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Editorial

Cancer cells have higher glucose requirements. Glucose is transport to the cells by glucose transporters (GLUTs) in cells. GSK-3 is enzyme which regulates the activity of GLUTs in tumerogenic HeLa hybrid cells and change in the enzyme may leads to cell apoptosis. Inhibitors of GSK-3 were useful for the selective killing of GLUT3-expressing tumor cells. Researchers also identified several commercially available GSK-3 inhibitors with selective killing activity in tumor cell growth.

Drinking water quality is a major problem in developing countries. For the water quality improvement interventions include boiling, chlorination, flocculation, filtration, or solar disinfection, mainly conducted at home. As evidence suggests effectiveness improves with adherence, studies assessing programmatic approaches to optimising coverage and long-term utilization of these interventions among vulnerable populations could also help strategies to improve health outcomes.

Glutamate is neurotransmitter and it plays roles in many physiological brain functions including memory. Memory dysfunction can affect our cognitive function, and peripheral blood glutamate levels have been reported to be altered in many cognitive function disorders, such as Asperger's syndrome and schizophrenia. Research demonstrate the significant relationships among plasma transaminases (AST and ALT), plasma glutamate levels, and memory functions in *homo sapiens*. Considering that research findings, scientists hypothesize that plasma transaminase elevation in obesity induced fatty liver patients evokes memory function disorders and leads to food dependency, which results in further obesity.

Probiotics are considering as beneficial microorganism. In the review article authors mention Challenges and Opportunities of Probiotic Microencapsulation Technology. Microencapsulations enhance the shelf life and potency of probiotics. However, there are many challenges to overcome with respect to the microencapsulation process and the conditions prevailing in the gut. This review focuses mainly on the methodological approach of probiotic encapsu-

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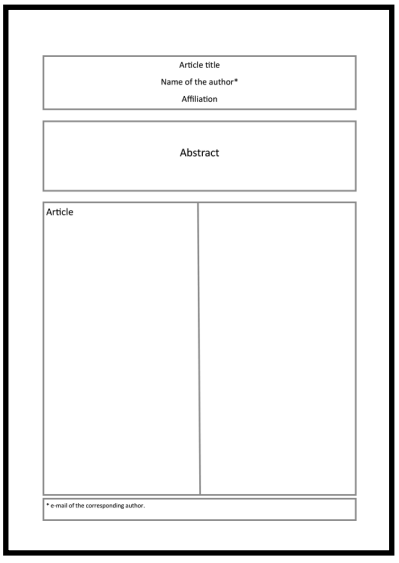

Selective growth inhibition by glycogen synthase kinase-3 inhibitors in tumorigenic HeLa hybrid cells

One of the most important considerations in cancer chemotherapy is selective killing of cancer cells without significant toxicity to normal cells. Cancer cells are well known to have accelerated metabolism, higher glucose requirements and increased glucose uptake. Glucose transport is a rate-limiting step for glucose metabolism and is mediated by glucose transporters (GLUTs) in mammalian cells. GLUT3 expression affect sensitivity in cancer cells. Increased glucose uptake is fundamental to many solid tumors and well associated with increases in glycolysis and the overexpression of glucose transporters (GLUTs) such as GLUT1 and GLUT3.
Adriamycin (ADR), camptothecin (CPT), ETOP, GSK-3 inhibitor IX and kenpaullone are some inhibitors that used for the inhibition of GSK-3 . GSK-3 is enzyme which regulates the activity of GLUTs in tumerogenic HeLa hybrid cells and change in the enzyme may leads to cell apoptosis. Because of this nature enzyme can be used as specific target to kill tumerogenic cells.

Chemical screening with a pair of HeLa cell hybrids identified GSK-3 inhibitors as useful for the selective killing of GLUT3-expressing tumor cells. Researchers also identified several commercially available GSK-3 inhibitors with selective killing activity, demonstrating a novel role for GSK-3 in the control of GLUT3 expression in tumor cell growth.

These types of inhibitors can be used as medicine of cancer and some of them are also on clinical trials. So from that we can conclude treating glucose metabolism can be help as therapy for tumor and cancers.

By

Shivam patel

IGBT IV

Interventions to improve water quality for preventing diarrhea

Diarrhoea is a major cause of death and disease, especially among young children in low-income countries. In these settings, many infectious agents associated with diarrhoea are spread through water contaminated with faeces. In remote and low-income settings, source-based water quality improvement includes providing protected groundwater (springs, wells, and bore holes), or harvested rainwater as an alternative to surface sources (rivers and lakes). Point-of-use water quality improvement interventions include boiling, chlorination, flocculation, filtration, or solar disinfection, mainly conducted at home.

Diarrhoea is a major cause of death and disease, especially among young children in low-income countries where the most common causes are faecally contaminated water and food, or poor hygiene practices.
In remote and low-income settings, source-based water quality improvement may include providing protected groundwater (springs, wells, andbore holes) or harvestedrainwater asanalternative tosurface sources (riversandlakes). Alternatively water may be treatedatthe point-of-use in people's homes by boiling, chlorination, flocculation, filtration, or solar disinfection. These point-of-use interventions have the potential to overcome both contaminated sources and recontamination of safe water in the home.

Interventions that address the microbial contamination of water at the point-of-use may be important interim measures to improve drinking water quality until homes can be reached with safe, reliable, piped-in water connections. The average estimates of effect for each individual point-of-use intervention generally show important effects. Comparisons between these estimates do not provide evidence of superiority of one intervention over another,

as such comparisons are confounded by the study setting, design, and population. Further studies assessing the effects of household connections and chlorination at the point of delivery will help improve our knowledge base. As evidence suggests effectiveness improves with adherence, studies assessing programmatic approaches to optimising coverage and long-term utilization of these interventions among vulnerable populations could also help strategies to improve health outcomes.

