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ISOLATION AND IDENTIFICATION ISOLATES OF BIFIDOBACTERIUM FROM HUMAN BREAST MILK AND THEIR USE AS PROBIOTICS

NAYAK A[1], UPADHAYAY D[2], MARCHAWALA F[2], BHATTACHARYA I[2] AND ANDHARE P[2*]

1: Student, MSc Microbiology, Parul Institute of Applied Sciences, Parul University, Post Limda, Waghodia, Gujarat, 391760

2: Assistant Professor, Parul Institute of Applied Sciences, Parul University, Post Limda, Waghodia, Gujarat, 391760

***Corresponding Author: E Mail: Dr. Prasad Andhare: prasad.andhare82145@paruluniversity.ac.in; Tel: +918200614350**

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ABSTRACT

Several studies have demonstrated a diversity of bacterial species in human milk, even in aseptically collected samples. The present study evaluated potential probiotic bacteria isolated from human milk and associated maternal variables. Milk samples were collected from healthy women and cultured on selective and universal agar media under aerobic and anaerobic conditions. Traditionally, human milk was thought to be a sterile fluid, but recent findings detected the presence of biotic component in human milk, such as bacteria, including the potentially protective genus of Bifidobacterium **[1, 2]**. Interestingly, these bacteria are present in maternal milk in sufficient population levels to allow the colonization of the newborn intestine after its ingestion, but not to induce an inflammatory response in the mammary glands. Additionally, maternal milk contains protein for *bifidobacteria*, the so-called *Bifidus* factors which are constituted of a spread of oligosaccharides in quite high concentrations (14 to 20 g/L). In this way, human milk can be considered as a natural symbiotic containing both probiotics *(Bifidobacterium*) and prebiotics (human milk oligosaccharides: HMO) in its constitution **[3]**. For this reason, breastfeeding is nutritional, immunologically and microbiologically essential for newborn.

Keywords: ***Bifidobacterium dentium, Streptococcus thermophilus, Lactobacillus bulgaricus, Bifidobacterium bifidus***

INTRODUCTION

Members of the genus Bifidobacterium are among the first microbes to colonize the human gastrointestinal tract and are believed to exert positive health benefits on their host. Due to their purported health-promoting properties, *Bifidobacteria* are incorporated into many functional foods as active ingredients. *Bifidobacteria* naturally occur during a range of ecological niches that are either directly or indirectly connected to the animalalimentary canal, such as the human mouth, the insect gut and sewage.

Unfortunately, breastfeeding is not always possible, there is a need to develop a new formula of substitutive products, that simulatehuman milk in the best possible way. The most common microorganisms found naturally in human milk are commences, and some of them could be potential probiotics to be used in the formulation of maternal-like milk. Among these potential probiotics, the genera *Bifidobacterium* are commonly isolated from human milk **[4–6]**. In the present study, we investigated some desirable traits in probiotic candidates of bacteria isolated from human milk, as well as some associated maternal variables

Bifidobacterium is a genus of gram-positive, non motile, often branched anaerobic bacteria. They are ubiquitous inhabitants of the alimentary canal, vagina and mouth (*B. dentium*) of mammals, including humans.

Bifidobacteria are a group of bacteria called probiotics that normally live in your intestines and stomach. They help your body perform essential functions like digestion and staving off harmful bacteria**.**

IMPACT ON HEALTH AND DISEASES

A diverse microbial community has evolved to adapt and survive within the human GIT and is usually referred to as the gut microbiota.

The past 20 years has seen a search specialise in those members of the gut microbiota that exhibit health-promoting or probiotic effects like protection of the host against pathogens by competitive exclusion modulation of the immune system and provision of nutrients through the breakdown of non-digestible dietary carbohydrates. Furthermore, compositional alterations of the alimentary canal (GIT) microbiota are linked to certain gastrointestinal diseases like inflammatory bowel disease and necrotizing enterocolitis.

PROBIOTICS

Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host." One of the most significant groups of probiotic organisms are the lactic acid bacteria, commonly used in fermented

dairy products. These bacteria have a long history of safe use in food. There is an upsurge in interest in these species as research is beginning to reveal the many possible health benefits associated with lactic acid bacteria**.**

THE PROBIOTIC EFFECTS OF LACTIC ACID BACTERIA

The actions of lactic acid bacteria are species and strain specific, and depend on sufficient numbers of bacteria being available in the intestines. The difficulty in identifying and classifying strains has complicated research, since benefits may only pertain to particular strains.

Nevertheless, lactic acid bacteria have a number of well-established benefits. They can improve lactose digestion, play a role in preventing and treating diarrhea and act on the immune system, helping the body to resist and fight infection.

