“One Never Notices What Has Been Done: One Can Only See What Remains To Be Done.”

MARIE CURIE

Polish-French Physicist
Isolation, Characterization, Determination of Probiotic Properties of Lactic Acid Bacteria from Human Milk

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Abstract: Probiotics mean live microorganisms that have beneficial effects on their host's health. Although probiotic strains can be isolated from many sources, for human applications the main criteria is being human origin. Breast milk is an important nutrient source for neonates. Lots of studies showed that the fluid has beneficial effects on the health of neonates. One reason of being beneficial is explained by the micro flora of human breast milk including beneficial lactic acid bacteria. In this study, isolates were identified by biochemical and molecular characterization and also probiotic properties of lactic acid bacteria. Isolated from human milk were investigated. Two of the isolates were observed as potential probiotics. Two of them are bacilli. These isolates showed resistance to stomach pH 3.0, tolerance against 0.396% bile concentration and antagonistic activity against Escherichia coli. Bacillus cereus and Staphylococcus aureus and. After investigating the probiotic properties of these isolates, they were identified by biochemical and molecular identification techniques and molecular identification by using Polymerase chain Reaction (PCR) of 165 ribosomal RNA (rRNA) and 165 sequencing. Two lactobacilli were identified as Lactobacillus rhamnosus (KF477233) and Lactobacillus casei (KF477232). In the light of this study, it is observed that human milk is a source of potential probiotic strains.

Keywords: PCR, Polymerase chain reaction, RNA, ribosomal RNA

I. Introduction

The word ‘probiotic’ comes from Greek language “pro bios” which means ‘for life’ opposed to ‘antibiotics’ which means ‘against life’. The history of probiotics began with the history of man by consuming fermented foods that is well known Greek and Romans consume very much (Giannecchi et al., 1999, Queiroz et al., 2005). In 1906 a Russian researcher Elie Metchnikoff, who has a nobel prize, firstly proposed the beneficial effects of probiotic microorganisms on human health. Metchnikoff hypothesized that Bulgarians are healthy and long lived people because of the consumption of fermented milk products which consists of rod shaped bacteria (Lactobacillus spp). Therefore, these bacteria affect the gut microflora positively and decrease the microbial toxic activity (Giannecchi et al., 1999, Carrión, 2003, Queiroz et al., 2005).

Probiotics are also challenge for the industrial applications. The probiotic concept is open to lots of different applications in a large variety of fields relevant for human and animal health. Probiotic products consist of different enzymes, vitamins, capsules or tablets and some fermented foods contain microorganisms which have beneficial effects on the health of host. They can contain one or several species of probiotic bacteria.

Most of products which destine human consumption are produced in fermented milk or given in powders or tablets. These capsules or tablets are not used for medicinal applications. They are just used as health supporting products. The oral consumption of probiotic microorganisms produces a protective effect on the gut flora.

Lots of studies suggest that probiotics have beneficial effects on microbial disorders of the gut, but it is really difficult to show the clinical effects of such products. The probiotics preparations use for traveler’s diarrhea, antibiotic associated diarrhea and acute diarrhea which is known that they have positive therapeutic effect (Giannecchi et al., 1999, Carrión, 2003, Queiroz et al., 2005).

The studies on the microbiology of human milk are restricted to the identification of potential pathogenic bacteria in clinical cases of mastitis or infant infections. However, it is clear that the prevention of infant from infectious diseases owing to the natural flora of human milk (Martin et al., 2004). Although there are limited knowledge about the commensal or probiotic bacteria that breast milk contain, bacteria commonly isolated from this biological fluid include staphylococci, streptococci, micrococci, lactobacilli and enterococci (Martin et al., 2004, Martin et al., 2005, Martin et al., 2005, Martin et al., 2005, Martin et al., 2005, Martin et al., 2005). Bacteria from these genera can be easily isolated from fresh milk of healthy women. So, these groups of bacteria should be considered the natural microflora of human milk rather than more contaminant bacteria (Martin et al., 2004, Martin et al., 2005).

