GROWTH STABILITY OF POTENTIAL PROBIOTIC, LACTOBACILLUSFERMENTUMCM5 ISOLATED FROM CAMEL MILK DURING STORAGE

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ABSTRACT

The study of this research work aimed to evaluate the survival and preservation of the cell viability of the (LAB) lactic acid bacteria *lactobacillus fermentum*. The microorganism was isolated specifically *lactobacillus fermentumCM5*. The culture was inoculated at the concentrations of 1% in skim milk and incubated for (37°C) for 24 h. Viability was determined after 24 h which was saved as control. Then the viability was checked after 1, 3, and 7 days of storage with different temperatures. Viability was determined by using the standard plate count method using de Man, Rogosa, and Sharpe (MRS) agar In this technique, samples were serially diluted with NaCl (saline solution) 0.85% w/v ranging from 10 -1 to 10 -10 serial dilutions. An aliquot of 100µl of each dilution was then switching to Petri plat and mixed with MRS agar and plats were incubated at (37°C) for 24 h. The viability was determined by counting the number of colonies determined after the incubation and was expressed as CFU/ml.

Keywords: *Lactobacillus fermentum*, viability, skim milk, colony-forming unit

1. INTRODUCTION

The World Health Organization and Food and Agriculture Organization of the United Nations (WHO/FAO) the definition of probiotics as living organism. To grown the expected health benefits as WHO/FAO describes as potential probiotic bacteria must be consumed.(Gorbach, 1987). Many researchers consider the viable number of cells required to affect the gastrointestinal environment is between 106 to 109 CFU/ml or CFU/g of the food and dairy products. This accepted dose is named as the therapeutic minimum, (Shah and Lahtinen, *et al*., 2000-2006).

However, a probiotic dairy and food product is considered useful only, if it consists of 107 CFU/ml at the time of consumption (Charalampopoulos *et al.*, 2002).In choosing probiotic microorganisms for industrial production approaches safety and technological characteristics to have to be considered. Functional properties consist of the viability of cells, balance of the cells within a food matrix, persistence activity in the gastrointestinal tract, species, and strain characteristics, fermentation technology, and availability of prebiotics (Tropcheva,R. *et al.*, 2013).

Prebiotics are described as non-digestible food substances that, when consumed in enough amounts, selectively stimulate the increase and/or activity of one or a constrained number of microbes in the colon resulting in documented health benefits, (Baele*et al.,* 2002). Prebiotics has introduced in functional probiotic food 2 formulations to increase the viability of probiotics and the shelf life of the product. Prebiotics can beaded separately or sometimes the food matrix itself has components with prebiotic properties including beta-glucan in oats. Hence, the use of such food matrices for fermentation can be more effective to supply probiotics than capsules (Sanders and Marco *et al.,* 2010). Mostly, Bifidobacteria growth is promoted by prebiotics and products that preserve the combination of probiotics and prebiotics are called “synbiotics”.(Orla-Jensen 1919).

The probiotic bacteria, which are used industrially as a food ingredient, can be obtained as freeze-dried cultures. The probiotics undergo a great load during processing and storage conditions of the lyophilized cultures and also in the nutrient matrix thereafter production process. Survival of probiotics and an appropriate nutritional matrix is also affected by an apesta factor, including pH, post-acidification (during storage) and fermented products, hydrogen peroxide production, oxygen poisoning (packaging via packaging), storage temperatures, stability in dried or frozen form, and lack of proteases (Kailasapathty, 2002).All these factors increase the stress on the microorganisms and their physiological and biological functions or the functionality of cells in the matrix. Stress conditions can cause probiotic cells to cause "sublethal injury", preventing cell division (Oliver *et al*., 2005). Lactic acid bacteria have defense mechanisms such as stress-induced protein production to regulate stress conditions and maintain the viability in food matrices under such conditions, cells cannot be cultured, but remain alive.

For commercial production processes, starter cultures are selected that contain large number of viable and undamaged cells. Usually, freeze-dried cultures of lactic acid bacteria containing more than 10^{11} CFU / g are used commercially (Mattila-Sandholm, 2002). Selecting a nutritional matrix that can support a probiotic culture at effective levels is one thing challenging. Most probiotic products have been dairy-based since ancient times. Dairy is a very substrate nutritious, The demand for foods with low fat and low cholesterol have led to a growing demand for non-dairy probiotics Products. (Kandler,O 1986). Consequently, whole grains, which contain prebiotic components in the bran are receiving more attention as potential substrates. While granola bars and oats contain drinks are available in the market as well as many other grains such as wheat, barley and malt have also been tested experimentally as food matrices for probiotics Puerto de viability of the inoculated probiotic strains (Charalampopoulos, 2002).

For commercial probiotic products, it is very important to maintain life stability. microorganisms during the shelf life of the products. Although there are some opinionsthat even dead / inactive cells can act as probiotics, viability is a key factor to get started culture. The carrier food must contain the expected viability. probiotics count until the best before date.