Contributed By

Malika Jha

IGBT V

Impact of plasma transaminase levels on the peripheral blood glutamate levels and memory functions in humans

Peripheral blood levels of aspartate aminotransferase (AST) and alanine transaminase (ALT) have been consider simply as liver injury biomarkers. Specifically, AST and ALT catalyze transamination from aspartate or alanine to glutamate and are significant positive regulators of tissue glutamate levels. Transaminases in human blood have efficient enzymatic transamination activity. Glutamate plays roles in many physiological brain functions including memory. Glutamate is a main excitatory neurotransmitter of the central nervous system. Memory dysfunction can affect our cognitive function, and peripheral blood glutamate levels have been reported to be altered in many cognitive function disorders, such as Asperger's syndrome and schizophrenia. The glutamate concentration in the blood is belief to have effects on brain function despite the existence of the blood brain barrier. Peripheral blood glutamate levels positively correlate with the concentration of glutamate in cerebrospinal fluid. There have been such a reports that have demonstrated that glutamate and glutamatergic receptor have significant effects on memory functions. One of the process by which glutamate affects memory function is through glutamatergic induction of long term potentiation (LTP) of synaptic strength thorough activation of calcium/calmodulin-dependent protein kinase II. Research demonstrate the significant relationships among plasma transaminases (AST and ALT), plasma glutamate levels, and memory functions in *homo sapiens*. Considering that research findings, scientists hypothesize that plasma transaminase elevation in obesityinduced fatty liver patients evokes memory function disorders and leads to food dependency, which results in further obesity (vicious liver-brain cycle). Violation of this vicious cycle should ameliorate metabolic syndrome progression.

Contributed By

Shivam Patel,

IGBT V

Challenges and Opportunities of Probiotic Microencapsulation Technology

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Abstract:

The administration of probiotic microbes for health benefit has rapidly expanded in recent years, with a global market worth $32.6 billion predicted by 2014. In order to confer health benefits, probiotic strains should remain in live form during the shelf life until consumption and maintain high viability throughout the gastrointestinal tract of humans. Therefore, an approach currently receiving considerable interest is to provide the probiotic live cells with a physical barrier against adverse environmental conditions. Probiotic encapsulation technology has the potential to protect microorganisms and to deliver them into the gut. However, there are many challenges to overcome with respect to the microencapsulation process and the conditions prevailing in the gut. This review focuses mainly on the methodological approach of probiotic encapsulation including biomaterials selection and choice of appropriate technology.

Keywords: Probiotics, Encapsulation, Immobilization, Biomaterials

Introduction:

Food and Agriculture Association of the United Nations (FAO) and World Health Organization (WHO) describes probiotic are a group of live microorganisms that, when administered in adequate amounts, confer a health benefit on the host[1].The history of probiotics begins with the history of man by consuming fermented food, but in 1908 a Russian researcher Ellie Metchnikoff, first proposed the beneficial effects of probiotic microorganisms on human health. Probiotics commonly are isolated from human and animal intestinal tracts. It is essential that probiotics must be considered as "generally recognized as safe" (GRAS) organisms for human use according to the US Food and Drug Administration. *Lactobacillus* and *Bifidobacterium spp*. are widely studied probiotic bacteria and prominent members of the intestinal flora[2, 3]. Survival of probiotics is based on their viability through acid tolerance, bile tolerance, gastric condition, intestinal condition. Apart from these preliminary characteristics, certain other criteria also set for microorganism to be used as "Probiotics", such as they must be non toxic, non pathogenic, non hypersensitive, stable and should show health benefit. Moreover, they should be compatible with product format to maintain desired sensory properties and are labelled in a truthful and informative manner to the consumer. Apart from high survival rates, the probiotic cultures should also not have a detrimental effect on sensory characteristics, for example, provide unpleasant flavours or textures. However, the oral administration of most of the probiotics results in the lack of ability to survive in a high proportion of the harsh conditions of acidity and bile concentration commonly encountered in the GI tract of human. Therefore, an approach which currently receiving considerable interest is Immobilization/Encapsulation of probiotics to solve the problems associated with storage and long term preservation of probiotics in food products.

1.1 Need of microencapsulation

In all cases, probiotic bacteria should remain alive from the time they are consumed until their settlement in the intestine. Providing probiotic living cells with a physical barrier has been

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proposed as an efficient technology to improve viability and preserve metabolic activity in the gastrointestinal tract[4], and to ensure viability during long-term storage[5]. Viability is defined as the number of trapped (encapsulated) probiotic cells (cfu g^{-1}) that remain viable in their site of action to produce a beneficial health effect to the host[6,7]. Encapsulation has been successfully used to improve cell viability during storage of several LAB including *Lactobacillus paracasei* NFBC 338 by spray-drying[8], *Lactobacillus casei* NCDC-298 by emulsification[9] and *L. casei* by extrusion[10].

1.2 Immobilization and Encapsulation

The terms immobilization and encapsulation are used interchangeably in most reported literature. The encapsulation is the process of forming a continuous coating around an inner matrix that is wholly contained within the capsule wall as a core of encapsulated material, while immobilization refers to the trapping of material within or throughout a matrix. In both cases, the bidirectional diffusion of molecules, such as the influx of oxygen, nutrients, and growth factors, essential for cell metabolism and the outward diffusion of waste products should be permitted.

In other words, encapsulation is defined as a process that entraps a substance into another substance, producing particles in the nanometer (nanoencapsulation), micrometer (microencapsulation) or millimeter scale[11,12]. The encapsulated substance is usually called core material, active agent, filler agent, internal phase, or payload phase. A substance used to encapsulate is called as coating membrane, shell, carrier or wall material, external phase or matrix. The carrier material used in food products or processes should be of food grade and must be able to form a barrier between the active agent and its surroundings[5].

1.3 Factors Affecting Microencapsulation process

The effectiveness of probiotic encapsulation process can be evaluated using different parameters such as viability maintenance after encountering detrimental environmental conditions, cell release/recovery ability, and hardening time. Different factors affecting the microencapsulation are discussed below.

1.3.1 Effect of Various Biomaterials on Viability of Probiotics

A wide variety of biomaterials have been used by researchers in order to check their effects on the process of microencapsulation as well as on the viability of probiotics.