More work needs to be done to confirm the role lactic acid bacteria might play in preventing or slowing the growth of colon cancer, lowering cholesterol levels, preventing urogenital infections, alleviating constipation and treating food allergy.

PROBIOTICS A SPECIAL CASE

In the quest for discovering how food can enhance health or prevent chronic diseases, researchers have stumbled on to another range of components in foods besides nutrients. This growing area of information has resulted in a new label for foods that have added benefits: functional foods.

Functional foods contain significant levels of biologically active components that provide health benefits beyond basic nutrition. Other terms for functional foods include nutraceuticals, pharmafoods, designer foods, mood foods...Some examples of functional foods are:

A. Oats and barley, which contain beta-glucans, and can improve blood sugar control and reduce blood cholesterol.

B. Cooked tomatoes, which contain the phytochemical lycopene, and may reduce risk of prostate and cervical cancer.

C. Yogurt and other cultured milk products, which contain lactic acid bacteria, and can enhance gastrointestinal system function. In fact, the potential benefits of yogurt and other foods containing living organisms are so numerous that they form a special sub-group of functional foods: probiotic foods.

WHAT ARE LACTIC ACID BACTERIA

Lactic acid bacteria produce lactic acid as their main product. They are widespread in nature—in soil, vegetables, meat, milk and the human body. Many are used in fermented dairy products. *Streptococcus thermophilus (S. thermophilus)* and

Lactobacillus bulgaricus (L. bulgaricus) are the two bacteria required to make yogurt. Many commercial yogurts in Canada also contain Lactobacillus acidophilus *(L. acidophilus*) and *Bifidobacterium bifidus (B. bifidus). Lactobacillus casei (L. casei*) is frequently found in cheeses. All these species of bacteria are accepted by Canada as probiotic **[7]**.

These species can be further classified into subspecies, variants and strains. The difficulty in identifying and classifying strains has complicated research, since benefits may only pertain to particular strains.

Long before the term 'probiotic' was coined, Elie Metchnikoff, the Nobel laureate immunologist, suggested in 1908 that the reason Balkan peasants lived long lives was because they drank milk fermented with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* **[8]**. He suggested that these bacteria would suppress "putrefactive fermentation", leading to better health and longevity. His ideas generated great interest in the role gut micro flora play in health, an interest that persists to this day.

Today, many potential health benefits of probiotic bacteria are under investigation, from improving the microbial balance in the intestine to enhancing immune system function.

Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" **[9]**. One of the most significant groups of probiotic organisms are the lactic acid bacteria.

WHAT MAKES A BACTERIA PROBIOTICS

For bacteria to exert any probiotic effect, they have to be able to survive both the stomach acids (pH as low as 1.5) and the bile acids (pH as low as 2). This is true of most lactobacilli.

Secondly, the bacteria must arrive in the intestines in sufficient quantities to have an effect. The amount required depends on the strain and the health benefit being studied. The minimum effective level for individual bacteria and specific health benefits is actively under research.

The bacteria may need to adhere to the wall of the intestine (i.e. "implant") and colonize in order for there to be an effect. Sherwood Gorbach, one of the discoverers of *Lactobacillus* GG, states, "Our research over the previous 20 years had established beyond doubt that implantation in the gut was the critical feature that a strain must possess to influence the intestinal milieu..." **[10]**. However, others contend that continuous transit (e.g. continually eating a probiotic food) is an alternative to the organism implanting and colonizing **[11]**.

Finally, the bacteria must show some beneficial effects on human health. Some examples of beneficial effects under investigation include alleviation of lactose intolerance, prevention and treatment of diarrhea, maintenance of normal intestinal flora, antagonism against pathogens, stimulation of the immune system, anticarcinogenic activity, and reduction of serum cholesterol levels.

EFFECTS OF PROBIOTCS: WHAT'S THE EVIDENCE

Lactose digestion

It is well-known that the presence of lactic acid bacteria, specifically *L. bulgaricus* and *S.thermophilus* in yogurt, improves lactose digestion **[11]**. It appears that the cell walls of the bacteria have to be intact (as is the case when the bacteria are alive) for the effect to occur. Some possible mechanisms for the improved lactose digestion include:

A. The lactase activity of the bacteria actually does the work of digesting lactose in the product once it reaches the intestine.

B. The slower transit time of yogurt may permit more time for the residual intestinal lactase and the yogurt bacteria to digest the lactose **[11]**.

C. Something in the yogurt may inhibit fermentation of lactose and thus reduce symptoms. Sweet acidophilus milk (milk with *L. acidophilus* which has not been allowed to ferment) does not seem to alleviate the symptoms of lactose mal digestion. Although some work shows a small effect, most work shows no effect.