2. MATERIALS AND METHODS

2.1 Sample Source and maintenance:

Sample Source of *Lactobacillus fermentum* CM5. The research work was carried out in the Molecular Microbiology Laboratory of Mohanlal Sukhadia University Udaipur to provide

Lactobacillus fermentum CM5 isolated from camel milk. Details of the materials and method used in the study are given below.

The culture was grown in *Lactobacillus* MRS Broth for 48 hours at 37 ° C, the culture was inoculated at a concentration of 1% in skim milk and incubated for 48 hours at 37 ° C, and the incubation process maintains the accurate temperature for bacterial growth and results in curdling.

2.2 Medium Compositions:

All media components and prepared media were used in the study of analytical quality, obtained from Hi-media (India), Merck (India) and chemical company Sigma (US).The media was prepared in double distilled water and sterilized by autoclaving technique at 12psi for 15 minutes, except skim milk which was sterilized at 12psi for 15 minutes.

2.3 Viability of *Lactobacillus fermentum* CM5 in skimmed milk during storage:

The culture was inoculated into skim milk at concentrations of 1% and incubated at 37 ° C for 24 hours. Viability was initially determined after 24 hours it was kept as a control. Subsequently, the viability was checked after 1, 3 and 5 days of storage. The viability was determined by the standard plate count method using de Man, Rogosa, and Sharpe (MRS) agar. In this method, the samples were serially diluted with saline. 0.85% w / v, ranging from 10 -1 to 10 -10 serial dilutions. A 100 µl aliquot of each dilution was then transferred to a Petri dish and mixed MRS agar and plates were incubated at 37 ° C for 24 hours. Viability was observed by the counting number of colonies. After incubation and was expressed as CFU/ ml.

Influence of some factors: temperature, prebiotics, sweeteners, and vitamins on the viability of skimmed milk during storage examine as follows:-

2.4 Effect of Sweeteners on Viability during Storage:

The effect of sweeteners such as sorbitol and sucrose on storage viability was studied using each sweetener in milk in two different concentrations (1 and 2%). The cultures were inoculated at a concentration of 1% and incubated at 37 ° C for 24 hours. The cultures were then stored in the refrigerator. Reliability was determined as indicated in the table.

2.5 Effect of Prebiotics on Viability during Storage:

The effect of prebiotics such as raffinose and inulin was studied by adding each prebiotic in two different concentrations 1 and 2% in skim milk and sterilized at 12 psi for 15 minutes. The culture was inoculated at a concentration of 1% in skim milk containing prebiotics. Incubated at 37 ° C for 24 hours. The culture was then kept in the refrigerator. Viability was determined as shown in the table.

2.6 Effect of vitamins on storage viability:

The effect of vitamins, namely thiamine and riboflavin, on storage viability was determined by adding each vitamin to skim milk at two different concentrations 1 and 2%. Culture inoculated at a concentration of 1% and incubated at 37 ° C for 24 hours. Culture then stored in the refrigerator. Viability was determined as shown in the table.

3.1 RESULT AND DISCUSSION

The survival of probiotic bacteria in dairy products and the viability of *lactobacilli* are important in the provision of a number of therapeutic agents. Consumer benefits many dairy products have now been reformulated with *Lactobacillus* strains to provide important health benefits. Probiotic cultures in food must be well defined and named correct, taxonomic, and sufficiently available in viable state quantities over the entire shelf life.

The use of lactobacilli as additives in human food is traditionally known. Research on its characterization and the beneficial effects are still active since the lactobacilli used as probiotics are isolated from different sources and it is quite difficult for everyone. Strains that possess all the desired properties require a thorough analysis of their functional properties. Based on the above facts, the current research aimed to isolate and identify the viability of lactobacilli and was studied under the influence of several factors. Like vitamins, sweeteners, temperature, and prebiotics. *Lactobacillus CM5* was tested for viability during storage. Effect of The viability of several was studied, such as temperature, sweeteners, prebiotics, and vitamins.

The viability of *Lactobacillus fermentum CM5* was investigated in skim milk during 5 days of storage. The effect of some factors such as Temperature, sweeteners, vitamins, and prebiotics was studied on the viability of the organism in skimmed milk during different storage. The viability of *Lactobacillus fermentum CM5* after an incubation period of 24 hours before any storage was determined in CFU/ ml and was used as a control.

