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Viability of Potential Probiotic *Lactobacillus Fermentum* CM6 Isolated from Camel Skim Milk during Storage

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Abstract: ***This study aimed to evaluate the survival and maintenance of the cell viability of the lactic acid bacteria lactobacillus fermentum. The bacteria were isolated namely lactobacillus fermentum CM6. Culture was inoculated at the concentrations of 1% in skim milk and incubated at 37°C for 24 h. Viability was determined initially after 24 h which was kept as control. Then the viability was checked after 1, 3 and 5 days of storage period. Viability was determined by standard plate count method using MRS agar. In this procedure samples were serially diluted with saline solution (0.85% w/v) ranging from 10^{-1} to 10^{-10} dilitions. An aliquot of 100µl of each dilution was then transfer to petri plat and mixed MRS agar. The plats were incubated at 37°C for 24 hour. The viability was determined by counting the number of colonies observed after incubation and was expressed as cfu/ml.***
Keywords: *lactobacillus fermentum, viability, skim milk, colony forming unit*

I. INTRODUCTION

Lactic acid bacteria (LAB) are a group of Gram positive, low GC, catalase negative, acid tolerant, nonrespiring, nonsporulating rod or cocci that synthesise lactic acid as the major metabolic endproduct during the fermentation of carbohydrates Axelsson. These bacteria are a heterogeneous group of organisms with a diverse metabolic capacity. For this reason, they are highly adapted to a wide range of conditions making them extremely successful in food and feed fermentations. They are responsible for the fermentation of, for example, sauerkraut, sourdough, cassava, all fermented milks, pickled vegetables and silage. They are heterotrophic and generally have complex nutritional requirements due to the lack of some biosynthetic pathways. Most species have several requirements for amino acids and vitamins. Because of this, they can only be found where these requirements can be met Charalampopoulos. In sum, they are a group of organisms that are diverse but physiologically similar, specialized in nutrient rich environments, limited in biosynthetic ability, and with a metabolism aimed at acid production. Lactobacilli are the largest genera in LAB. They are a very heterogeneous group, widespread in nature and containing the most acid tolerant species. Species such as Lactobacillus plantarum and L. casei can be found in a number of different environments whereas other species such as L. sanfransiscensis and L. delbrueckii are found only in certain habitats Axelsson. Lactobacilli can be divided into three groups according to the principal carbohydrate pathway employed by the species Axelsson. Lactobacilli are found in where rich, carbohydrates-containing substrate are available, and thus, in a variety of habitats such as mucosal membranes of humans and animals, (mainly in oral cavity, intestine, and vagina) and on plant material and fermenting food. The organisms are widely distributed in animal feeds, silage, manure, and milk and milk products. According to the proposed concept on the efficacy of probiotic application, the probiotic bacteria must be viable at the time of consumption to achieve beneficial function. Official standards for the minimum suggested level for probiotics in the food to attain this viability require a minimum of 106-107 cfu/g, which have been introduced by several food organizations worldwide. Drying is widely used as a means of preservation of bacterial cells. Because high water activity dramatically decreases the viability of probiotics, removal of water can effectively extend the shelf life of probiotic products. Drying is widely used as a means of preservation of bacterial cells. Because high water activity dramatically decreases the viability of probiotics, removal of water can effectively extend the shelf life of probiotic products. Freeze-drying is another practicable method to improve the viability of probiotics is to immobilize the bacteria in an external protective matrix, which can improve their resistance to adverse conditions and facilitate better survival in specific food products . The technique of microencapsulation based on complex (w/o/w) dispersion offers several advantages for the immobilization of probiotics. The condition of lactobacilli such as grown aerobically and anaerobically at 37°C in MRS medium. oxygen, and the type of neutralizer, have a large effect on the growth activity of lactobacillus.

