Chapter 1: Introduction & Literature Reviewed

1.1. Breast anatomy

Lactiferous ducts are formed by a lining of columnar epithelium and sustained by myoepithelial cells. The columnar epithelium layer display a significant role in controlling milk stasis, fabrication, as well as resorption. Arrangement of this columnar epithelium cells is regulated by local factors (casein and pressure) and hormones (Nguyen and Neville 1998). In non-lactating woman, their presence a keratin plug that is responsible for blocking of lactiferous duct and thus provide protection against the entering of bacteria into duct. At the time of lactation due to breastfeeding areola and lactiferous duct expand leading to formation of lactiferous sinus when milk is supposedly accrues at the time of breastfeeding sessions (Figure 1.1).

![Anatomy of mammary gland](image)

**Figure 1.1: Anatomy of mammary gland** (Macea, et al. 2006).

1.2 Human milk

The human microbiome project was taken up via 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 part and provide vital role for prediction of human health status. However, one of the key system ignored, was the human milk microbiome. 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 (Morrow and Rangel 2004). Bioactive molecules like polyamines, oligosaccharides, fatty acids, lactoferrin, lysozyme, immunoglobulin, immune-competent cells and antimicrobial peptides are present in colostrum and milk (Newburg 2005). They are the main constituent that providing defense to the infants. 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 of infant (Martín Rocío et al. 2009). Daily consumption of breast milk by an infant is 800 ml/day, this in fact contributes to transport of $1 \times 10^5$ to $1 \times 10^7$ bacteria each day leading to their colonization in gut and finally built up gut microflora (Heikkilä and Saris 2003). Human milk protect against gastrointestinal infections (Duijts et al. 2010), respiratory infections (Nishimura et al. 2009) and allergic diseases (Greer et al. 2008, Ip et al. 2008). According to the American Academy of Pediatrics (AAP, 2012) it also trimmed down possibility of diseases like Inflammatory Bowel Disease (IBD), obesity and diabetes (AAP, 2012).

As the neonate are born with immature immune system they are more prone to get infected. In such situation breast feeding can help in building up the immune system of infant as it contains fatty acids, $\alpha$-lactalbumin, IgA, oligosaccharides, lactoferrin, lysozyme, antioxidants and cytokines molecules bearing immune protective role (Chirico et al. 2008, Goldman 2007). Human milk proteome consist of 976 proteins out of which plentiful possess immunogenic property (Gao Xinliu et al. 2012, Molinari et al. 2013). Beside the immune molecules, breast milk also consists of blood derived leukocytes which gets transported to the milk via Paracelullar 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 microbiota play vital roles in the neonates gut, they decrease occurrence and severity of infections, involve in production of antimicrobial compounds and improve intestinal barrier role by enhancing mucine formation and decreasing intestinal permeability (Olivares et al. 2014). Studies revealed that *Lactobacillus* from human milk get accumulate in the gut of infant thus reducing in the incidence of gastrointestinal
infections (up to 46%), upper respiratory tract infections (up to 27%) and total number of infections (up to 30%) (Maldonado et al. 2012). The same microorganisms also contributes in digestion by breaking down sugars and proteins; and all together participated in overall development of neonate immune system.

1.3. Importance of breastfeeding

Health benefits of breastfeeding on lactating mother and new-born are conferred as below (Figure 1.2).

![Figure 1.2: Influence of breast milk on lactating mother and infants’ health](image)

A. Human milk is the best food for infants.

Human milk is easily digested by the new born babies and consists of all the required nutrients which is essential for the babies in the initial months after birth. Breast milk have several vital components that assist growth and maturation of neonate. Also it consist of components which defends infants from illnesses. Human milk consist of readymade antibodies that are gain from the mother these antibody protect the baby during initial stage of growth. Beside antibody, Fatty acids are also present in the human milk that
play a key role in development of infant brain and eye visual capacity. Base up on cognitive and neurological tests, it is found that IQ level of breast fed new born babies is higher than the babies who consumed artificially available fed.

**B. Breastfeeding protects lives.**

Sudden Infant Death Syndrome (SIDS) may occur breastfeeding was not done in certain cases. Also it can premature fetus to the fatal gastrointestinal illness.