1.3.2 Capsule Characteristics with Respect to the Surrounding Environment

Selection of capsular material with respect to the surrounding environment is very important. The probiotic cells are to be targeted in the small intestine, and selection of capsule material(s) should be such that their decomposition occurs after subjecting them to the small intestine pH or pancreatic enzymes. If the beads are to be retained in the large intestine, it is preferable to be tolerant against the pancreas and small intestine conditions. However, this is not always easily achievable due to the restrictions in the chemical characteristics of encapsulation materials. Generally, all the capsules must be resistant to the acidic conditions of gastric juices and sometimes it is necessary to use special types of hydrophobic components to make the beads tolerant against moisture.

1.3.3 Coating of the Capsule

Efficient coating of capsule improves its physicochemical property. For example, shell coating on the alginate capsules makes them resistant to the chelating agents of calcium ions and also increases their mechanical strength.

1.3.4 Concentration of Capsule material and Bead Diameter

Concentration of capsule preparing material and final bead diameter are factors which affect encapsulation efficiency. As bead diameter in-

creases, it causes inappropriate mouth feel and flavor. Furthermore, increasing capsule diameter decreases digestibility by pancreatic enzyme.

1.3.5 Modification of Capsule Materials

Chemical modification of capsular material improves encapsulation effectiveness. Structural modification of the capsule materials is by direct structural changes and/or by addition of special additives.

1.3.6 Initial Concentration of Microbial Cells

As concentration of microbial cells in the encapsulation solution increases, the number of entrapped cells in each bead (cell load) increases and, as a result, quantitative efficiency of encapsulation increases. If cell load exceeds the limit, softening of capsule structure occurs.

1.3.7 Conditions of Processing Factors

Microencapsulation processes such as freeze drying, spray drying, micro-ionization, and storage conditions are employed in order to avoid injuries to the beads and contained cells.

1.4 Biomaterials used for Microencapsulation of Probiotics

Biomaterial is defined as "Any natural material or not, which is in contact with a living structure and is intended to act with biological system." It includes natural and synthetic polymers which are directly in contact with living cell so they should be biocompatible and biodegradable. Encapsulation of probiotics in biodegradable polymer matrix has a number of advantages. Cryo- and osmoprotection agents can be incorporated into the matrix which enhances the survival of cell during storage and processing. It helps in the delayed release of cell by maintaining the dissolution properties of the coating layer. Apart from these properties carrier materials should have sufficient mechanical strength, nontoxic nature, easy availability and high loading capacity especially for its utilization in reactors and in industry.

1.4.1 Use of Alginate System for Encapsulation of Probiotics

Alginate is a naturally derived polysaccharide extracted from various species of algae and is composed of two monosaccharide units: α-L-guluronic acid (G) and β-D-mannuronic acid (M) linked from β (1–4) glycosidic bond[13]. High temperature (60°C to 80°C) is needed to dissolve alginate in water and usually concentration range of 0.5–4%. Alginate gels are insoluble in acidic media[14].

1.4.2 Use of Chitosan for Encapsulation of Probiotics

Chitosan is a linear polysaccharide with negative charge arising from its amine groups obtained by deacetylation of chitin. It can be isolated from crustacean shells, insect cuticles, and the membranes of fungi. It is a copolymer of two monomer residues anhydro-N-acetyl-D-glucosamine and anhydrous-D-glucosamine. It is soluble at pH < 6 and forms gel structure by ionotropic gelation. Chitosan can further polymerize by means of cross-linking formation in the presence of anions and poly anions[15]. It is used for coating of gelatin capsules, because its efficiency for the increasing viability of probiotic cells is not satisfactory; it is most often used as coat/shell but not as capsule.

1.4.3 Use of Starch for Encapsulation of Probiotics

Starch consists of D-glucose unit bind together with glycosidic bonds. High-amylose corn starch can be applied for enhancing functions of capsule or shell/coat formation. Lyophilized corn starch has been reported to be used as capsule-forming material; however, it decomposes after being subjected to pancreatic enzymes[16] while resistant starch is not degraded by the pancreatic amylase and it can be used by the probiotic bacteria in the intestine.

1.4.4 Use of Xanthan-Gellan Gum for Encapsulation of Probiotics

Gellan gum is an anionic polysaccharide derived from *Sphingomonas elodea* which is constituted of a repeating unit of four monomers, glucose, glucuronic acid, glucose, and rhamnose. Xanthan is also an exopolysaccharide available from *Xanthomonas campestris* and a effective ratio of xanthan-gellan gum is 1:0.75 which is resistant to acidic conditions.

1.4.5 Use of *κ*-Carrageenan for Encapsulation of Probiotics

Carrageenan is polymer having linear structure consisting of D-galactose units alternatively linked by *α*-(1–3) and *β* (1–4) bonds. Types of Carrageenan are kappa (*κ*), iota (*ι*), and lambda (*λ*)[17]. Carrageenan gelatin is induced by temperature changes. A rise in temperature (60–80°C) is required to dissolve it, and gelation occurs by cooling to room temperature, and then microparticles are stabilized by adding potassium ion. It is commonly used as a food additive. The encapsulation of probiotic cell in *κ*-carrageenan beads keeps the bacteria in a viable state, but the produced gels are brittle and do not withstand stresses[18].

1.4.6 Use of Gelatin for Encapsulation of Probiotics

Gelatin is a protein-based coating material used for encapsulation of probiotics. Because of its amphoteric nature, it is an excellent candidate to incorporate with anionic-gel-forming polysaccharides. It is frequently used in food and pharmaceutical industries[19]. It is a protein derived by partial hydrolysis of collagen of animal origin. It has versatile functional properties, and forms a solution of high viscosity in water which set to a gel on cooling.

1.5 Immobilization and Encapsulation Technologies

Immobilization techniques often mimic nature, as naturally many microorganisms own the ability to adhere to and survive on different kinds of surfaces, and thus cells may grow within natural structures. The selection of the encapsulation method depends on the required particle average size, the physical and chemical properties of the carrier material, the applications of the encapsulated material, the required release mechanism and overall cost. These parameters need to be studied for each specific organism and process[12, 20].