Among these, the types of growth media used play an impor-tantroleinthe growth activity. Freeze-dried probiotic microorganisms that were stored at refrigerated conditions had lower viability than those stored at frozen temperature due to slight metabolic and cellular activity at the refrigerated temperature. The types of growth media, Various growth media for lactic acid bacteria, such as MRS broth, M-17, Elliker 450C, lactococci and vagococci at 100C, but not at 450C. Streptococci do not grow at 100C, while growth at 450C in dependent on the species Axelsson (1993). Salt tolerance (6.5% NaCl) may also be used to distinguish among enterococci, lactococci/vagococci, and streptococci, although variable reactions can be found among streptococci Axelsson (1993). Extreme salt tolerance (18% NaCl) is confined to genus Tetragenococcus. Tolerances to acid and/or alkaline conditions are also useful characteristics.

Main Application of Lactobacillus are important in the production of foods that require lactic acid fermentation, notably dairy products (yogurt and cheese), fermented vegetables (olives., pickles, and sauerkraut), fermented meats (salami), and sourdough bread. The use of lactobacilli in the food industry has a long history. They are preventing diarrhea in hospitalized adults. Drinking a specific beverage containing Lactobacillus casei, Lactobacillus bulgaricus, during antibiotic treatment and for a week afterwards significantly decreases the risk of developing diarrhea. Clinical research shows certain strains of Lactobacillus might help treat bacterial vaginosis when applied inside the vagina. Researchers have found Lactobacillus acidophilus suppositories (Vivag, Pharma Vinci A/S, Denmark) and vaginal tablets (Gynoflor, Medinova, Switzerland) may be effective. Researchers also found that vaginal capsules Lactobacillus gasseri and Lactobacillus rhamnosus, seem to lengthen the time between infections. They also Helping prescription medications treat Helicobacter pylori (H pylori) infection, which causes stomach ulcers. In this genus are used for a wide variety of applications. These applications include food and feed fermentation. Have natural resistances to certain antibiotics and chemotherapeutics. They are considered potential vectors of antibiotic resistance genes from the environment to humans or animals to humans. Lactobacillus has been identified as potential probiotic. Lactobacillus species administered as a probiotic have been found be an effective treatment for irritable bowel disease and have no negative side effects L. acidophilus is used to prevent necrotizing entercolitis and other neonatal infections Some Lactobacillus species have been associated with cases of dental caries. Lactic acid can corrode teeth, and the Lactobacillus count in saliva has been used as a "caries test" for many years, they use as probiotics. Importance of probiotic consumption in humans : The number of food and other dietary adjuncts products containing live Bifidobacterium and Lactobacillus bacteria have significantly increased over the last 20 years due in part to the beneficial effects these probiotic organisms are believed to provide (Laroia and Martin, 1990). Although research is ongoing, the available evidence indicates that ingestion of probiotic bacteria may promote desirable changes in the gastrointestinal tract of humans (Kaplan and Hutkins, 2000). Probiotic foods are becoming increasingly popular. A number of health benefits have been claimed for Bifidobacterium sp. and therefore inclusion of these organisms in the diet is considered to be important in maintaining good health, Champagne (1996). Probiotics have anticarcinogenic properties, a specific probiotic effect, which are of three types: (1) elimination of procarcinogens; (2) modulation of procarcinogenic enzymes; and (3) tumour suppression (Wollowski et al., 2001 and Grill et al., 1995a). Furthermore, consumption of these organisms is an ideal method to re-establish the balance in the intestinal flora after antibiotic treatment (Gibson et al., 1994). There is a growing agreement relating to the beneficial aspects of specific dairy products such as fermented milk and yoghurt and of bacterial cultures that ferment the dairy products in human and animal nutrition. Experimental and epidemiological studies provide evidence that fermented milk and bacterial cultures that are routinely used to ferment the milk reduce the risk of certain types of cancer and inhibit the growth of certain tumours and tumour cells (Reddy and Rivenson, 1987).

II. MATERIAL AND METHODS

Material and methods used in the study are as follows :

A. Source of Lactobacillus fermentum CM6

The research work was conducted in Molecular Microbiology Laboratory at University of Mohanlal Sukhadia Udaipur, to provide isolated *Lactobacillus fermentum* CM6 from camel milk. The detail of materials and method used in the study, are given below :-

B. Maintenance of Lactobacillus fermentum CM6

The culture was grown in MRS broth at 37° C for 48 hours ,culture was inoculated a concentration 1% in skim milk and incubated at 37 ° C for 48 hours, the incubation maintains optimal temperature for growth and result in curding.

