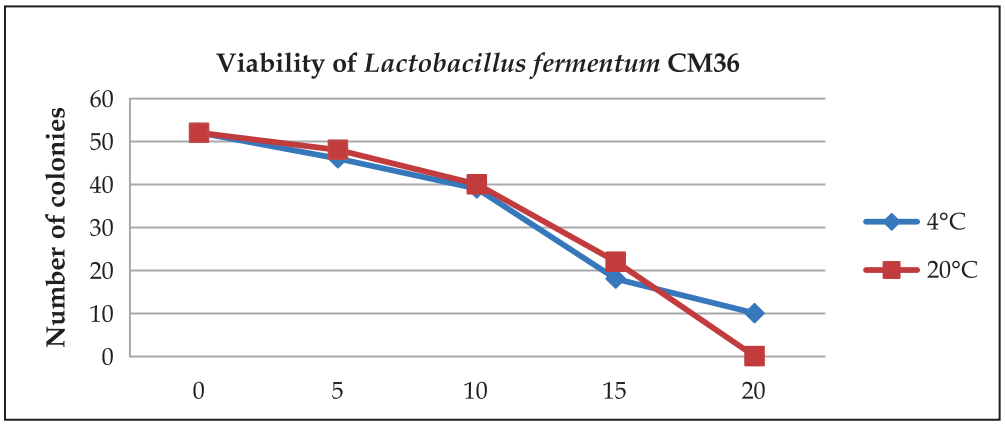

Fig 1. Number of colonies of *Lactobacillus fermentum* CM36 in skim milk during storage at 4°C and -20°C during 20 days of storage.

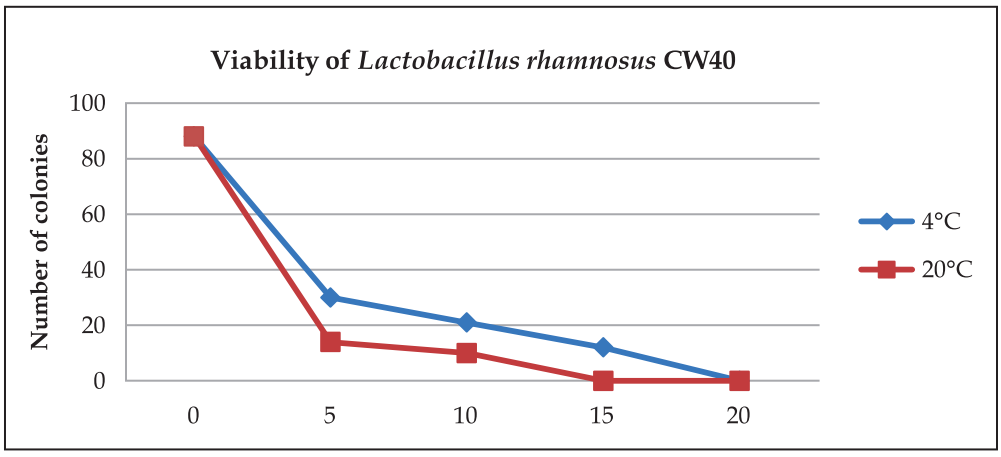

Fig 2. Number of colonies of *Lactobacillus rhamnosus* CW40 in skim milk during storage at 4°C and -20°C during 20 days of storage.

Table 1. Percentage viability loss of CM36 and CW40 during 20 days of storage at 4°C and -20°C, at a 5 days interval (Compared to the reference point of initial viable count after inoculation in skim milk and 24 h incubation without storage).

S.No.	Lactobacilli isolates	Storage temperature	Storage period (days)			
			5	10	15	20
1	*Lactobacillus fermentum* CM36	4°C	11.53%	25.0%	65.38%	80.76%
		-20°C	26.92%	42.30%	57.69%	100%
2	*Lactobacillus rhamnosus* CW40	4°C	77.27%	88.63%	97.72%	100%
		-20°C	84.09%	88.63%	100%	100%

viable cells were present at 4°C for 20 days and at -20°C for 15 days. For *L. rhamnosus* CW40 viable cells were present at 4°C for 15 days and at -20°C for 10 days. There was an adequate number of probiotic bacteria needed to be consumed in order to avail their health benefits and the minimal number of probiotic bacteria in a product should be above 10^6 or 10^7 per gram or ml to exhibit health benefits (Lahteinen *et al*, 2010). Results of the present investigation were in accordance with the above mentioned study. The rate of survival of both *L. fermentum* CM 36 and *L. rhamnosus* CW 40 in skimmed milk was significantly greater at 4°C than -20°C. Similar findings suggesting better survival rate of lactobacilli at 4°C was given by Canganella *et al* (2000).

It was concluded that *Lactobacillus fermentum* CM36 and *Lactobacillus rhamnosus* CW40 showed good viability during refrigeration and also possessed antibacterial activity, bile tolerance and antibiotic resistance. Therefore, both isolates proved to be potential strains and can be used as a starter culture for the production of fermented food products to impart health benefits to the consumer.

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