Diarrhea

Lactic acid bacteria may be useful in preventing and shortening the duration of several types of diarrhea **[11]**.

A number of well-designed studies have noted that fermented milk products effectively prevent or treat infantile diarrhea **[12]**. Effects have been noted with *L. casei* and *B. bifidum*.

A few small studies show that lactic acid bacteria can reduce the incidence of antibiotic-related diarrhea **[13]**. This suggests a role for lactic acid bacteria in immunosuppressed patients who routinely use antibiotics **[14]**.

A few studies of traveller's diarrhea have demonstrated the effectiveness of lactic acid bacteria in decreasing the incidence of diarrhea **[15]**.

Lactic acid bacteria can probably reduce diarrhea in several ways:

Lactic acid bacteria compete with pathogens for nutrients and space in the intestines .

By-products of metabolism may have a direct effect against the pathogens. For example, in vitro work shows that *L. casei, L. acidophilus* and *L. bulgaricus* can all produce antimicrobial agents such as *acidophilin* and *bulgarican* that can inhibit growth of pathogens.

Lactic acid bacteria may be effective against diarrhea due to effects on the immune system.

EFFECTS ON THE IMMUNE SYSTEM

Lactic acid bacteria enhance immune system function at the intestinal and systemic levels. In humans, lactic acid bacteria have been shown to increase:

A. B-lymphocytes or B cells, which recognize foreign matter **[16]**,

B. phagocytic activity, helping to destroy foreign matter **[17]**,

C. IgA-, IgG- and IgM-secreting cells and serum IgA levels, which would increase antibody activity **[18]**, and D-γ-interferon levels, which help white blood cells fight disease.

Another way the body's defences work is by the barrier provided by the mucus layers of the intestine. The mucosa provides a physical barrier, usually preventing foreign substances from passing through the gut. As well, a large variety of immune cells are found in the gut mucosa. This allows the gut to interact with the immune system. Lactic acid bacteria can stimulate immune activity in the intestinal mucosa.

In conditions such as allergy or auto-brewery syndrome (abnormal gut fermentation resulting in increased levels of blood ethanol), the permeability of the small intestine can increase, allowing undigested protein molecules to pass through. Lactobacillus GG has been shown to reverse gut permeability **[19]**.

Probiotic bacteria may be able to play a role in treating food allergy. This was demonstrated in an experiment with infants known to have excema due to a cow milk allergy. Infants in the experimental group got hydrolyzed whey formula fortified with LGG, while those in the control group just got whey formula. The skin condition of the infants getting the LGG improved significantly compared to the control group. In addition, the experimental group had improved levels of factors associated with inflammation of the intestine.

POTENTIAL EFFECTS

Hypocholesterolemic effects

Some strains of *L. acidophilus* can take up cholesterol in the presence of bile **[20]**. Other in vitro research shows that cholesterol can precipitate with free bile salts in the presence of *L.acidophilus*, especially in an acid environment **[21]**. Thus, it has been hypothesized that one or both of these actions would take place in vivo and help lower serum cholesterol in humans.

Various studies with fermented milk products have shown either no effect or a reduction in cholesterol levels. In conclusion, there is not yet good evidence to confirm a cholesterol-lowering effect of fermented milk products.

Constipation

Milk products fermented with some strains of *L. acidophilus* and *bifidobacteria* shorten intestinal transit time. This effect may be useful for those with constipation, such as the elderly **[22]**. A well-controlled human study is needed to confirm this.

Colon cancer

Several lactic acid bacteria may help prevent initiation of colon cancer. It has also been demonstrated that lactic acid bacteria slow the growth of experimental cancers, although the results are not long-term **[23]**. It appears that lactic acid bacteria can reduce the levels of colon enzymes that convert procarcinogens to carcinogens. Specifically, lactic acid bacteria can reduce levels of the enzymes β-glucuronidase, nitroreductase, and azoreductase. Lactic acid bacteria may also be involved in the direct reduction of procarcinogens, for example, by taking up nitrites and by reducing the levels of secondary bile salts. In most reports, these effects only occur during the period of time that the bacteria are consumed.

Urogenital infections

Lactic acid bacteria may reduce candidal vaginal infections. This is still speculative; however, it would be research worth pursuing. One small study showed that women with recurrent vaginal candidiasis who ate 8 oz. daily of a yogurt containing L acidophilus had fewer occurrences of vaginal candidiasis than during the control period in which they ate no yogurt **[24]**. This was a cross-over study which started with 21 women. Eight of those who started in the treatment group refused to cross over to the control phase since they experienced so many fewer infections.