**C. Breastfeeding infants are healthier.**

A breastfeeding for at least 4 months can protect infants against ear infections in the initial year of infant’s life. Breastfeeding decreases the occurrence of microbial infections for instance bacteraemia, meningitis and decreased occurrence of respiratory infections in new born. Breastfeeding is defensive against new born botulism. A study reveals that breastfeeding for as a minimum two months gave defends against Type I insulin dependent diabetes mellitus (IDDM). Breastfeeding infants might have lower a risk of childhood lymphoma and inflammatory bowel disease. Also they are less prone to have diarrhoea.

**D. Breastfeeding supports mothers for easy recover after delivery.**

Breastfeeding supports mother for uterus contract to its prior pregnancy form and also reductions in blood lost after childbirth. A lactating mothers who carry on breastfeed for as a minimum 3 months can reduce their weight sooner than bottle-feeding mothers. A lactating mothers are recovered from their menstrual cycles earlier than bottle-feeding moms.

**E. Breastfeeding retains mother’s health during their whole lives.**

Breastfeeding decreases the risk of osteoporosis, ovarian and breast cancer. During lactation periods, LDL cholesterol, triglyceride and total cholesterol levels drop while the favourable HDL cholesterol level is present in proper level which reduce risk of heart diseases (Hallgren et al. 2008).
1.4. Origin of microflora in the human milk

The occurrence of microflora in the various human body parts makes dynamic and interconnected network (Costello et al. 2009). Probability exists that the neonate’s mouth or mother skin may contributes in the development of breast milk microflora to some extent (Figure 1.3). Beside this physiological and hormonal alteration occurring during pregnancy and after pregnancy increased gut permeability which in turn transfer of gut microflora to the mammary gland. Dendritic cells and macrophages also play an important role in the migration of microbes to the mammary gland (Fernández et al. 2013). These bacteria are transferred from maternal community to breast milk via entero-mammary pathway (Figure 1.3). Along with above apparent mechanisms, the retrograde flux between the mother’s skin microbes and infant’s oral microbes may also help in the development of the human milk microbiome (Albesharat et al. 2011, Makino et al. 2011).

![Figure 1.3: Origin of microflora in human breast milk (Fernández et al. 2013).](image)

Some microbiota of the new-born’s oral cavity might contaminate breast milk during feeding due to milk flow back again into the milk ducts of the breast (Ramsay et al. 2004).
Still, this retrograde flux does not clarify why colostrum consist the microflora which characterizes breast milk (Martín et al. 2004). Though the human salivary microbiota is fully explored, Streptococcus species present dominantly in both adults (Aas et al. 2005, Nasidze et al. 2009) and in infants (Bearfield et al. 2002, Li et al. 1997). Streptococci are also predominantly found in breast milk (Jiménez et al. 2008) which reveals that salivary microbiota was significantly affect the breast milk microbiome.

Some of the common skin microflora like Corynebacterium, Staphylococcus, and Propionibacterium (Gao Zhan et al. 2007), are also found in human milk. But, it should be highlighted that the prevalence this group of microbiota are also occurred in the mucosal layer of the genitourinary track and gastrointestinal tracts. Streptococci and Staphylococci have gained attention about their role of initial colonization in infant gastrointestinal tract (Martín Virginia et al. 2012).

