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Biologically active components of human and bovine milk as potent antimicrobial agents

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Abstract: Colostrums and milk are rich source of antimicrobial proteins and peptides which plays a vital role in immunity of offspring and accelerate maturation of their immune system. These primary protective factors of milk do have potential to be explored as bio-therapeutic agents in prevention and therapy of multiple microbe mediate infections and food spoilage. Natural bioactive components of milk exhibit antibacterial, antiviral, antifungal, antiparasitic, anti-tumoral, anti-inflammatory and anti-oxidative properties that open new avenues for development of safe and cost effective therapies. This review provides a brief overview of the antimicrobial components of milk with their potential application as therapeutic agents. The term 'bovine' used in upcoming sections, represents Cow and Bufallo milk, unless until specified. A detailed understanding of these antimicrobial proteins and other components offers great potential to add value to the dairy industry. There is rich scope of identifying novel milk peptides having both nutritional and therapeutic values.

Keywords: Antimicrobial; Bio-therapeutic; Colostrums; Milk; Therapies; Bioactive Components

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Milk, defined as a dynamic bioactive fluid, contains distinct bioactive molecules responsible for nutrition, growth and protection against infection and contributes to immune maturation, organ development and establishment of healthy gut flora (Ballard and Morrow 2013). It is a complete and sole food for newborn mammals during the early stages of rapid development (Severin and Wenshui 2005). Nutritional value of milk and milk product is due to presence of lactose (5%), protein (3.2%), lipids (4%) and mineral salts (0.7%) (Severin and Wenshui 2005). Besides being enriched with the nutritional components, milk is a rich source of components providing immunological protection to both neonates and adults (Warner et al. 2001). Colostrums and milk, comprises the first natural host defense system, and are critical for survival (Clare et al. 2003). However, composition of both human and bovine milk changes from colostrums to late lactation, and also varies with the type of food and feed and between mothers (Ballard and Morrow 2013).

Several of the milk proteins with antimicrobial potential are relatively resistant to proteolysis in gastrointestinal tract and contribute to the defense against bacteria, viruses and yeasts (Lonnerdal 2003). Milk proteins, among the total protein content of human diet have highest immune stimulation potential (Ambroziak and Cichosz 2014). Identification and characterization of the anti infectious and antimicrobial components of both colostrums and milk, has been among the early scientific discoveries and this group of proteins are being explored and studied for their health benefits. Results from some recent studies supports the assumption that milk and dairy product consumption is associated with lower incidence of type 2 diabetes and insulin resistance, suggesting their anti-diabetic and anti-inflammatory potential (Hirahatake et al. 2014). Presently there is major focus on discovery of novel, antimicrobial milk peptides that can be generated from existing milk proteins via proteolysis or during microbial fermentation (Clare et al. 2003; Dallas et al. 2013). Pathogenic organisms pose a threat to

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health and well-being of both humans and animals. These threat organisms are evolving at a faster rate than our therapeutic regimes and are easily transmitted through environment and un-hygienic practices followed during preparation of food products. Food poisoning outbreaks are on rise, especially in developing countries like India (Dikid et al. 2013).

Besides alarming increase in pathogen led infections, increasing trend of development of antimicrobial resistance in pathogens, especially against traditional and present day antibiotics is posing a serious threat globally (Koluman and Dikici 2013; Raghunath 2008). There is strong need to develop alternative treatment regimes, especially non-toxic and cost effective interventions having novel mode of action and cellular targets different from current day therapeutics (Ibrahim et al. 2002). Antimicrobial components of milk provide a potential alternative to current day therapeutics. The present article reviews the antimicrobial components of both bovine and human milk with brief introduction to other nutritional and bioactive components, and their potential application as bio-therapeutic agents. Future research developments in the current field can open new and promising avenues against common life style disorders, microbial derived infections along with associated health benefits.