C. Medium Compositions

All medium components and ready prepared media used in the study were of analytical grade obtained from Hi-media (India), Merck (India) and sigma chemical company (USA).

The media were prepared in double distilled water and sterilized by autoclaving at 15psi 15 minutes except skim milk which was sterilized at 12psi for 15 miniutes.

D. Viability of Lactobacillus fermentum CM6 in Skim milk During Storage

Culture was inoculated at the concentrations of 1% in skim milk and incubated at 37°C for 24 h. Viability was determined initially after 24 h which was kept as control. Then the viability was checked after 1, 3 and 5 days of storage period. Viability was determined by standard plate count method using MRS agar. In this procedure samples were serially diluted with saline solution (0.85% w/v) ranging from 10^{-1} to 10^{-10} dilitions. An aliquot of 100µl of each dilution was then transfer to petri plat and mixed MRS agar. The plats were incubated at 37°C for 24 hour. The viability was determined by counting the number of colonies observed after incubation and was expressed as cfu/ml.

E. Factors Affecting Viability during Storage

Influence of various factors such as temperature, sweeteners, prebiotics, and vitamins on viability in skim milk during storage was determined as follows :-

F. Effect of Sweeteners on viability during Storage

Effect of sweeteners such as sucrose and sorbitol on viability during storage was studied by supplementing each sweeteners in skim milk at two different concentration (1and 2%) . cultures were inoculated at 1% concentration and inocubated at 37°C for 24 h . The cultures were then stored in refrigerator .Viability was determined as given in table

G. Effect of Prebiotics on Viability during Storage

The effect of prebiotics such as inullin and raffinose was studied by adding each prebiotic at two different concentration (1 and 2%) in skim milk and sterilized at 12psi for 15 min. Culture was inoculated at 1% concentration in skim milk containing prebiotics and incubated at 37°C for 24 h. The culture was then stored in refrigerator. Viability was determined as given in table .

H. Effect of Vitamins on Viability During Storage

Effect of vitamins namely thiamine and riboflavin on viability during storage was determined by adding each vitamin into skim milk at two different concentration (1 and 2%). Culture inoculated at 1% concentration and incubated at 37°C for 24 h. The culture then stored in refrigerator. Viability was determined as given in table.

III. RESULT AND DISCUSSION

The survival of probiotic bacteria in dairy products and viability of lactobacilli is important in providing a number of therapeutic benefits to consumers many dairy products have now been reformulated to include *Lactobacillus* strains to provide health benefits. Probiotic cultures in food should be well defined and correctly and taxonomically named, available in a viable state in sufficient quantities for the entire period of shelf life.

Use of lactobacilli as additives in human diets is known from long back. Research investigation related to their characterization and beneficial effects is still running as lactobacilli used as probiotics are isolated from different sources and it is quite difficult that all strains possess all the desired characteristics requires extensive analysis of their functional properties. Based on the above facts, the present investigation was aimed to isolate and identify viability of lactobacilli was studied under the influence of various factors such as vitamins, sweeteners, temperature and prebiotics. The *lactobacillus* CM6 tested for viability during storage. Effect of various such as temperature, sweeteners, prebiotics, and vitamins on viability was studied.

A. Viability of Skim Milk during Storage

Viability of *Lactobacillus fermentum* CM6 in skim milk during storage of 5 days was studied. Effect of various factors such as temperature, sweeteners, vitamins and prebiotics on viability of the organism in skim milk during storage was studied . The viability of *Lactobacillus fermentum* CM6 after 24 h of incubation period before any storage was determined in cfu/ml and used as control.

B. Effect of Temperature on viability During Storage

Lactobacillus fermentum CM6 was tested for viability in skim milk during for 5 days of storage under the effect of two different temperature *i.e* 4°C and -20°C.