Remarkably, the studies reveals that abundance of Staphylococcus epidermidis was significantly different between the feces of healthy breast-fed newborns to formula-fed newborns (Balmer and Wharton 1989, Lundequist et al. 1985) this shows that such microbes are already present in mammary glands. Regardless of sharing of few phylum, the prevalence microbiota in breast milk and breast skin microbiome are significantly differed to each other (Hunt et al. 2011a) e.g. bacterial belongs to the Bifidobacterium genus are strictly anaerobic so they can’t grow on breast skin. Similarly, Bifidobacterium longum DNA was shared by breast milk, maternal and infants feces (Gueimonde et al. 2007). Jost et al. reported that anaerobic genera, like Bacteroides, members of the Clostridia class Bifidobacterium and Parabacteroides was shared among human milk, maternal and neonatal feces using pyrosequencing approach (Jost et al. 2014). Disadvantage of metagenomics studies is that it doesn’t give data in regarding of the viability of the identified microbes also strain level can’t be obtain identification. Therefore, without confirming the occurrence of these microbes by culture dependent method, it is remains indistinct whether human milk is a resource of viable gut-incorporated anaerobes or dead cells (Jost et al. 2014). However, transmission of lactobacilli, bifidobacteria and other bacterial strain from the mother gut to the infant gut (Kulagina et al. 2010), from the mother gut to human milk (Abrahamsson et al. 2009), from human milk to the infant gut (Martín Virginia et al. 2012) has also been confirmed using
bacterial strain specific study. Such studies support the hypothesis which stated that microbes might be vertically transmitted from lactating women to their infant through breastfeeding.

1.5. Somatic cells in Human milk

Human milk which is consider to be dynamic and bioactive fluid undergoes variations in composition i.e. colostrum to late lactation and also it varies from mother to mother. Amazingly, the lactating breast is merely one of the metabolically vital organs in human but still there is no any medical test to prove its health status. Somatic cell count is a key factor normally used in the dairy industries to check the bovine milk quality and occurrence of intramammary infection. At the time of intramammary infection, significant rises in total somatic cell counts is observed in human milk, this parameters can be consider as an essential tool for initial prediction of breast conditions in women. Somatic cells mostly consist of epithelial cell and white blood cell. An epithelial cell gets shaded for the lining of the gland at the time of infection and also leukocytes number increase in the mammary glands due to infection or injury (Dairyman’s digest, 2009). The somatic cells consists of 25% epithelial cells and 75% leukocytes, i.e. neutrophils, erythrocytes, macrophages, lymphocytes (Paape and Weinland 1988). The white blood cells work as a defending element which fights against the infection and assist repair of damaged tissue. At the time of inflammation, the main increase in SCC is owing to the influx of neutrophils in the milk which have been estimated above 90% (Harmon 1994, Miller et al. 1985). Somatic cell count from milk of healthy mammary gland is found to be lesser than 1×10^5 cells/ml, this number can higher than 1×10^6 cells/ml under the infectious condition (Bytyqi et al. 2010).

The presence of cellular component in milk helps in predicting the health of the mammary gland. During attack of pathogens, the number of leukocytes increases specially neutrophils, their number also depends on lactational stages and breast health. (Boutinaud and Jammes 2002, Cregan 2002, Hassiotou F et al. 2012, HO et al. 1979). Recent studies illustrate that increase in number of leukocytes and macrophage is directly associated with breast infection and that their number decreases significantly upon recovery (Riskin et al. 2011). Infants with either respiratory or gastrointestinal infection transfers infection to their mother and this leads to build up of immune response thereby increasing subsequently the number of
leukocytes and other immune components (Hassiotou Foteini and Geddes 2013, Riskin et al. 2011).

1.6. Microbial profiling of human milk

Until the last eras, studies of breast milk was restricted to the explore pathogenic microbes in stored milk or milk retrieved from maternal infected human milk but microbes present in healthy human milk remain unexplored (Table 1.1) (El-Mohandes et al., 1993, Wright et al. 1998 and Le Thomas et al. 2001). Standard microbiological based culturing methods can only detect small proportion of bacteria because great majority of bacteria on the earth are not cultivable in laboratory condition. To identify these unculturables and estimate real bacterial diversity, culture independent method is required. Sequence based identification of microbial species through sequencing has overcome the limitation. The nine hypervariable region of 16S rRNA can be used for identification of bacterial species. Amplification of 16S rRNA region using universal primer are useful for estimation of bacterial diversity.