1.5.1 Extrusion technique

Extrusion is the oldest and the most common approach to make capsules with hydrocolloids (e.g., alginate and starch) and might be achieved by simply dropping an aqueous solution of probiotics into a gelling bath. The size and shape of the beads usually range 2–5 mm and depend on the diameter of the needle and the distance of free fall. It offers a small range size (smaller than emulsion), but it does not provide particles under 300 *μ*m. Extrusion is more popular than emulsion technology due to its simplicity, easy handling, low cost at least at small scale, and gentle formulation conditions, which ensure maintenance of high cell viability (80–95%). The technology does not use harmful solvents and can be done under both aerobic and anaerobic conditions. Application of jet cutter technology allows today large-scale production of the micro-beads. The survival of the probiotic microorganisms *L. acidophilus* 547, *B. bifidum* ATCC 1994, and *L. casei* 01 microencapsulated in chitosan-coated alginate pearls was evaluated in yogurt made with UHT milk and stored at 4°C for 4 weeks. Sodium alginate ($20gL^{-1}$) and chitosan ($4gL^{-1}$) were used to prepare the pearls. The results showed that the survival of the encapsulated probiotic bacteria was greater vs. free cells in approximately one log cycle[21].

1.5.2 Emulsion technique

An emulsion is the dispersion of two immiscible liquids in the presence of a stabilizing compound or emulsifier. When the core phase is in an aqueous phase termed a water-in-oil emulsion (w/o)

while a hydrophobic core phase is termed an oil-in-water emulsion (o/w). Entrapment of cells of lactic bacteria (*Lactobacillus delbrueckii* ssp. *bulgaricus*) in the droplets of reconstituted sesame oil body emulsions increased approximately 10^4 times their survival rate compared to free cells when subjected to simulated GI tract conditions[22]. The particle size formed by this method is smaller (25 µm- 2 µm) than the size produced by the extrusion method (2 to 5 mm). The need for vegetable oil in the formulation may increase operation costs when compared with the extrusion method[6].

1.5.3 Lyophilization or Freeze drying method

Lyophilization is done by freezing the probiotic together with the carrier material (typically between -30 and -20°C), followed by vacuum sublimation of water at absolute pressure between 0.05 to 0.1 mBar and temperature between -50 to -30°C. Once lyophilized, cryoprotectants are added to preserve and stabilize the probiotic activity during storage. The most common cryo protectants are lactose, trehalose, sorbitol, sucrose, milk protein and skim milk[23]. Encapsulated probiotics by lyophilization have better storage stability, especially at low temperatures and inert atmosphere (nitrogen or vacuum)[5]. Unfortunately, lyophilization is 4 to 7 times more expensive than spray drying[24].

1.6 Conclusion & Future perspective

In the present article principle, methods, and materials used in the encapsulation process of probiotic cells are discussed. The delivery of viable microencapsulated probiotic bacteria will have tremendous demand in the field of nuetraceuticals in near future. It gives structure and innovative system to the core material for the probiotic food product and also enhances the productivity and efficiency. It protects biological cells against surrounding environment which destruct the core. However, microencapsulation method is promising on a laboratory scale, the developed technologies for producing gel beads still present serious difficulties for large -scale production of food grade microencapsulated microorganisms. Another major challenge is to improve the viability of probiotics during the manufacturing processes, particularly heat processin.

References

1. FAO/WHO. *Guidelines for the Evaluation of Probiotics in Food. London*, UK: Food and Agriculture Organization of the United Nations/ World Health Organization; (2002).
2. Fuller R. Probiotics in man and animals. *Journal of Applied Bacteriology*. **66 (5)**:365–378 (1989).
3. Solanki, HK, Pawar, DD, Shah, DA, Prajapati, VD, Jani, GK, Mulla, AM, and Thakar, PM Development of Microencapsulation Delivery System for Long-Term Preservation of Probiotics as Biotherapeutics Agent, Biomededical Reseach International, doi**10**.1155/2013/620719, (2013).
4. Picot A, Lacroix, C Encapsulation of Bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt, International Dairy Journal. **14(6**), 505–515,(2004). .
5. Zuidam NJ, Nedovic VA Encapsulation Technologies for Active Food Ingredients and Food Processing. Springer. USA, (2010).
6. Krasaekoopt, W., Bhandari, W. and Deeth, HC. Evaluation of encapsulation techniques of probiotics for youghurt, International Dairy Journal. **13**, 3-13, (2003).
7. De Vos P, Faas MM, Spasojevic M, Sikkema J. Encapsulation for preservation of functionality and targeted delivery of bioactive food components. International Dairy Journal. **20**, 292-302(2010).
8. Desmond C, Ross RP, O'Callaghan E, Fitzgerald G, Stanton C. Improved survival of *Lactobacillus paracasei* NFBC 338 in spray-dried powders containing gum acacia. Journal of Applied Microbiology. **93(6**), 1003–1011(2002).