There are surprisingly not so much studies on the isolation and analysis of commensal or potential probiotic bacteria from breast milk (Martin et al., 2003). However, if the bacteria with the ability to provide
Lactation that is formation of milk, is possibly initiated in two ways, first the act of sucking evokes a nervous reflex, the secretion of prolactin, GH and ACTH by the anterior pituitary. These are all essential to lactation. Secondly delivery of the placenta causes a sudden fall in concentration of progesterone in the blood, and this allows the release of prolactin. Forty lactating women who came to Kovai Medical Centre and Hospital, Coimbatore for regular check up were selected for this study, their ages ranged between 20 to 40 years. In the same age group in healthy non lactating women were selected. Serum samples were subjected to analyze prolactin hormone. Total protein, albumin, Vit-A, Vit-C, Iron, Calcium, and Vit-B12, IgA. This study was done in lactating women who were healthy. It was observed that their results showed an increase in the level of Vit-A, Prolactin and Vit-C, a decrease in the Iron, Calcium and Total Protein, albumin and normal level of IgA and Vit-B12. Milk samples were subjected to analyze Lactose, Vit-A, Vit-C, Iron, Calcium and Total Protein, albumin, Vit B12, IgG and IgA, Glucose, Sodium, Chloride, C-reactive Protein, Lactoferrin. Antibodies are abundant in human milk throughout lactation. There is abundant evidence that women are able to produce milk with adequate content of protein, fat, carbohydrate, and most minerals even when their supply of nutrients is limited. The nutrients in human milk most likely to be present in lower than normal concentrations in response to chronically low maternal intakes are the vitamins, especially vitamins B₆, B₁₂, A, and D. Those maintained at the expense of maternal stores or tissues include the macronutrients, most minerals, and folate. To measure urinary free deoxypyridinoline (Dpd) excretion in non-lactating and lactating women.
UNUSUAL CHANGES IN BREAST MILK COMPOSITION IN LACTATING WOMEN

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ABSTRACT

Lactation is the production of milk. The mammary glands contain milk glands. In the glands, special epithelial cells line small sacs called alveoli. These cells secrete milk. The alveoli are surrounded by a layer of tissue containing smooth muscle fibers. When the muscle contracts it causes milk to be released. Forty lactating women who came to KHMS and Government Hospital, Kodaikanal for regular check up were selected for this study, their ages ranged between 20 to 40 years. In the same age group in healthy non lactating women were selected. Serum samples were subjected to analyze prolactin hormone, Total protein, albumin, Vit-A, Vit-C, Iron, Calcium, and Vit-B12. IgA. This study was done in lactating women who were healthy. It was observed that their results showed an increase in the level of Vit-A, Prolactin and Vit-C, a decrease in the Iron, Calcium and Total Protein, albumin and normal level of IgA and Vit- B12 Pregnancy induced hypertension (PIH), glucose intolerance and anemia are common medical complications observed during pregnancy in Indian women and their effects were studied on the composition of colostrum collected within 24 hours of delivery from lactating women included in the study. PIH during pregnancy significantly decreased colostrum IgA and total proteins, but showed a significant increase in K+ levels, where as women with glucose intolerance showed a significant decrease in total lipids and lactose and an increase in Na+ levels in colostrum compared to normal controls. The group with anemia also showed a significant decrease in colostrum IgA and total protein levels when compared with the control group. Thus, an increase in pH level with age can result in a proportional decrease in the protein level of the human breast milk. Variation in the vitamin A content of colostrum, matured milk and transitional milk exhibited the same pattern as protein, calcium and sugar content. Colostrum had the highest level of vitamin, which declined in matured milk, with a minimum of transitional milk. However, vitamin A level generally decreased with age. Vitamin A is one of the most effective antimicrobial factors in human breast milk that protects infants against microbial infectious agents, particularly cytomegalovirus.