At the temperature 4°C, viability of *Lactobacillus fermentum* CM6 was studied, after inoculation and incubation of 24 hrs without storage the viability was determined as control 10^{-9} × 2.05. After 24 hr of storage at 4°C viability was found to be 10^{-9} ×2.26, after 3 days of storage 10^{-9} ×2.11, and the 5 days of storage is 10^{-9} ×1.19 cfu/ml, viability decrease as the storage period increase. Table 3.2(a).

At the temperature -20°C, viability of *Lactobacillus fermentum* CM6 was studied, after inoculation and incubation of 24 hrs without storage the viability was determined as control 10^{-9} × 2.05. After 24 hr of storage at -20°C viability was found to be 10^{-9} ×2.20, after 3 days of storage 10^{-9} ×1.51 And the 5 days of storage period is 10^{-9} ×0.98 cfu/ml. The viability was gradually decreased as storage period was increased at the temperature of -20°C. Table 3.3

A remarkable decrease was found in the viability as storage periods was increased at 4°C and -20°C. After the storage period of 1day, 3 days, and 5 days of storage viability was observed. The viability of the isolated *Lactobacillus fermentum CM6* was found better at -20°C storage as compared to that of 4°C. The effect of rest of the factors such as sweeteners, vitamins, and prebiotics on viability in skim milk was studied during storage at -20°C.

C. Effect of Different Temperature on Viability of Lactobacillus fermentum CM6 in skim Miik During Storage

S.No	Storage (In days)	Control	Viability at different temperature	
		10^9 × 2.83	4°C	-20°C
1	1		10^9 – 0. 58	10^9 – 2.20
2	3		10^9 – 0. 44	10^9 - 1.51
3	5		10^9 – 0.39	10^9 - 0.98

D. Effect of Different Sweeteners on Viability of Lactobacillus fermentum CM6 in skim Milk During Storage

1) Effect of sorbitol on viability of Lactobacillus fermentum CM6

S.No	Storage (In days)	Control	Viability at different temperature	
		10^9 × 2.05	4°C	-20°C
1	1		10^9 – 2.26	10^9 - 1.50
2	3		10^9 – 2.11	10^9 - 0.60
3	5		10^9 – 1.19	10^9 - 0 .49

2) Effect of Sucrose on viability of Lactobacillus fermentum CM6

S.No	Storage (In days)	Control	Viability at different temperature	
		10^9 × 1.50	4°C	-20°C
1	1		10^9 – 0.41	10^9 – 0.48
2	3		10^9 – 0.38	10^9 - 0.34
3	5		10^9 – 0.33	10^9 - 0.32

E. Effect of Different Prebiotics viability of Lactobacillus fermentum CM6 in Skim Milk During Storage

1) Effect of refiinose on viability of Lactobacillus fermentum CM6

S.No	Storage (In days)	Control	Viability at different temperature	
		10^9 × 1.28	4°C	-20°C
1	1		10^9 – 0.01	10^9 – 2.30
2	3		10^9 – 1.82	10^9 - 0.33
3	5		10^9 – 0.42	10^9 - 0.31

2) Effect of inulin on viability of Lactobacillus fermentum CM6

S.No	Storage (In days)	Control	Viability at different temperature	
		10^9 × 2.10	4°C	-20°C
1	1		10^9 – 2.70	10^9 – 0.36
2	3		10^9 – 0.40	10^9 - 0.34
3	5		10^9 – 0.38	10^9 - 0 .31

IV. CONCLUSION

The health of gastrointestinal tract of human being is affected by numerous factors. One of the most importent factors is probiotics. Today, is a strong increase in the consumption of probiotic bacteria using food products, mainly probiotic dairy products. The nutritional and therapeutic impacts of food on health increased the consumers awareness toward the functional food and demand for functional food is growing rapidly around the world.

In the present study the lactobacilli was isolated from milk , the isolated was screened for different probiotic attributes so that they could be applied for promotion of the host health .The result revealed the potential probiotic properties of the *Lactobacillus fermentum* CM6. isolated from camel milk sample. The *Lactobacillus fermentum* CM6 survived in the MRS broth supplemented with the different concentration. The *Lactobacillus fermentum* CM6 maintained their total cell count at varied degree. This strain useful for the preparation of functional food products for prevention of highly prevalent disease.