Ulcers

Lactic acid bacteria show some promise against stomach ulcers. Work with a specific strain of *L. acidophilus* demonstrated that *L. acidophilus* competes effectively (in vitro) against Heliobacter pylori for attachment sites, limiting the number of H. pylori that can attach to the cell wall. Infection with H. pylori is a risk factor for stomach ulcers. A small study of patients with ulcers showed that *Bifidobacteria bifidum* promoted healing of gastric ulcers in 50% of the patients and eradication of H. pylori from the mucous membranes in 30% of the patients.

CONCLUSION

The results of this study confirm that *lactobacilli* and *bifidobacteria* are common members of the human milk microbiota of women who did not receive antibiotics during pregnancy or lactation. Therefore, the presence of such bacteria may be a marker of a healthy non–antibiotic-altered human milk microbiota, and this should be taken into account when defining a criterion standard of breast milk. As a consequence, administration of selected

human milk lactobacilli or bifidobacteria to pregnant or lactating womenreceiving antibiotics, or to their infants, may constitute an attractive approach to restore the natural bacterial ecosystem existing in human milk.

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REFERENCES

[1] Metchnikoff E. The Prolongation of Life, 1st edition. G.P. Putnam's Sons, NY 1908.

[2] Hill C. *et al*. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and approproiate use of the term probiotic 2014.

[3] Health Canada Accepted claims about the nature of probiotic microorganisms in food 2009.

[4] Martini M.C., Kukielka D., Savaiano D. Lactose digestion from yogurt: influence of a meal and additional lactose 1991.

[5] Kuhn C., Titze A., Lorenz C.A. Are viable microorganisms essential for the enhancement of intestinal hydrolysis of lactose by the β-galactosidase of fermented milk products 1996.

[6] Martini M.C., Bollweg G.L, Levitt M.D, *et al*. Lactose digestion by yogurt β-galactosidase: influence of pH and microbial cell integrity 1987.

[7] Schaafsma G. Lactose intolerance and consumption of cultured dairy products — a review 1993.

[8] Relative efficiency of yogurt, sweet acidophilus milk, hydrolyzed-lactose milk, and a commercial lactase tablet in alleviating lactose maldigestion.

[9] Sanders M.E. Lactic acid bacteria as promoters of human health 1994. In Functional Foods, ed. Goldberg I. London: Chapman & Hall. 294–322.

[10] Saloff-Coste C.J. Diarrhea and fermented milks 1995.

[11] Salminen S., Deighton M. Lactic acid bacteria in the gut in normal and disordered states 1992.

[12] Aronsson B., Barany P., Nord C.E. *et al*. Clostridium difficile-associated diarrhea in uremic patients 1987.

[13] Black F.T., Andersen P.L., Orskov J., *et al*. Prophylactic efficacy of

bifidobaterium on traveller's diarrhea. Travel. Med. 1989.

[14] Oksanen P.J., Salminen S., Saxelin M., *et al*. Prevention of traveller's diarrhea by bifidobacterium GG. Ann. Med. 22:53–56 1990.

[15] De Simone C., Vesely R., Bianchi Salvadori B., *et al*. The role of probiotics in modulation of the immune system in man and in animal 1993.

[16] Schiffrin E.J., Rochat F., Link-Amster H., *et al*. Immunomodulation of human blood cells following the ingestion of lactic acid bacteria. J. of Dairy Science 78:491–497 1995.

[17] Kaila M., Isolauri E., Soppi E., *et al*. Enhancement of the circulating antibody secreting cell response on human diarrhea by a human lactobacillus strain 1992.

[18] Halpern G.M., Vruwink K.G., Van de Water J., *et al*. Influence of long-term yoghurt consumption in young adults 1991.

[19] Perdigón G., Medici M., Bibas Bonet de Jorat M.E., *et al*. Immunomodulating effects of lactic acid bacteria on mucosal and tumoral immunity. Int. J. Immunother. IX: 29–52 1993.

[20] Majamaa, H., Isolauri E. Probiotics: A novel approach in the management of food allergy. J. Allergy and Clin. Immunol. Feb: 179–185 1997.

[21] Buck L.M., Gilliland S.E. Comparison of freshly isolated strains of Lactobacillus acidophilus of human intestinal origin for ability to assimilate cholesterol during growth 1994.

[22] Klaver F.A.M., van der Meer R. The assumed assimilation of cholesterol by lactobacilli and *Bifidobacterium bifidum* is due to their bile salt-deconjugating activity 1993.

[23] Hitchins A.D., McDonough F.C. Prophylactic and therapeutic aspects of fermented milk 1989.

[24] Fernandes C.F., Shahani K.M. Anticarcinogenic and immunological properties of dietary lactobacilli 1990.