SPECTRA OF ANTIMICROBIAL AGENTS IN MILK

Antimicrobial components of milk have been broadly differentiated into specific and non-specific factors, based on the mode of their action. Specific components viz. Immunoglobulins, Complement system, Macrophages and Bifidus factor; provide protection against specific antigens**.** However, non specific ones, including Lactenins, Lactoferrin, Lysozyme and Lactoperoxidase have broad reactivity against multiple pathogens. Both the specific and non specific antimicrobial components are discussed herein below and summarized in tabular form (Table 1 and 2) for quick comparison.

Immunoglobulin (Ig)

Immunoglobulins constitute an important component of the immunological activity found in colostrum and mature milk. They are transferred from mother to offspring as passive immunity during early stages of life (Hurley and Theil 2011). Immunoglobulins function as antibodies, the antigen binding proteins that are expressed over B cell membrane and are also secreted by plasma cells. In ruminants in particular, where no exchange of immune factors occurs in utero, colostrums and, to a lesser extent, milk provide protection through a high immunoglobulin content, without which the ruminant would not survive (Larson et al. 1980). The concentration of immunoglobulins is particularly high in colostrum, with IgG being the major immunoglobulin class in ruminants (47.6 mg/ml) and IgA being the major immunoglobulin present in human colostrum (17.35 mg/ml). In contrary to colostrums, concentrations of IgG and IgA in mature milk of ruminant and human are 0.59 and 1 mg/ml respectively (Stelwagen et al. 2008).

Immunoglobulins has been divided into five classes viz. IgG, IgM, IgA, IgE and IgD, determined by the type of heavy chain molecule. In brief, IgG is the most abundant (80 %) class in serum, divided into four subclasses (IgG1-IgG4). IgM accounts for 5-10% of total serum Ig, secreted by plasma cells as pentamer. It is the first Ig molecule to be synthesized by the neonate and to be produced as primary response to antigen. IgE, responsible for allergic reactions, is present in extremely low concentration in serum. IgD, together with IgM, is the major membrane bound Ig functioning in

Table 1 Specific antimicrobial components of milk

	Immunoglobulins	Complement	Macrophages	Bifidus factor
Source	Human and Bovine	Human and Bovine	Human and Bovine	Prominent in human with very low activity in ruminants (Gyorgy et al. 1954)
Activity	Bactericidal	Bactericidal	Bactericidal	Growth promotion of specific bacteria
Thermal Stability	Stable at Pasteurization temperature, reduced activity at boiling	Heat labile at pasteurization temperatures (Goldsby et al. 2000)	Stable at Pasteurization temperature, decreased survival at freezing conditions (Cress and Paxson 1977)	Stable at autoclaving conditions (Kunz 2012)
Mechanism of action	Activation of B cells; prevents attachment of pathogenic bacteria to the walls of gut (Goldsby et al. 2000)	Opsonization and Phagocytosis (Frank and Fries 1991; Goldsby et al. 2000)	Phagocytosis mediated lysis (Stelwagen et al. 2008)	Creates acidic pH and prevents pathogenic bacteria (Kunz 2012); specifically promotes *Bifidobacteria* (Kitaoka 2012)

Table 2 Non specific antimicrobial components of milk

	Lactenins	Lysozyme	Lactoferrin	Lactoperoxidase
Distribution	--	Whey	Whey (70%) and Casein (30%)	--
Source	Human, Bovine	Human > Bovine	Human > Cow > Buffalo	Bovine, negligible in human
Activity	Bactericidal	Bacteriostatic and Bactericidal	Bacteriostatic and Bactericidal	Bactericidal
Thermal Stability	Partial Inactivation at 65°C/30 min; Inactivation at 70°C (Yadav et al. 1993)	Stable at acid and neutral pH but labile at alkaline (Venkataramani et al. 2013)	Stable at 100°C/5 min. Degraded at 120°C/5 min but retains antibacterial activity (Abe et al. 1991)	Stable at pasteurization (63°C/30 min and 72°C/15 sec) but destroyed at 80°C/ 2.5 sec (Ludikhuyze et al. 2001)
Mechanism of action	Bacterial agglutination (Singhal et al. 1997)	Hydrolysis of beta 1-4 glycosidic linkage between NAM-NAG of gram positive peptidoglycan (Masschalck and Michiels 2003)	Iron deprivation (Levy 1996) and cell membrane disruption (Ellison et al. 1988; Leitch and Willcox 1999)	Hypothiocynate mediated oxidation (Sharma et al. 2013)