1.6.1. Culture dependent assessment of human milk microbial diversity

Dr. Juan Rodriguez with his associate researcher R. Martin in 2003, was the first who study microbial diversity of human milk using Culture dependent methods. They isolated a total of 178 isolates from each mother and infant pair that is from human milk, nipple areola, infant’s mouth and feces and subjected it to Randomly Amplified Polymorphic DNA (RAPD) analysis. They employed 16S rDNA sequencing to identify the isolates. Enterococcus faecium and Lactobacillus gasseri were found to be prevalent in both lactating women and their newborn, but no the lactic acid producing bacteria from breast skin was identified base up on RAPD analysis (Martín Rocío et al. 2003). After that Gronlund et al. (2007) studied association of maternal fecal and breast milk Bifidobacteria and infant fecal Bifidobacteria using real time PCR from sixty-one mother-infant pair. They found that Bifidobacterium longum was the high abundant species isolated form breast milk. Moreover, they concluded that Bifidobacterium bifidum and Bifidobacterium adolescentis colonization frequency and count correlated significantly between mother and its infant (Grönlund et al. 2007).

Collado et al. in 2009, in their study examined 50 breast milk samples for the presence of differential bacterial genera by using qPCR technique. They found that Staphylococcus,
Streptococcus, Bifidobacterium and Lactobacillus were the most abundant genera in all the samples. In addition, Maria Carmen Collado et al. (2012) studied effect of pregnant women weight on milk microbiota of 56 mother (34 normal weight and 22 overweight) using qPCR. They observed higher level of Staphylococcus group of bacteria and lower levels of Bifidobacterium group of bacteria in overweight mother as compared to normal weight mother. Moreover, they found higher prevalence of Akkermansia muciniphila in breast milk of overweight mothers (Collado MC et al. 2009). Solis et al. (2010) studied establishment and development of lactic acid bacteria and Bifidobacterium during initial three month of life in 20 vaginally delivered breastfed infant and mother. Streptococcus, Lactobacillus and Bifidobacterium were the most dominant genus in breast milk contributing to the initial establishment of microbiota in newborn (Solís et al. 2010).

Albesharat et al. (2011) isolated a about 700 isolates of LAB from fecal sample of breastfeeding mother, feces of their infant, from breast milk and fermented food, normally consumed in Syria, and characterized it by RAPD and Matrix Assisted Laser Desorption Ionization-Time-Of-Flight Mass Spectrometry (MALDI-TOF-MS). They found thirty six different species of Lactobacillus, Pediococcus, Streptococcus, Weissella and Enterococcus. Interestingly, they found identical RAPD genotype of P. pentosaceus, L. plantarum, L. brevis, L. fermentum, Enterococcus faecium, and Enterococcus faecalis in feces of mother, breast milk of mother and in feces of their babies (Albesharat et al. 2011).