9. Mandal S, Puniya A, Singh Y. Effect of alginate concentrations on survival of microencapsulated *Lactobacillus casei* NCDC-298. International Dairy Journal. **16**, 1190-1195(2006).
10. Sandoval and Castilla O Textural properties of alginate-pectin beads and survivability of entrapped *Lb. Casei* in simulated gastrointestinal conditions and in yoghurt, food research international **43(1**), 111-117 (2010). .
11. Lakkis JM. Encapsulation and Controlled Release Technologies in Food Systems. Blackwell Publishing (2007).
12. Burgain C, Gaiani C, Linder M, Scher J. . Encapsulation of probiotic living cells: From laboratory scale to industrial applications. Journal of Food Engineering, **104**:467-483 (2011).
13. Dong Z, Wang Q, Du Y. Alginate/gelatin blend films and their properties for drug controlled release*. Journal of Membrane Science.* **280**(1-2), 37–44(2006).
14. Hansen LT, Allan-Wojtas PM, Jin Y-L, Paulson AT. Survival of Ca-alginate microencapsulated *Bifidobacterium* spp. in milk and simulated gastrointestinal conditions. *Food Microbiology,* **19(1):**35–45 (2002).
15. Klein J, Stock J, Vorlop K-D. Pore size and properties of spherical Ca-alginate biocatalysts. *European Journal of Applied Microbiology and Biotechnology.* **18(2**):86–91 (1983).
16. Fanta GF, Knutson CA, Eskins KS, Felker FC. Starch microcapsules for delivery of active agents. US patent, **6**, 238, 677 (2001). .
17. Gaaloul, S, Turgeon SL, Corredig M. Influence of shearing on the physical characteristics and rheological behaviour of an aqueous whey protein isolate-*κ*appa-carrageenan mixture. *Food Hydrocolloids.***23(5**), 1243–1252 (2009).
18. Chen MJ, Chen KN. Applications of probiotic encapsulation in dairy products. In: Lakkis JM, editor. *Encapsulation and Controlled Release Technologies in Food Systems.* New York, NY, USA: Wiley-Blackwell; pp. 83–107 (2007).
19. Rokka S, Rantamäki P, Protecting probiotic bacteria by microencapsulation: challenges for industrial applications. *European Food Research and Technology*, **231(1),** 1–12(2010).
20. Parra-Huertas RA Revisión: Microencapsulación de Alimentos. Revista Facultad Nacional de Agronomía, Medellín, **63(2)**, 5669-5684 (2010).
21. Krasaekoopt, W., Bhandari, W. and Deeth, HC. Survival of probiotics encapsulated in chitosan-coated alginate beads in yoghurt from UHT and conventionally treated milk during storage, Food Science and Technology, **39(2),** 177–183(2006).
22. Hou, RCW, Lin, MY, Wang, MMC and Tzen, JTC. Increase of viability of entrapped cells of *Lactobacillus delbrueckii* ssp. *bulgaricus* in artificial sesame oil emulsions. Journal of Dairy Science. **86(2),** 424–428 (2003). .
23. Semyonov D, Ramon O, Kaplun Z, Levin-Brener L, Gurevich N, Shimoni E Microencapsulation of *Lactobacillus paracasei* by spray freeze drying. Food Ressearch International. **43**, 193-202 (2010).
24. Chavez BE and Ledeboer AM, Drying of Probiotics: Optimization of Formulation and Process to Enhance Storage Survival, Drying Technology: An International Journal,**25(7-8),** 1193-1201 (2007).

Human Milk: A source of Microflora

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Abstract: Breast milk is a vital source of nutrient as well as bacteria. Bacteria that are present in milk aid to initiation and development of infant gut microflora. These bacteria play an important role in reduction of incidences and severity of infection to the child due to their probiotic properties. Breast milk protects the newborn against infectious diseases, as it consist of breast milk components like different antimicrobial compounds, immunoglobulin, immune component cells and bacteriocin secreted by probioic bacteria which all together stimulate the growth of the beneficial bacteria in neonate gut. In the present review effort will be made to understand a development of milk microflora and also the microbial diversity in breast. Information

Human milk

The human microbiome project was taken up by National Institutes of Health in the year 1991 with a goal to conduct survey of microbes present within the body and those resting on human body and the potential impact these communities may have on health. However, one of the key system ignored, was the human milk. Human milk is an intricate biological fluid which fulfills nutritional supplies of new born baby, helps in the development of infant immune system and provide defense against pathogens [1]. Bioactive molecules like polyamines, oligosaccharides, fatty acids, lactoferrin, lysozyme, immunoglobulin, immune-competent cells and antimicrobial peptides present in colostrums and milk[2]are the main constituent involved in providing defense. Recent studies articulate the presence of not only the environmental bacteria but also the symbiotic and probiotic bacteria in the milk which are transmitted through milk to the infant and hence contribute in constructing gut microflora ofinfant [3].Daily consumption of breast milk by an infant is 800 ml/day, this in fact contributes to transport 1×10^5 to 1×10^7 bacteria each day leading to their colonization in gut and finally built up gut microflora [4].Human milk protect against gastrointestinal infections[5], respiratory infections[6] and allergic diseases.According to the American Academy of Pediatrics (AAP, 2012) it also trimmed down possibility of diseases like Inflammatory Bowel Disease (IBD), obesity or diabetes(AAP, 2012).

As the neonate are born with immature immune system theyare more prone to get infected. In such situation breast feeding can help in building up the immune system of infant as it containsfatty acids, α-lactalbumin, sIgA, oligosaccharides, lactoferrin, lysozyme, antioxidants and cytokines molecules bearing immunoprotective role. Human milk proteome consist of 976 proteins out of which plentiful possess immunogenic property. In addition to immune molecules, breast milk also consists of blood derived leukocytes which gets transported to the milk via paracellular pathway. Often, cellular and biochemical milk components work synergistically having direct or indirect effect (e.g. modifying the microenvironment of the infant gut) on infant immunity. Human milk bacteria play several roles in the infant gut, they reduce incidence and severity of infections, involve in production of antimicrobial compounds or improve intestinal barrier function by enhancing mucine production and reducing intestinal permeability [7]. Studies have shown accumulation of*Lactobacillus* strain from human milk to the gut of infant which leads to 46%, 27%, and 30% reductions in the incidence rates of gastrointestinal infections, upper respiratory tract infections and total number of