activation of B cells. Bovine serum and milk contains three major immunoglobulins viz. IgG, IgM and IgA; that are selectively transported from serum to bovine mammary gland. Colostrums contains very high doses of total Igs (40-200 mg/ ml); out of which IgG1 accounts for over 75% of Igs in colostral whey, followed by IgM, IgA and IgG2. Total concentration of Ig reduces to around 0.7-1.0 mg/ml in mature milk (Korhonen et al. 2000).

Immunoglobulin A (IgA) represents 10-15% of total Immunoglobulin in serum. It is predominant Ig class in external secretions such as breast milk, saliva, tears and mucus of the bronchial, genitourinary and digestive tracts. IgA of external secretions, called secretory IgA, consists of a dimer or tetramer, a J chain polypeptide, and a polypeptide chain called secretory component. Daily production of SIgA is greater than that of any other Immunoglobulin class. IgA secreting plasma cells are concentrated along mucus membrane surfaces. Along the jejunam of the small intestine, there are more than $2.5x10^{10}$ IgA secreting plasma cells, a number that exceeds total plasma cell population of bone marrow + lymph + Spleen. SIgA provide defense against bacteria such as *Salmonella*, *V. cholera* and *Neisseria gonorrhoeae* and viruses such as polio, influenza and reovirus. Secretory IgA binds to microbes in infant's digestive tract and thereby prevent their attachment to the walls of the gut and their subsequent passage into the body's tissues (Goldsby et al. 2000).

Complement system

Complement system was first discovered by Jules Bordet in 1894, as the activity of blood serum that completes the action of antibody (Ogundele 2001). Complement system of milk plays a significant role in providing innate immunity against udder microorganisms, through its bactericidal, opsonic, and phlogistic functions (Rainard 2003). Complement system, which is a complex of several distinct protein fragments (C1-C9), acts mainly through recognition, ingestion and lysis of pathogens by macrophages and by controlling inflammation (Frank and Fries 1991). This complex system promotes lysis of cells, bacteria and viruses; promotes phagocytosis of particulate antigens via. Opsonization; trigger activation of immune responses such as inflammation, manage secretion of immuno-regulatory molecules and removes immune complexes from circulation and deposit them in spleen and liver (Immune clearance) (Goldsby et al. 2000).

All the known components of complement system have yet not been detected in colostrums or mature milk of ruminants. However, few of them viz. C3 and C5 are present in significant amount in mastitic milk (Rainard 2003). Few individual components of complement system have also been detected in human breast milk (Ogundele 2001), with relatively low values in mature milk, as compared to colostrums (Goldman et al. 1986). Human breast milk complement have suggested role against transmission of viral diseases including HIV (Ogundele 2001), protection against allergic diseases (Saarinen et al. 1995), immuno-modulation and regulation of gut motility (Sanderson et al. 1994).

Macrophages

Macrophages are the phagocytic cells of one's immune system. Granulocyte monocyte progenitor cells of bone marrow differentiate into pro-monocytes, which are transported to blood stream, where they differentiate into monocytes. Monocytes circulate in blood for a period of around eight hours during which

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they get mature and enlarged. Mature monocytes migrate to tissues and get differentiated into specific tissue macrophages (Labro 2000). Like other tissue macrophages, those in milk are also thought to generate from peripheral blood monocytes, which migrate into milk through mammary gland epithelium (Pitt 1979). After gaining entry into breast milk, blood monocytes produce granulocyte macrophage colony stimulating factor (GM-CSF) and differentiates into dendritic cells (Ichikawa et al. 2003). Human colostrums is packed with about 10^5-10^7 cells/ml of living leukocytes, comprising 40-50% of macrophages, 40-50% of poly morphonuclear neutrophils and 5-10% lymphocytes (Xanthou 1997). Milk, especially human breast milk is unique in that it harbors large number of macrophages (Pitt 1979), which comprises 80% of total cells present in colostrums and early human milk (Ichikawa et al. 2003).

