European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 07, Issue 11, 2020

ISOLATIONOF PROBIOTICS FROM DAIRY SAMPLE

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ABSTRACT

Probiotics are refers to as microorganisms which have many health benefits.The most probiotics are lactic acid bacteria and some other groups. These probiotics are present in higher amount in milk and milk products. In this study the numerous groups of probiotic bacteria have been identified and isolated in food and food products especially in dairy products such as milk, cheese, kefir, acidophilus milk, yogurt etc… Probiotics are gram positive rods or cocci aerotolerant anaerobes / facultative anaerobes, non – motile, non -sporing and non motile. They have high tolerance to stomach pH than other microbes. These strains were isolated using De Mann Rogosa Sharpe (MRS) agar medium and incubated at 37°C for 4i hours separately under aerobic and anaerobic condition. After growth they were isolated, purified and characterization were done based on morphology analysis such as gram staining and biochemical analysis. The probiotics are useful for both humans and animals. They have several health benefits such as improve immune response, many antibiotic activity, antioxidant activity etc. Probiotics contains more B vitamins and also helps in lactose metabolism and food digestion.

KEYWORDS: Probiotics-Lactic acid bacteria-De Mann Rogosa Sharpe agar medium-High pH tolerance- Morphology and Biochemical Analysis.

I INTRODUCTION

Probiotics are live microorganisms which bring about health benefits when administered in adequate amounts, and hence, the consumption of probiotic food is very popular worldwide [Brian's V.R., 2006]. Probiotics are defined as 'live microorganisms which when administered in adequate amount of conference health benefits to the host'. Alternatively, probiotics have been defined as live microbial feed supplement that Beneficially affect the host by improving its intestinal microbial balance [S.Fijan, 2014]. Probiotics was originally used to improve the health of both animals and humans through the modulation of the intestinal microbiota [Salminen et al., 2005].A number of health benefits have been claimed for probiotic bacteria such as *Lactobacillusacidophilus*, *Bifidobacteriumspp*., *Lactobacilluscasei*, etc. Because of potential health benefits, organisms are increasingly incorporated into dairy foods [Fuller R. 1989].Lactic Acid Bacteria (LAB) are considered as a major group of probiotic bacteria. LAB play an important role in food applications. Fermented foods are produced through fermentation of certain sugars by LAB and the origins of them are the lost in antiquity. The most commonly

LAB are used as starter culture in food fermentations [Reuter G 2001].Lactic Acid Bacteria is well known for the greatest proportion of them belongs to the category of dairy products, namely fermented milk, cheese, yogurt, butter, ghee, Kefir, Acidophilus milk etc… [Parente E, 2002].Lactic Acid Bacteria have been found to produce bacteriocins namely polypeptide synthesized ribosomally by bacteria that can have a bacteriocidal or bacteriostatic effect on the other bacteria. In general bacteriocins lead to cell death by inhibiting cell wall biosynthesis or by disrupting the membrane through pore formation [Zapparoli G, 2017]. Bacteriocins are important in food fermentations where they can prevent food spoilage or the inhibition of food pathogens. The best known bacteriocin is nisin, which gained widespread application in the food industry and used as food additive particularly in processed cheese, dairy products [Delves – Broughton J, 1996]. Bacteriocins produced by LAB are Lacticin from Lactococci, Macedovicin from Streptococcus macedonicus, Reuterin from Lactobacillus reuteri, Sakacin from Lactobacillus sake, Curvaticin and Lactocin from Lactobacillus curvatus, Pediocin from Pediococcus acidilactici [Castellano P, 2006].

IIMATERIALS AND METHODS

The study was performed by the experimental analysis using different chemicals and materials following sequential procedure to isolate and characterize the probiotic bacteria from dairy sample.

SAMPLECOLLECTION:

The desired milk sample was collected from the local cattle farm in kovilambakkam and the milk was kept outside one day for rotten (Fig 1.1). The expired ghee sample was collected. Both the sample was transferred to the laboratory.

FIG1.1 ROTTEN MILK SAMPLE

PREPARATIONOFTHESAMPLE:

The suspected microbial colonies were isolated from the collected milk and ghee sample by serial dilution method. Twelve saline tubes were prepared and sterilized in autoclave. 1ml of milk sample were diluted in 10ml of distilled water in test tubes 10^{-1}. Then 1ml from 1st tube was transferred to 9ml containing test tube (10^{-2}). Repeat the process in 10^{-3} to 10-6 dilution tubes. Last 1ml of sample was discarded from 10-6 tube. The same procedure was repeated for the ghee

sample (Fig1.2).Nutrient agar medium plates were prepared and sterilization was done. Medium was poured on the sterile petriplates and allowed to solidify. Then 100µl of sample from 10^{-3} to 10-6 were taken and spread plated. The plates were incubated at 37°C for 18-24hours.

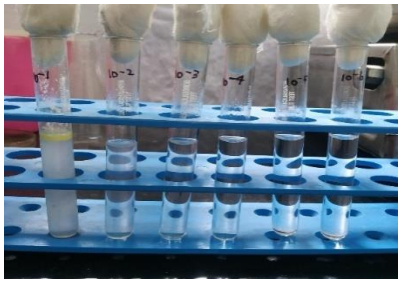


Fig 1.2 Serial Dilution Of Ghee Sample

SCREENINGOFPROBIOTICBACTERIA

The suspected colonies from nutrient agar plate were screened for the probiotic bacteria (latic acid bacteria) using MRS (deMan Rogosa sharpe) agar plates. deMan Rogosa sharpe broth and the other ingredients were prepared and mixed in distilled water in conical flask.The medium was sterilized in autoclave and poured on the sterile petriplates. After solidification zig zag streaking were done on the plate. The plated were then incubated at 37°C for 18-24 hours. The pure lactic acid producing colonies were observed in the MRS agar medium.