In 2014, Khodayar-Pardo et al. studied the bacterial community present in 32 Spanish breastfeeding mothers by quantitative PCR and evaluated effect of lactational stage, delivery mode and gestational age on milk microbiota. They identified Enterococcus, Lactobacillus and Streptococcus spp. as the dominant bacterial group. They also concluded that Bifidobacterium found most frequently in vaginal than the cesarean delivery mode (Khodayar-Pardo et al. 2014). In 2014, Ana Soto et al isolated Bifidobacterium, Lactobacillus, Enterococcus and Staphylococcus species from breast milk of 47 Slovenian lactating mother(Soto et al. 2014). Moreover, Gonzalez et al. (Gonzalez et al. 2013) also found Staphylococcus, Streptococcus and Lactobacillus genera in breast milk collected from 121 Mozambique women (Albesharat et al. 2011).
<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Country and sample size</th>
<th>Experimental techniques</th>
<th>Identified Microbial profiles</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Spain</td>
<td>Culturing and identification of LAB using RAPD study</td>
<td>Lactic acid bacteria, specifically <em>Lactobacillus gasseri</em> and <em>Enterococcus faecium</em> were present in all the milk samples</td>
<td>(Martín Rocío et al. 2003)</td>
</tr>
<tr>
<td>2</td>
<td>Finland</td>
<td>Real-time PCR</td>
<td>Bifidobacteria were detected in all milk samples with the <em>Bifidobacterium longum</em> being most abundant</td>
<td>(Grönlund et al. 2007)</td>
</tr>
<tr>
<td>3</td>
<td>Spain</td>
<td>qPCR</td>
<td><em>Lactobacillus, Bifidobacterium, Staphylococcus, Streptococcus, Enterococcus</em> and <em>Clostridium</em> cluster XIVa-XIVb were the most abundant</td>
<td>(Collado MC et al. 2009)</td>
</tr>
<tr>
<td>4</td>
<td>Spain</td>
<td>Culturing and identification of lactic acid bacteria and bifidobacteria using 16S rRNA sequencing and RAPD</td>
<td><em>Streptococcus</em> mainly <em>Streptococcus salivarius</em> was predominant followed by <em>Lactobacillus</em> and <em>Bifidobacterium.</em></td>
<td>(Solís et al. 2010)</td>
</tr>
<tr>
<td>5</td>
<td>Syria</td>
<td>Culturing and identification of lactic acid bacteria using RAPD, 16S rRNA gene sequencing &amp; Matrix Assisted Laser Desorption Ionization (MALDI)</td>
<td>Lactic acid bacteria like <em>Lactobacillus, Enterococcus, Pediococcus, Streptococcus, Staphylococcus,</em> and <em>Weisella</em> were isolated.</td>
<td>(Albesharat et al. 2011)</td>
</tr>
<tr>
<td>No.</td>
<td>Country</td>
<td>Sample details</td>
<td>Methodology</td>
<td>Dominant genera</td>
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</tr>
<tr>
<td>6</td>
<td>United States</td>
<td>16 samples collected from each subject, 22–26 weeks postpartum</td>
<td>Pyrosequencing</td>
<td>Staphylococcus, Streptococcus, Corynebacteria, Serratia, Pseudomonas, Serratia,</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Staphylococcus, Streptococcus, Corynebacterium, Propionibacterium, Sphingomonas,</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Bradyrhizobium</td>
</tr>
<tr>
<td>7</td>
<td>Finland</td>
<td>56 mothers (22 overweight &amp; 34 normal weight) &amp; their infants, 1–2 d (colostrum), 1 month, and 6 month postpartum</td>
<td>qPCR</td>
<td>Lactobacillus, Bifidobacterium, Staphylococcus, Bifidobacterium, Lactobacillus,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Staphylococcus occurred in higher abundance and Bifidobacterium, Lactobacillus were observed in lower abundance in overweight mother</td>
</tr>
<tr>
<td>8</td>
<td>Finland</td>
<td>18 samples collected, 0–2 d, 1 month, and 6 month postpartum</td>
<td>Pyrosequencing, qPCR</td>
<td>Weisella, Leuconostoc, Staphylococcus, Streptococcus, and Lactococcus dominant in colostrum whereas Leuconostoc, Weisella, Lactococcus, &amp; Staphylococcus in mature milk</td>
</tr>
<tr>
<td>9</td>
<td>India</td>
<td>7 randomly milk samples collected</td>
<td>Cultured probiotic bacteria</td>