Major or chief activity of macrophages is the phagocytosis of foreign particle or molecule. Neutrophils and macrophages infiltrating the mammary gland are bactericidal, not only directly through phagocytosis, but also through production of cytokines, reactive oxygen species, and antimicrobial peptides (Stelwagen et al. 2008). After phagocytosis, the foreign molecule is lysed with the help of antimicrobial and cytotoxic substances in an oxygen dependent or oxygen independent method. Oxygen dependent killing activity is due to generation of toxic amount of either of Superoxide anion ($O2^{-}$), Hydroxyl radicals ($OH^{\cdot}$), H_2O_2, Hypochlorite anion (CLO^{-}), Nitric oxide (NO), Nitrogen-di-oxide (NO_2), Nitrous acid (HNO_2), and Monochloramine (NH_2Cl). However the oxygen independent activity is based on molecules like Defensins, TNF-alpha, Lysozyme and Hydrolytic enzymes. Additionally, milk macrophages have been reported to contain engulfed seceratory IgA molecules, which are released on contact with gut foreign molecule (Field 2005).

Bifidus factor

Bifidus factor or carbohydrate growth factor is defined as a compound that specifically enhances the growth of *Bifidobacteria* in either a product or in the intestine of humans and/or animals. Human milk oligosaccharides (HMO) are considered as the most promising source of bifidus factor in human milk (Bezkorovainy et al. 1989). HMO are not digestible by intestinal enzymes and reach the large intestine intact, where they are potentially used by bifidobacteria (Kitaoka 2012). *Bifidobacterium* sp. possess intracellular and extracellular enzymes (1,3-b-Galactosyl-N-acetyl-hexosamine phosphorylase) that metabolize human milk oligosaccharides into lacto-N-biose-I (LNB), that serves as a selective bifidus factor for infant type strains (Kitaoka 2012). Factor responsible for bifidus activity in human milk has been identified as *Methyl–N-acetyl-D-glucosamine* (Sardesai 2003). Methods have been standardized for in vitro synthesis of LNB from sucrose and GlcNAc by simultaneous action of four enzymes (Nishimoto and Kitaoka 2007). HMOs are being excessively explored for their beneficial health effects ranging from their probiotic potential to being antiadhesive antimicrobial, immune modulator and promoter of brain development (Bode 2012).

Bifidus factor has low protein and high lactose concentration. High lactose content assists bacterial fermentation in intestine, creating an acidic environment (Kunz 2012), that in particularly reduces the likelihood of pathogenic bacteria in the intestine e.g. inhibition of enteropathogenic *E. coli* and *Shigella* (Kobata 2013). Also, acidic pH is necessary for Ca, Fe and P absorption. Bifidus factor is not destroyed or altered in its activity under boiling and autoclaving (121°C/15 min) (Kunz 2012), and remain unchanged under refrigerated storage (Lawrence 1999). Cream, proteins and salts can be removed from human milk with only small loss of bifidus activity (Gauhe et al. 1954). Besides promoting growth of *Bifidobacteria,* bifidus factor also enhance synthesis of vitamin B complex (Onishi et al. 1995).

Lactenin

Lactenin is one among the non specific antimicrobial factor that preserves milk after first few hours of milking. It exists in two fractions viz. L1 and L2, whose concentration varies in colostrums and mature milk. Quantity of L1 fraction is higher in bovine colostrums with lower amounts of L2. In contrary to this, mature milk is rich in L2 fraction. These two components are more bactericidal, when present together, than either one alone. Higher lactenin concentration denotes negligible or nil amount of lysozyme. Lactenin 1 has been identified to be a glutenin and Lactenin 2 to be a peroxidase (Singhal et al. 1997). Presence of several components viz. Cysteine, H_2O_2, p-chloro-mercurobenzoate, whole blood, peptone, glutathione and other reducing substances destroy lactenin activity in milk. It is known since earlier days, that fat removal or rennet coagulation destroys lactenic activity of milk (Jones and Sims 1929). The antimicrobial efficacy of lactenins, is reduced at 22°C. Heating of milk to pasteurization temperature (65°C/30 min) results in partial inactivation of L1 fraction. However, its activity is completely destroyed at 70°C/20 min at neutral pH (Yadav et al. 1993). Lactenins are mostly active against *Streptococcus pyogenes*. However, the organism can thrive under udder conditions, due to the fact that lactenins has little activity under anaerobic conditions (Yadav et al. 1993).