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Hence, This strain can be used as probiotics after making *in vivo* trials by following standard procedures.

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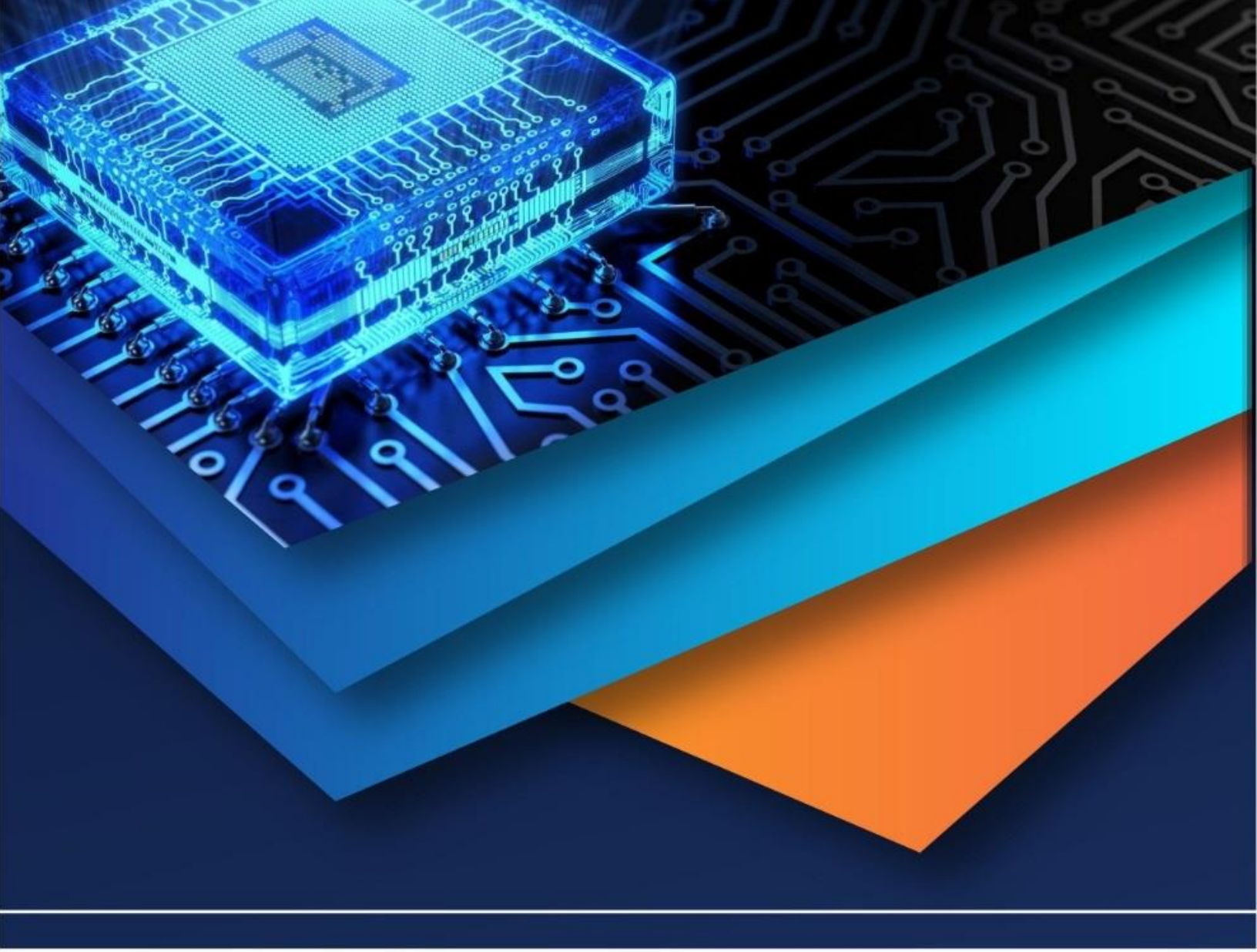

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