IDENTIFICATIONOFBACTERIA

The identification of bacteria was carried out by morphological studies.

Gramstaining: The smear on a glass slide was treated with primary stain (crystal violet) for one minute, then slide is washed in water. The smear is treated with few drops of gram's iodine for a minute. The slide is again washed with water. After the smear is decolourized, it is washed in water without any delay. The smear is finally treated with few drops of counter stain (safranin) for one minute and washed with water. The slide is dried in air and observe under microscope.

III: RESULTS

PROCESSING OF SAMPLE

Individual colonies were isolated from the milk sample plate (fig 2.1a) and Ghee sample (fig 2.1.b)

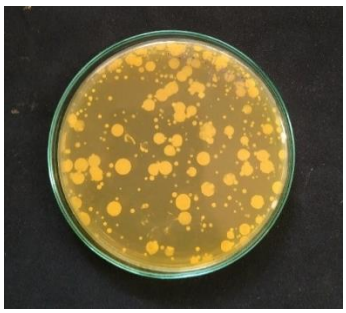


Fig 2.1.a.Nutrient agar 10^{-3} dilution (Milk sample)

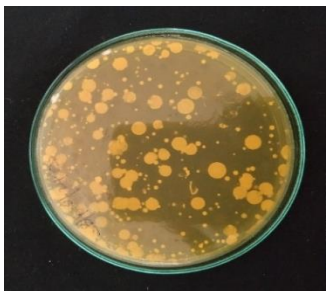


Fig 2.1.b.Nutrient agar 10^{-5}dilution (Ghee sample)

SCREENING OF PROBIOTIC BACTERIA

Isolated colonies from both sample serial dilution plate were inoculated into the MRSH for the identification of probiotic bacteria (lactic acid bacteria).fig2.2

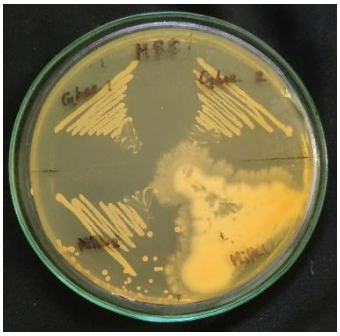
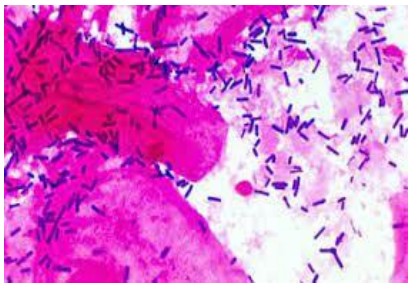


Fig.3.2. Colonies in MRS medium

IDENTIFICATION OF BACTERIA

Morphological staining of the colonies from the MRS agar plates were studied to identify the probiotic bacteria. (fig.2.3.a) and (fig 2.3.b)



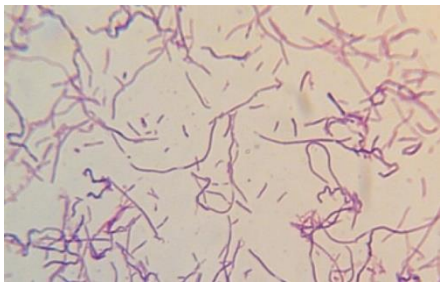


Fig3.3.a Gram staining Of culture from milk sample

Fig.2.3.b. Gram staining of culture from ghee sample

Table : Morphological characterization

S.NO	SAMPLE	RESULT
1.	Milk sample	1. Gram positive short rods
2.	Ghee sample	1. Gram positive long rods

IV DISCUSSION

Various isolates were observed on the nutrient agar plate. Morphology studies were also studied on the isolates.

In the cow milk sample and the ghee sample serial dilution it had showed 2 different colonies in the nutrient agar plates respectively. Whether this was earlier been reported by some workers [Vanniyasingam.J et al.,2019; Broome. M.C et al., 1990].

The cow milk may contain several probiotic bacteria and other gut microflora. Those gut microflora and the probiotic bacteria may have several health benefits. The probiotic bacteria may contains several species such gram positive shortand long bacterial colonies.This study was earlier been reported by the some workers [Wedajo. Bet al.,2015].

Among the various media developed for selective enumeration probiotic bacteria,MRS agar (pH 5.2) is suitable for *Lactobacillus spp.* and other lactic acid bacteria species. The MRS media had a several specific nutrients for the growth of LAB species . This study was also reported by some works . [N. P. Shahet al.,1998].

V CONCLUSION

LAB are the most commonly used microorganisms for the fermentation and preservation of foods. Their importance is associated mainly with their safe metabolic activity while growing in foods utilizing available sugar for the production of organic acids and other metabolites. Advances in the genetics, molecular biology, physiology, and biochemistry of LAB have provided new insights and applications for these bacteria.

Among the various media developed for selective enumeration of probiotic bacteria, MRS agar is mostly suitable for the Lactobacillus spp. The probiotic bacteria have good response in immune system and it also play an important role in human health development. Because of its Anti- oxidant activity, Anti- bacterial activity and Anti – fungal activity and great wound healing activity also.

Thus the consumption of probiotic food products has a great effect in human daily life.

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