Lysozyme

Lyso-zyme, also known as, N-acetylmuramidase or muramidase is a hydrolytic enzyme (15 KDa single chain protein) which has been purified from cells, secretions and tissues of virtually all living organisms and viruses (Benkerroum et al. 2008). This novel protein, discovered by Flemming in 1922, is found in colostrums and normal milk of both human and bovine animals and is major component of human milk whey fraction. Human milk harbours around 30 mg/ml of lysozyme, which is over 3000 times more than that present in

bovine milk. It is generally admitted that the level of lysozyme is highest in colostrums with lower concentration in mature milk. However, several studies reported that there is no significant difference in lysozyme concentration between colostrums and mature milk (Priyadarshini and Kansal 2003; Benkerroum et al. 2008).

Lysozyme specifically acts at the NAM-NAG linkage (N-acetylmuramic acid and N-acetylglucosamine) of cell wall peptidoglycan, resulting in hydrolysis of beta 1-4 glycosidic linkage (N-acetylmuramoylhydrolase enzymatic activity), rendering gram positive bacteria (peptidoglycan ~80 %) more sensitive to lysozyme action (Masschalck and Michiels 2003). Gram negative bacteria are under normal condition, considered lysozyme resistant. However, synergistic action of lactoferrin and lysozyme results in bactericidal effect, where former damage outer membrane of gram negative bacteria and organism become sensitive to lysis by later. Alternate strategies are being explored for enhancing the activity spectrum of lysozyme by modern day techniques (include denaturation, covalent attachment of polysaccharides, fatty acids and hydrophobic peptides). Co-treatment with permeabilizing agents such as EDTA or polycations or high hydrostatic pressure treatments are also being studied (Masschalck and Michiels 2003). Incorporation of lysozyme into active packaging film has been shown to significantly prevent *Micrococcus* infection in foodstuffs, without any loss of activity (Buonocore et al. 2005). On similar lines, immobilized lysozyme in poly vinyl alcohol inhibited growth of microorganisms directly from the film without being released into the foodstuffs (Conte et al. 2007).

Lysozyme is thermally stable to boiling temperature (100°C) at both acidic and neutral pH. However, its thermal stability is lost under alkaline conditions. Bovine milk looses only around 40% of its lysozyme activity at 100°C for 20 min at pH4 (Venkataramani et al. 2013).

Lactoferrin

Lactoferrin is a highly conserved, iron binding red glycoprotein, resembling blood serum transferrin. Lactoferrin is found in varying concentrations in milk of different species with highest in human breast milk followed by cow and buffalo. It is also second predominant protein in human milk (0.2 – 2.6 mg/ml) (Chierici et al. 1994), with higher concentration in colostrums (5.0 to 6.7 mg/ml) (Queiroz et al. 2013). In contrast, cow milk has lower amount, with 0.83 mg/ml in colostrums and 0.09 mg/ml in mature milk (Sanchez et al. 1988). Lactoferrin plays a wide variety of roles related to protection against microbial infection along with promotion of nutritional status (Embleton et al. 2013).