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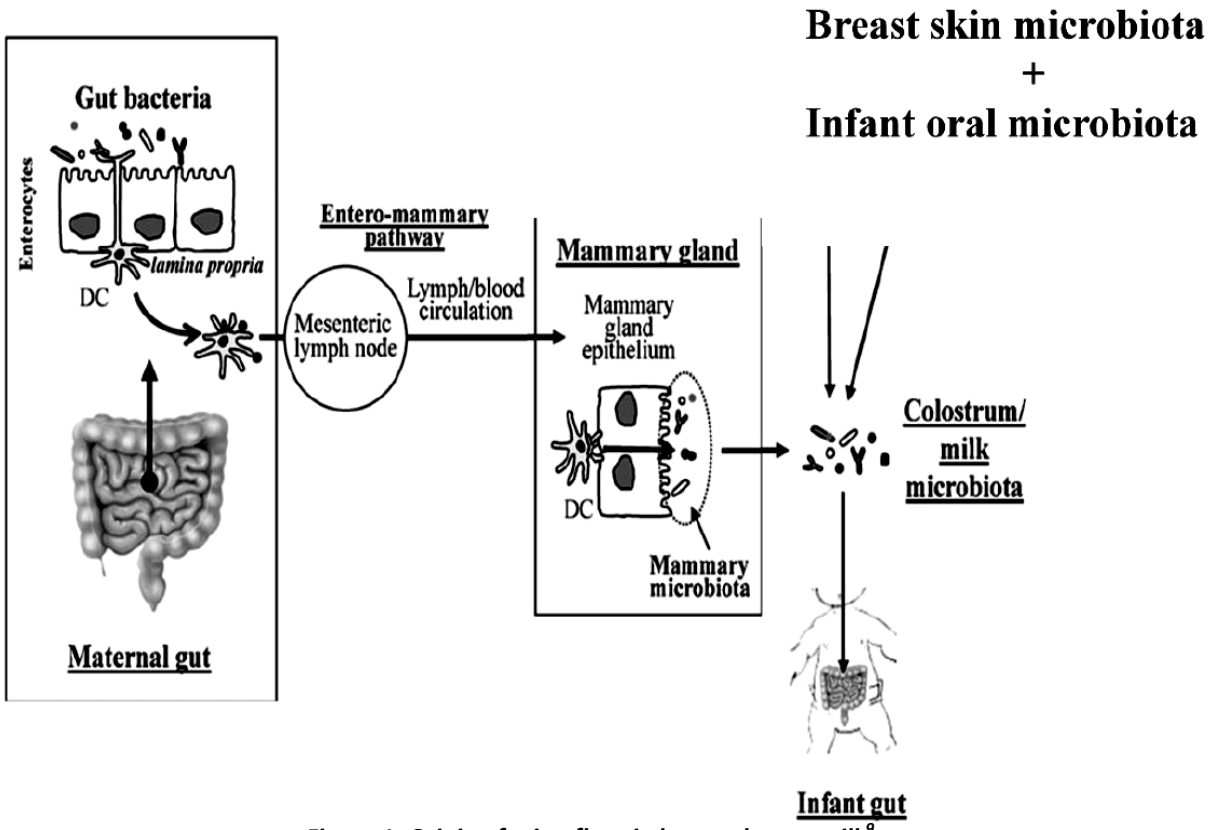

Figure 1: Origin of microflora in human breast milk[9].

infections, respectively[8]. These microorganisms also contribute in digestion by breaking down sugars and proteins; participate in the right maturation of the infant immune system.

Origin of microflora in the human milk

There exists an ordered mechanism which leads to establishment of human milk microbiome (figure1). During pregnancy it is observed that physiological and hormonal changes leads to increased permeability of gut as a consequence of which microbes get drift towards mammary gland.

Reports says that dendritic cells and macrophages plays key role in the transfer of microbes to the mammary gland [9]. Also the retrograde flux between the mother's skin microbes and infant's oral microbes to some extent contribute in the development of the human milk microbiome[10,11].

Mechanisms of Heath Promoting Bacteria – Probiotic against Pathogenic Infection

The milk microbiota plays a considerable role in decreasing frequency of infection in new born baby because of their probiotic properties (figure 2). Probiotics have potential to produce antimicrobial substance like bacteriocin, acetic acid, lactic acid, hydrogen peroxide and diacetyl, each work in its particular manner to wipe out the unwanted bacteria and generates the antagonist effect towards the pathogenic bacteria. Bacteriocins are important antimicrobial peptide. They causes increased secretion of mucus thereby demolish intestinal pathogens [12-14], on the other had acetic acids and lactic acids reduces the intestinal pH and makes the environment advisable for the survival of pathogens [15]. Probiotics can eradicate pathogens via competitive exclusion and/or blocking the attachment of them to the intestinal epithelium cells while competing for the glycoconjugate receptors [16]. Moreover, a competition for essential nutrient occur between probiotics and pathogens, which is on the bases of nutrient absorptions, the innate metabolic ability, growth rate and the secretion of inhibitors [17].

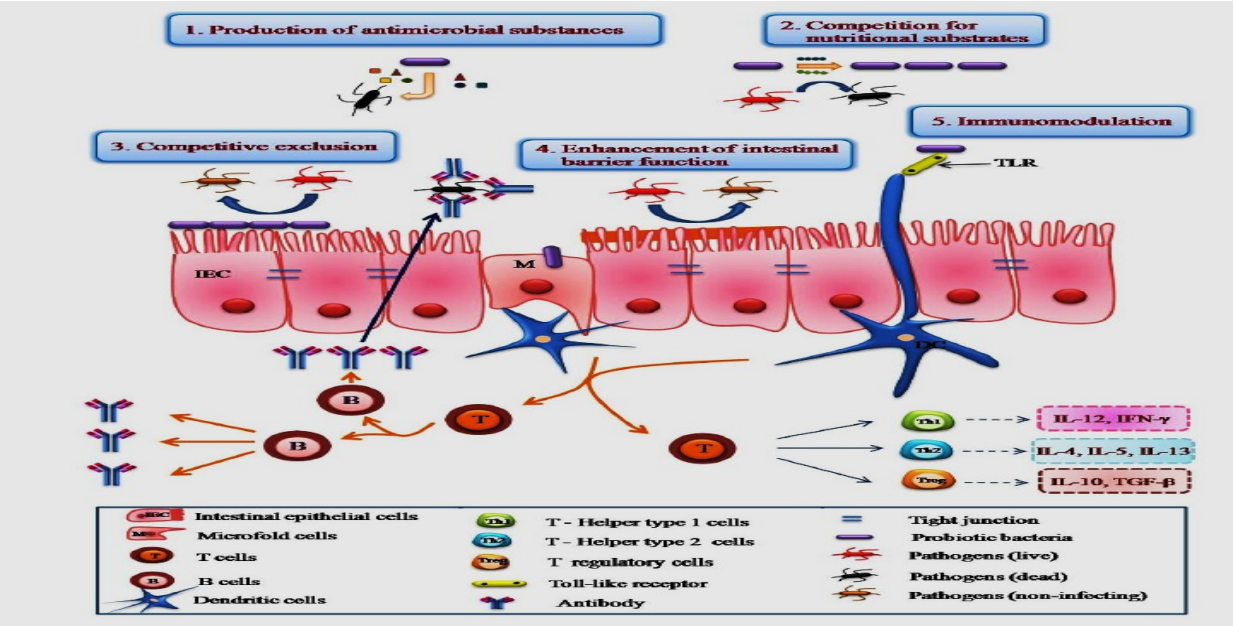

Figure 2: mechanisms of probiotic bacteria

Microbial profileof human milk.