<td>Lactobacillus fermentum, Enterococcus mudtii, Enterococcus faecium, Lactobacillus reuteri and Bacillus subtilis were identified by 16S approach.</td>
</tr>
</tbody>
</table>
| 10 | **Mozambique**  
No. of sample 55 (29 of whom tested positive for HIV) 14 d, 15-90 d, 91-180 d, and 181-360 d postpartum | Culturing of nonfastidious bacteria, yeasts, molds, qPCR | 44 genera and 124 species were identified; Commonly cultured isolates were belonged to *Staphylococci, Streptococci*, and *Lactobacilli* | (Gonzalez et al. 2013) |
|---|---|---|---|---|
| 11 | **Switzerland**  
No. of sample 7  
3–6 d, 9–14 d, & 25–30 d postpartum | pyrosequencing; RAPD; Sanger sequencing | *Firmicutes* and *Proteobacteria* dominated. *Staphylococcus, Streptococcus, Pseudomonas,* and *Ralstonia* were most abundant genus | (Jost et al. 2013) |
| 12 | **Canada**  
No. of sample 1 (10 milk samples pooled)  
9–30 d postpartum | Metagenomic sequencing on Illumina | 360 genera were identified. *Proteobacteria* (65%) and *Firmicutes* (34%) dominated; *Pseudomonas* and *Staphylococcus* were most abundant genus | (Khodayar-Pardo et al. 2014, Ward et al. 2013) |
| 13 | **Spain**  
No. of sample = 32  
1–5 d, 6–15 d, and 17–18 d postpartum | qPCR | *Lactobacillus, Streptococcus,* and *Enterococcus* spp. Were most prevalent | (Khodayar-Pardo et al. 2014) |
| 14 | **Spain**  
No. of sample 24 (half with celiac disease)  
· 1 month postpartum | qPCR | *Bifidobacterium* spp. was observed in all milk samples. *Bifidobacterium bifidum* and *Bifidobacterium breve* were most abundant | (Olivares et al. 2014) |
| 15 | **Canada**  
No. of sample 9 (1 undergoing chemotherapy related to Hodgkin lymphoma) | Ion Torrent sequencing | Chemotherapy was associated with lower microbial assortment and changed in bacterial outlines: reduced percentage abundances of *Xanthomonadaceae* and *Acinetobacter* with chemotherapy. | (Urbaniak et al. 2014) |
<table>
<thead>
<tr>
<th>Country</th>
<th>No. of samples</th>
<th>Collection Method</th>
<th>Identification Method</th>
<th>Findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germany and Austria</td>
<td>160</td>
<td>Culturing of <em>Lactobacilli</em> and <em>Bifidobacteria</em> and its identification by 16S sequencing</td>
<td><em>Lactobacilli</em> and <em>bifidobacteria</em> were isolated and identified.</td>
<td>(Soto et al. 2014)</td>
<td></td>
</tr>
<tr>
<td>India</td>
<td>32</td>
<td>Culture-dependent method, Sanger sequencing</td>
<td>At species level, <em>Enterococcus facealis</em>, <em>Lactococcus lactis</em>, <em>Bacillus litoralis</em>, <em>Bacillus licheniformis</em>, <em>Bacillus safensis</em>, <em>Lactobacillus Oris</em>, <em>Pseudomonas aeruginosa</em>, <em>Staphylococcus aureus</em>, <em>Staphylococcus epidermis</em>, <em>Lysinibacillus sp.</em> were identified.</td>
<td>(Vaidya et al. 2015)</td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>10 (6 vaginally and 4 caesarian delivered mother)</td>
<td>Pyrosequencing qPCR</td>
<td>Alteration in microbiome of human milk based on mode of delivery. <em>Streptococcus</em>, <em>Staphylococcus</em>, <em>Enterobacteriaceae</em> and <em>Pseudomonas</em> were most abundant genera.</td>
<td>(Cabrera-Rubio R. et al. 2015)</td>
<td></td>
</tr>
<tr>
<td>Canada</td>
<td>39</td>
<td>Illumina sequencing</td>
<td>No statistical difference was observed in human milk microbiome based on birthing method, gestation time and infant gender. <em>Staphylococcus</em>, <em>Pseudomonas</em>, <em>Streptococcus</em> and <em>Lactobacillus</em> were most abundant genera.</td>
<td>(Urbaniak et al. 2016)</td>
<td></td>
</tr>
<tr>
<td>China</td>
<td>90 (30 samples without aseptic cleansing and 60 samples collected aseptically)</td>
<td>Illumina 16S sequencing qPCR</td>
<td><em>Streptococci</em> and <em>Staphylococci</em> dominated in both collection procedure. <em>Acinetobacter</em> was predominant in milk collected without aseptic cleansing.</td>
<td>(Sakwinska et al. 2016)</td>
<td></td>
</tr>
</tbody>
</table>
1.6.2. Culture independent method to assess of human milk microbial diversity