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Keywords: K-Potassium, Vit-vitamins

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Molecular and Bacteriological Examination of Cow Milk in Coliform Mastitis

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Abstract: In the present study, 20 samples of milk of cow, pasteurized milk were collected from the local market and were analyzed for microbial count and IMVC tests to determine the coliform load in the sample. Further, the presence of E. coli (EC79587) was confirmed by using PCR and 16S rRNA sequencing. Majority of the milk samples of different origin were found to be contaminated by the coliform group of bacteria. The samples were found to be positive for E. coli by PCR analysis and 16S rRNA sequencing. Pasteurized milk samples did not show presence of E. coli by PCR, but they showed considerable count of bacterial growth by total plate count method. The results indicated that analyzed milk could contribute a potential risk for public health in the cases that it was consumed or used in the production of dairy products without being pasteurized or being subjected to a sufficient heat process. Moreover, PCR is less labor intensive, more rapid for bacterial identification and it further confirmed in 16S rRNA sequencing.

Keywords: PCR-Polymerase chain Reaction, IMVC-Indole methyl red, vogue propanol, citrate utilization

I. Introduction

Mastitis is the most economically significant disease of dairy animals. This condition is widespread in dairy herds and is associated with a significant reduction in milk yield, increased costs of production and deteriorated milk quality. These costs are borne directly by milk producers and indirectly by the consumers of dairy products. The disease also results in partial or complete damage to udder tissues and decreases the productive life span of the animal.

Mastitis is caused by many bacteria, which include the coliform group (specifically Escherichia coli, Enterobacter, Klebsiella species), Streptococci, Staphylococci, Corynebacteria, Pasteurella, Mycoplasma, Leptospira, Yersinia, Mycobacteria, Proteus, Enterobacter, and other organisms like fungi, yeasts and virus. (Kotowaki, 1988 and Gonzalez et al., 1980).

The most important species in the coliform group of organisms is Escherichia coli (E.coli), a rod-shaped member of the coliform group, can be distinguished from most other coliforms by its ability to ferment lactose at 44°C, and by its growth and color reaction on certain types of culture media. It is the lactose fermenter and produces pink colonies in MacConkey agar. When cultured on an EM agar plate, a positive result for E.coli is a metallic green sheen with dark purple colonies. Unlike the general coliform group, E.coli are almost exclusively of fecal origin and their presence is thus an effective confirmation of fecal contamination.

Typically, E.coli are about 11% of the coliforms in human feces. (Burns et al., 1996).

Optimal growth of E.coli occurs at 37°C, but some laboratory strains can multiply at temperatures of up to 49°C. Growth can be driven by aerobic or anaerobic respiration, using a large variety of redox pairs, including the oxidation of pyruvic acid, formic acid, hydrogen and amino acids, and reduction of substrates such as oxygen, nitrate, dimethyl sulfoxide and trimethylamine N-oxide. (Bradley et al., 2001).

Mastitis is an inflammation of udder which is mainly caused by coliforms and other microorganisms. It is a most economically important disease in cattle’s. It is mainly caused by E.coli in dairy cattle’s. The major reason for the disease is environmental factors like contaminated water. In the present study virulent E.coli strains were isolated from Bovine mastitis and the virulent genes were identified using Multiples PCR, and 16S rRNA sequencing to identify the coliform organisms. Identification of Virulent E.coli from Bovine mastitis was carried out in Mother Teresa Women’s University, Kottayam.

Identification of Virulent E.coli from Bovine mastitis was carried out in Mother Teresa Women’s University, Kottayam, and 2006 - 2009. The part of the work was carried out in Tamil Nadu Veterinary and Animal Sciences University, Chennai. The results are produced and discussed in this chapter.

The aim of this project is the isolation of E.coli from the mastitis milk sample. Mac conkey agar and Eosine Methylene blue agar were used in the preliminary identification of E.coli further identification was done by biochemical and sugar fermentation test. Mastitis is an inflammation of udder which is mainly caused by coliforms and other organisms. It is a major disease caused by E.coli in dairy cattle. 10 isolates of E.coli were obtained from 10 samples of mastitis milk showing 50% incidence rate.