Untilllast decades, microbiological studies, focused on human milk, were restricted to identification of potential pathogenic bacteria in stored milk or milk retrieved from infected breast milk however microbes present in healthy mother breast milk remained unexplored. Identification of microbes present in the human milk using culture base and culture independent methods was for the first time reported in the year 2003 where 8 healthy lactating Spanish mothers along with their infants were selected to study prevalence of lactic acid bacteria.[18]. They reported presence of *Enterococcus faecium* and *Lactobacillus gasseri*inhealthy breast milk, areola, the infant's mouth and feces. However lactic acid bacteria that happened to be present in the milk where dissimilar to those present on the skin of the breast. As an outcome, it can be concluded that human milk is a vital source of lactic acid bacteria which are transferred to the neonates at the time of breast feeding and that these microbes are not result of skin contamination [19].Their study also revealed that the possible amount of lactic acid bacteria and other health promotingbacteriareduces the risk of lactation mastitis to great extent [20].

In 2011, Hunt et al. used new approach i.e. 454-pyrosequencing in which specific primer targeting the V1–V2 hypervariable regionof 16S rRNA-gene were used. They characterized the microbial diversity and temporal stability of bacterial in 16 healthy milk samplescollected 3 times over a 4-wk period [21]. Data analysis at the genus level demonstrated high species richness and diversity of bacteria, while only 9 genera were found frequent in all the samples. A total of 52% of all operational taxonomic units were allocated to "core" bacterial species which included *Pseudomonas, Staphylococcus, Serratia, Corynebacterium,Ralstonia, Streptococcus, Sphingomonas, Bradyrhizobium*and*Propionibacterium*. Limitation of culture dependent method isthat we cannot know the precise number of bacteria but only comparative abundances of microbial taxacan be forecasted. This was a first type of report which exposed characterization of microbial community present in human milk using next-generation sequencing techniques. Similar type of study was also performed on 18 Finnish women by Cabrera-Rubio et al. who studied the human milk microbial at three different time point i.e. colostrum and 1 &6 month postpartum)[22]. For ecological study at the family

and genus levels, they used 454-pyrosequencing and quantitative PCR using 16S rRNA gene primer which can amplify V1 to V3 region of the 16S rRNA gene.Data analysis articulate high abundance of lactic acid bacteria,(mainly in colostrum) beside this higherabundance of *Staphylococcus, Veillonella, Streptococcus, Prevotella*and*Leptotrichia*was also observed in the milk samples[22].

Extensive characterization of the human-milk microbiota was done by Jost et al. using both culture-dependentand culture-independent techniques.They used primer specified for V5–V6 region of 16S rDNA.The study was carried on milk collected from 7 healthy breast of Swiss women, each samples was collected during the first month of delivery. Phyla which were foundto predominant were*Bacteroidetes,Actinobacteria,Proteobacteria* and *Firmicutes,*along withgenes *Pseudomonas, Staphylococcus, Ralstonia, Streptococcus, Bacteroides,Blautia,* and *Bifidobacterium.*However *Lactobacillus* and *Enterococcus* genera were present in 15% and 9.5% of thespecies richness in healthy human milk samples, respectively.

In 2013, Ward et al. used illumina plat form to study 10 milk sample collected from 10 different lactating women between 9 to 30 days after delivery[23]. Microbes present in milk were identified at the phylum and genus levels. In addition, microbial community present in the milk was compare with microbes present in themother and neonate fecal samples atthe phylum level. Above 360 bacterial genera were identified, in addition it was also observed that breast milk is less diverse compared to diversity of bacteria in infantsfeces. Mainly the variation among the findings of colleagues, Huntand Ward was observed which may be due to variation in samplingmethods (e.g., sanitized vs. uncleanbreast), DNA extraction techniques (e.g., using bead beating or chemical base rupture of bacterial cell membranes), next generation sequencing platforms (pyrosequencing vs. Illumina), and environmental differences (e.g., diet, geographical region).

In 2014, Khodayar-Pardo et al.also studied the bacterial community present in 32 Spanish breastfeeding women. They identified *Enterococcus, Lactobacillus* and *Streptococcus spp.* as the dominant bacterial species[24].Later,Tu_sar et al. also found *Bifidobacterium Lactobacillus, Enterococcus*and *Staphylococcus* species from 47 Slovenian lactating mother[25]. Gonzalez et al. found presence of *Staphylococcus, Streptococcus* and *Lactobacillus* genera from milk collected from Mozambique mother[26]. Albesharat et al., used an well-designed combination of culture-dependent and culture-independent method for identification of lactic acid bacteria present in Syrian lactating mother milk[11].

In conclusion there is strongevidence that human milk consists of a diverse and feasiblemicrobial community, although it is secreted by healthy lactating mother without any signs and symptom of mastitis or other mammary gland disease. Somehow, variations of microbial profiling in studies wereresulted due to behavioral,environmental or genetic differences or as a consequence of methodological variation.Occurrence of bacteria is not limited to humans only, thereare studies which sustain presence of bacterial populations in milk of ovine, bovine, and caprine taxa. As such, the era has come to move away from the past belief that breast milk is sterile tothe current study that it is comprises of rich microbial community. This acknowledgment is the primaryobligatory step in allowing thatbreast milk should be considered as a probiotic food.