Inhibitory action of lactoferrin is primarily based on its activity to squeeze iron from the medium, making it unavailable to bacteria, which compete with lactoferrin for iron (Levy 1996). Some, if not all of its activity is ascribed to Lactoferricin, a bactericidal peptide formed during digestion of lactoferrin. Lectoferricin, derived from N-terminal of lactoferrin, has stronger activity against both gram positive and gram negative bacteria (Newburg and Walker 2007). Lactoferrin activity is also potentiated by the presence of specific antibodies inhibited or abolished by adding iron *in vitro* or due to the high citrate and low bicarbonate concentration as in bovine milk. Second mode of action of lactoferrin is through destruction of the cell membrane of organisms. Lactoferrin directly interects with lipid A content of lipo-polysaccharide of gram negative bacteria, resulting in damage of cell membrane and promoting the release of lipo-polysaccharide (Ellison et al. 1988). Similar action is observed in gram positive cells, where cell wall lipotechoic acid, serves as site of attachment (Leitch and Willcox 1999). Such interaction based alterations of cell wall and membrane components, facilitates the action of other natural antibacterial components viz. LPS and lysozyme (Gonzalez-Chavez et al. 2009). Bacteriostatic potential of lactoferrin is also based over its degree of saturation. Lesser the iron saturation more is iron sequestration and antibacterial activity. Human lactoferrin is more effective, owing to the fact that its degree of saturation (5-8%) is much below than cow's milk lactoferrin (15-20%) (Steijns and van Hooijdonk 2000; Yen et al. 2011).

Antibacterial property of lactoferrin has been reported against *S. aureus*, *P. aeruginosa*, *Clostridium tyrobutyricum*, *S. albus*, *V. cholera*, *B. subtilis* and *B. stearothermophilus*. Lactoferrin also shows significant anti-viral (against both HIV and HCMV) and anti-parasitic (*Entamoeba histolytica*) activity (Leon-Sicairos et al. 2006). Several other milk proteins tested showed strong antiviral effects only after chemical modification e.g. by making them polyanionic (for anti HIV activity) or polycationic (anti HCMV) (Florisa et al. 2003). Besides antimicrobial properties, lactoferrin in milk do have anti-inflammatory effects, associated with its ability to penetrate the core of the leucocytes and blockade of nuclear factor kappa B (NF-κB), the master regulator or major transcription factor of inflammation (Queiroz et al. 2013). Lactoferrin also shows anti-oxidative, anti-cancer, Immune stimulative and chemopreventive properties (Ambroziak and Cichosz 2014).

Lactoperoxidase

Lactoperoxidase (LP) is a heme containing chain glycoprotein of milk and other secretions such as saliva, tears and airways (Sharma et al. 2013). It is present in cow milk in higher concentrations, with only milder concentration in human milk and secretions such as saliva and tears. It is present in cow milk at 30 mg/ml. Inhibitory action of this enzyme is due to formation of an antimicrobial system, termed as LP system (lactoperoxidase-thiocynate-hydrogen peroxidase system). Lactoperoxidase is natural component of milk, thiocynate is obtained from nutrition/diet of animal, and H_2O_2 comes from polymorpho-nuclear-leucocytes or activity of udder microorganisms. The enzyme, in the

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presence of H_2O_2, catalyses the oxidation of thiocynate (SCN-) and produces an intermediate product, hypothiocynate, a potent oxidant with both bacteriostatic (Group B and N Streptococci; *S. aureus*; *S. faecalis*; *E. coli*; *P. aeruginosa*; *S. agalactiae*) and bactericidal (Group A Streptococci; *E. coli* and *S. typhimurium*; *Plasmodium falciparum* etc.) (Touch et al. 2004; Sharma et al. 2013) properties. LPS enhance the thermal destruction of *L. monocytogenes* and *S. aureus* (Siragusa et al. 1989; Kamau et al. 1990) and has also been shown to have anti-carcinogenic (Tenovuo et al. 1985), anti-viral (Pourtois et al. 1990; Mikola et al. 1995) and antifungal (Benoy et al. 2000; Ahariz et al. 2010; Kho et al. 2012) properties.