3.2 Effect of Temperature on viability during Storage:

The viability of *Lactobacillus fermentum CM5* was tested in skimmed milk for 5 to 7 days of storage under the influence of two different temperatures, (4°C and -20°C). At a temperature of 4 °C, the viability of *Lactobacillus fermentum CM5* was studied, after inoculation of 24 hours without storage viability was determined after storage at 4°C for 24 hours, the viability was found to be 10^9× 2.26, after 3 day of storage 10^9× 2.11, and the 5 days storage is 10^9× 1.19 CFU / ml, viability increasing with decreases storage period. Table 3.2 (a).At -20°C, the viability of *Lactobacillus fermentum CM5* was studied, after inoculation and incubation of 24 hours without storage viability was determined. After storage at -20°C for 24 hours, the viability was found to be 10^9× 2.20, up to 3 days storage 10^9× 1.51 And the storage period of 5 days is 10^9× 0.98 CFU / ml. Viability gradually decreased as storage. The period was extended at the temperature of -20° C. Table 3.3. A marked decrease in viability was found as the storage times were extended to 4° C and -20°C. After the storage period of Storage viability of 1 day, 3 days and 5 days was observed. The isolated *Lactobacillus fermentum CM5* viability was found better at -20°C storage compared to 4°C. The effect of other factors such as sweeteners, vitamins, and prebiotics on Viability in skim milk was examined during storage at -20°C.

3.2.1 Effect of Different Temperature on Viability of *Lactobacillus fermentum CM5*:

S. No	Storage (In days)	Control	Viability at different temperature	
		10ˆ9×2.83	4°C	20°C
1			10ˆ9–0.58	10ˆ9 – 2.20
2			10ˆ9–0.44	10ˆ9 - 1.51
3			10ˆ9– 0.39	10ˆ9 - 0.98

3.2.3 Effect of Different Sweeteners on Viability of *Lactobacillus fermentum CM5*

1. Effect of sorbitol on viability of *Lactobacillus fermentum CM5*:

S. No	Storage (In days)	Control	Viability at different temperature	
		10ˆ9×2.05	4°C	20°C
1			10ˆ9–2.26	10ˆ9 – 1.50
2			10ˆ9–2.12	10ˆ9 - 0.59
3			10ˆ9– 1.20	10ˆ9 - 0.48

2. Effect of Sucrose on viability of *Lactobacillus fermentum CM5*

S. No	Storage (In days)	Control	Viability at different temperature	
		10ˆ9×1.50	4°C	20°C
1			10ˆ9–0.41	10ˆ9 – 0.48
2			10ˆ9–0.33	10ˆ9 - 0.34
3			10ˆ9– 0.38	10ˆ9 - 0.32

3.2.4 Effect of Different Prebiotics viability of *Lactobacillus fermentum CM5*

1) Effect of refiinose on viability of *Lactobacillus fermentum CM5*

S. No	Storage (In days)	Control	Viability at different temperature	
		10ˆ9×2.83	4°C	20°C
1			10ˆ9–0.58	10ˆ9 – 2.20
2			10ˆ9–0.44	10ˆ9 - 1.51
3			10ˆ9– 0.39	10ˆ9 - 0.98

2) Effect of inulin on viability of *Lactobacillus fermentum CM5*

S. No	Storage (In days)	Control	Viability at different temperature	
		10ˆ9×1.50	4°C	20°C
1			10ˆ9–0.41	10ˆ9 – 0.48
2			10ˆ9–0.33	10ˆ9 - 0.34
3			10ˆ9– 0.38	10ˆ9 - 0.32

CONCLUSION

One of the most important factors is probiotics. Today, there is a sharp increase in the consumption of probiotic bacteria with the help of food products, mainly probiotic dairy products. The nutritional and therapeutic effects of food on health have made consumers aware of functional foods and their demand. Functional foods are growing rapidly around the world.

In the present study, the lactobacilli were isolated from milk; the isolated was screened for different probiotic attributes so that they could be applied for the promotion of the host health. The result revealed the potential probiotic properties of the Lactobacillus fermentum CM5 isolated from camel milk. The Lactobacillus fermentum CM5 survived in the MRS broth supplemented with the different concentrations. The Lactobacillus fermentum CM5 maintained their total cell count at varying degree. This strain useful for the preparation of functional food products for the prevention of highly prevalent disease.

The result revealed the potential probiotic properties of Lactobacillus fermentum CM5 isolated from camel milk. The Lactobacillus fermentum CM5 survived in the MRS broth supplemented with the different concentrations. This strain is useful for the preparation of functional food products for the prevention of many common diseases. Lactobacillus fermentum CM5 showed viability during storage in skimmed milk. They meet the minimum viable counts to achieve beneficial health effects. Survival at two different temperatures 4 and -20 ° C when viability was found at an acceptable level 10ˆ8-10ˆ9 CFU / ml. The addition of sweeteners, prebiotics, vitamins improved the viability reaction of lactobacillus fermentum CM5 in skimmed milk for 5 to 7 days of storage at two different temperatures (4 °C and -20°C). Therefore, the inclusion of these factors will reduce the effectiveness. Skim milk enhances dairy products. With the right combination of factors, the strain increased during the product storage period, providing benefits to the consumer. Therefore, this strain can be used as probiotics after in vivo testing following standard procedures.

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