References

1. Morrow, A. L. & Rangel, J. M. in *Seminars in pediatric infectious diseases.* 221-228 (Elsevier) .
2. Newburg, D. S. Innate immunity and human

milk. *The Journal of nutrition* **135**, 1308-1312 (2005).
3. Martín, R. *et al.* Isolation of bifidobacteria from breast milk and assessment of the bifidobacterial population by PCR-denaturing gradient gel electrophoresis and quantitative real-time PCR. *Applied and Environmental Microbiology* **75**, 965-969 (2009).
4. Heikkilä, M. & Saris, P. Inhibition of Staphylococcus aureus by the commensal bacteria of human milk. *Journal of Applied Microbiology* **95**, 471-478 (2003).
5. Duijts, L., Jaddoe, V. W., Hofman, A. & Moll, H. A. Prolonged and exclusive breastfeeding reduces the risk of infectious diseases in infancy. *Pediatrics* **126**, e18-e25 (2010).
6. Nishimura, T., Suzue, J. & Kaji, H. Breastfeeding reduces the severity of respiratory syncytial virus infection among young infants: A multi-center prospective study. *Pediatrics International***51**, 812-816 (2009).
7. Olivares, M. *et al.* Human milk composition differs in healthy mothers and mothers with celiac disease. *European journal of nutrition* **54**, 119-128 (2014).
8. Maldonado, J. *et al.* Human milk probiotic *Lactobacillus fermentum* CECT5716 reduces the incidence of gastrointestinal and upper respiratory tract infections in infants. *Journal of pediatric gastroenterology and nutrition***54**, 55-61 (2012).
9. Fernández, L. *et al.* The human milk microbiota: origin and potential roles in health and disease. *Pharmacological Research* **69**, 1-10 (2013).
10. Makino, H. *et al.* Transmission of intestinal Bifidobacterium longum subsp. longum strains from mother to infant determined by multilocus sequencing typing and amplified fragment length polymorphism. *Applied and environmental microbiology*, AEM. 05346-05311 (2011).
11. Albesharat, R., Ehrmann, M. A., Korakli, M., Yazaji, S. & Vogel, R. F. Phenotypic and genotypic analyses of lactic acid bacteria in local fermented food, breast milk and faeces of mothers and their babies. *Systematic and Applied Microbiology* **34**, 148-155 (2011).
12. Thirabunyanon, M., Boonprasom, P. & Niamsup, P. Probiotic potential of lactic acid bacteria isolated from fermented dairy milks on antiproliferation of colon cancer cells. *Biotechnology letters* **31**, 571-576 (2009).
13. Verdenelli, M. C. *et al.* Probiotic properties of Lactobacillus rhamnosus and Lactobacillus paracasei isolated from human faeces. *European journal of nutrition* **48**, 355-363 (2009).
14. Gaudana, S. B., Dhanani, A. S. & Bagchi, T. Probiotic attributes of Lactobacillus strains isolated from food and of human origin. *British Journal of Nutrition* **103**, 1620-1628 (2010).
15. Ridwan, B. *et al.* Antimicrobial activity of a multispecies probiotic (Ecologic 641) against pathogens isolated from infected pancreatic necrosis. *Letters in applied microbiology* **46**, 61-67 (2008).
16. Vanderpool, C., Yan, F. & Polk, D. B. Mechanisms of probiotic action: implications for therapeutic applications in inflammatory bowel diseases. *Inflammatory bowel diseases* **14**, 1585-1596 (2008).
17. Nilsson, L., Gram, L. & Huss, H. H. Growth control of Listeria monocytogenes on cold-smoked salmon using a competitive lactic acid bacteria flora. *Journal of Food Protection®* **62**, 336-342 (1999).
18. Martín, R. *et al.* Human milk is a source of lactic acid bacteria for the infant gut. *The Journal of paediatrics* **143**, 754-758 (2003).
19. Rodríguez, J. M. The origin of human milk bacteria: is there a bacterial entero-mammary pathway during late pregnancy and lactation? *Advances in Nutrition: An International Review Journal* **5**, 779-784 (2014).
20. Arroyo, R. *et al.* Treatment of infectious mastitis during lactation: antibiotics versus oral administration of Lactobacilli isolated

from breast milk. *Clinical Infectious Diseases* **50**, 1551-1558 (2010).
21. Hunt, K. M. *et al.* Characterization of the diversity and temporal stability of bacterial communities in human milk. *PLoS One* **6**, e21313 (2011).
22. Cabrera-Rubio, R. *et al.* The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery. *The American journal of clinical nutrition* **96**, 544-551 (2012).
23. Ward, T. L., Hosid, S., Ioshikhes, I. & Altosaar, I. Human milk metagenome: a functional capacity analysis. *BMC microbiology* **13**, 116 (2013).
24. Khodayar-Pardo, P., Mira-Pascual, L., Collado, M. & Martinez-Costa, C. Impact of lactation stage, gestational age and mode of delivery on breast milk microbiota. *Journal of Perinatology* **34**, 599-605 (2014) .
25. Soto, A. *et al.* Lactobacilli and bifidobacteria in human breast milk: influence of antibiotherapy and other host and clinical factors. *Journal of pediatric gastroenterology and nutrition* **59**, 78 (2014).
26. González, R. *et al.* Breast milk and gut microbiota in African mothers and infants from an area of high HIV prevalence. (2013).


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lation including biomaterials selection and choice of appropriate technology. In near future microencapsulated probiotic bacteria will have tremendous demand in the nuetraceuticals.

Breast milk play vital role in providing immunity to the newborn against infectious diseases, as it consist of breast milk components like different antimicrobial compounds, immunoglobulin, immune component cells and bacteriocin. In the present review authors described that how milk microflora was development and also the microbial diversity in breast milk. Authors also find out the strong evidence that human milk consists of a diverse and feasible microbial community. The variations of microbial profiling in studies were resulted due to behavioral, environmental or genetic differences although it is secreted by healthy lactating mother without any signs and symptom of mastitis or other mammary gland disease. The era has come to move away from the past belief that breast milk is sterile to the current study that it is comprises of rich microbial community. This review also suggested that, breast milk can be considered as a probiotic food.