LP system is being exploited as a method of preservation i.e. cold sterilization for raw (Siragusa et al. 1989; Gaya et al. 1991; Zapico et al. 1998) as well as pasteurized milk (Barrett et al. 1999), where, thiocynate and H_2O_2 are being supplemented from outside in prescribed values. Cold sterilization is preferred over heat sterilization, owing to the fact that refrigerated temperature preserves antimicrobial substances and other heat labile constituents of raw milk from degradation. Other potential applications of LP system involves preservation of minimally processed fruit and vegetables (Touch et al. 2004); infant formulas (Gurtler et al. 2007); fish (Van Hooijdonk et al. 2000); beef (Kennedy et al. 2000) and in toothpaste for preventing dental caries and plaque accumulation (Hoogendoorn and Moorer 1973; Hugoson et al. 1974). In contrast to the earlier reports, lactoperoxidase has been reported in human milk. However, its physiologic significance is not yet known (Lonnerdal et al. 2003). LP in human milk is harmless to mammalian cells (Kussendrager and van Hooijdonk 2000) and may contribute to the defense against infection already in the mouth and upper gastrointestinal tract. LP has been reported to be thermally stable at pasteurization temperature (63°C/30 min and 72°C/15 sec). However, its activity is lost at higher temperatures and pressure (Ludikhuyze et al. 2001).

Microflora

Besides, antimicrobial factors, Breast milk is a rich source of viable and beneficial microbial diversity. This rich microbial diversity is being explored by advent of new culture independent molecular techniques including genome sequencing, DNA signatures etc. Human milk represents a continuous supply of commensal and probiotic bacteria to the infant gut (Fernandez et al. 2013). This inherent microbiota is known to enhance immunity, intestinal barrier function, liberate nutrients and strengthen functional gut brain axis (Latuga et al. 2014). Lactic acid bacteria viz. strains of *Lactobacillus*, *Bifidobacteria*; has been shown to have antimicrobial activities, besides, other bio-therapeutic effects. Few of the selected organisms are being termed as Probiotics (live, beneficial bacteria which when administered in adequate amount, administer health benefits to the most) and have rich antimicrobial activities against food borne pathogens viz. *Salmonella*, *Shigella*, *E. coli* etc.

Other biologically active components

Other biologically active components of natural raw milk include milk proteins (caseins, whey proteins, glycomacropeptide etc.), peptides (Casomorphins, a-Lactorphin, β-Lactorphin, Lactoferroxins, Casoxins, Casoplatelins, Immunopeptides, Phosphopeptides etc.), oligosaccharides, hormones, mucin and gangliosides and endogenous peptides (Cheison and Wang 2003; Severin and Wenshui 2005). Digestion of milk derived lipids, results in generation of anti-infective degradation products (van Hooijdonk et al. 2000). Recent peptid-omic studies carried out by Dallas and co-workers, identified over 300 milk peptides (in majority derived from β-casein) showing similarity with peptides with known antimicrobial and immuno-modulatory functions. This milk derived peptide mixture could inhibit growth of *E. coli* and *S. aureus*. This study suggested that the pre-digestion of milk proteins may provide selective advantage through evolution by protecting both mother's mammary gland and offsprings (Dallas et al. 2013). Another recent study fractionated 24 human milk peptides exhibiting antimicrobial, anti-oxidative and growth promoting activity. Two of the identified peptides viz. Lactoferrin derived peptide and kappa casein short chain peptide showed antimicrobial activities against both gram-positive and gram-negative bacteria (Mandal et al. 2014). Most of the valuable milk proteins discussed in preceding sections viz. Immunoglobulins, Lactoferrin, Lactoperoxidase, Lysozyme and other biologically active peptides are resistant to pepsin and trypsin activity, maintaining their biological activity within human organisms (Ambroziak and Cichosz 2014).

Conclusion

Milk is a complete fluid imparting both nutrition and health benefits to the infants, young and adults. Nutritional and bio-therapeutic components of milk have yet not been explored well. There is need to further explore milk through analytical, biochemical and cell biological research tools for presence of other novel biologically active molecules. Components such as growth and antimicrobial factors may be utilized for product development, functional foods, Infant foods, dietary supplements, nutraceuticals and bio-therapeutics.

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