In 2011, Hunt et al. used new approach (454-pyrosequencing), utilizing specific primer which mainly focused on the V1–V2 hypervariable region of 16S rRNA gene of bacteria. They described microbial assortment and temporal stability of microbial profiles in healthy human milk which was collected from 16 USA women over a 4-week period (Hunt et al. 2011b). Half of the bacterial sequences were contributed by 9 “core” OTUs which includes Pseudomonas, Staphylococcus, Corynebacterium, Ralstonia, Streptococcus, Sphingomonas Bradyrhizobium and Propionibacterium. Moreover, the proportion of these core OTUs varied greatly between subjects.

Similarly, Cabrera-Rubio et al. (2012) studied bacterial diversity in human milk over three different time periods (colostrum and 1 & 6 month postpartum) in 18 Finnish women (Cabrera-Rubio Raul et al. 2012). They found that human milk microbiome changes over lactation period. Bacteria belonging to Weisella, Leuconostoc, Staphylococcus, Streptococcus, and Lactococcus were more abundant in colostrum. While in 1 and 6 month milk samples Veillonella, Leptotrichia, and Prevotella, typical inhabitant of oral cavity increased significantly. Moreover, they concluded that milk from obese mother tends to be altered and less diverse than normal-weight mothers.

Jost et al. (2013), examined bacterial diversity in breast milk of 7 mothers at 3 different sampling stage (days 3-6, 9-14 and 25-30 postpartum) using culture dependent and independent techniques. They found that Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes were the most abundant phyla and includes representative from the genus Pseudomonas, Staphylococcus, Ralstonia, Streptococcus, Bacteroides, Blautia, and Bifidobacterium. Moreover, for the first time they also found bacterial belonging to Roseburia and Faecalibacterium, that are butyrate producers and essential for colonic health (Jost et al. 2013).

After that, Ward et al. (2013) performed metagenomic functional analysis of a pooled milk samples form 10 donor mother using Illumina sequencing. Over 360 bacterial genera were identified with predominance of sequences belonging to Proteobacteria and Firmicutes. In addition, they also concluded that human milk is less diverse than the feces of infant and mother at the phylum level. Human milk contained prominent amounts of genetic component
which link to stress response, nitrogen membrane transport, immunomodulatory functions and metabolism. (Ward et al. 2013).

In addition, Camilla Urbaniak et al. (2014) in their study examined bacterial diversity in human milk. Breast milk samples from lactating mother who undergoing chemotherapy of Hodgkin’s lymphoma was taken at each 2 weeks over a 4 month period. They found that chemotherapy causes substantial alteration in microbiome from a healthy-controls, with diminution of genera such as *Eubacterium*, *Cloacibacterium*, *Bifidobacterium* and *Staphylococcus* (Urbaniak et al. 2014).

A recent two independent studies by Cabrera-Rubio at al. (2015) and Camilla Urbaniak et al. (2016) studied milk microbiota composition of healthy women and correlated delivery method. In addition to method used for delivery, Camilla Urbaniak also studied alteration of milk microbiota with gestation time and infant gender. Camilla Urbaniak et al. (2016) in their study collected human milk from 39 Canadian mother and analyzed microbiomes by 16S rRNA gene sequencing with Illumina pyrosequencing. They found *Proteobacteria* and *Firmicutes* as most dominant phyla and *Staphylococcus, Pseudomonas, Streptococcus* and *Lactobacillus* as most abundant genus. However, comparison of bacterial profiles between term and preterm infant and vaginal and C section delivery mode revealed no statistical significant difference (Urbaniak et al. 2016). In contrast, Cabrera-Rubio et al. (2015), in their study, compared milk microbiome of 6 vaginally delivered mother and 4 Caesarian delivered mother and found significant separation of milk microbiome based on mode of delivery (Cabrera-Rubio R. et al. 2015).

The microbiota of breast milk from 90 Chinese lactating women was analyzed with two different collection procedure i.e. without aseptic cleansing and after aseptic cleaning by Olga Sakwinska et al. (2016). They found that *Streptococci* and *Staphylococci* were the most abundant in both the group and results were consistent with that of previous study. However, they revealed that breast milk collected without aseptic cleansing and rejection of foremilk had higher abundance of *Acinetobacter sp*. Moreover, *Bifidobacteria* and *Lactobacilli* were present in few samples but with low abundance (Sakwinska et al. 2016).
1.7. Probiotics as a health promoting bacteria in human milk

According to the currently accepted definition of probiotics are: "Live bacteria that are occurred in acceptable amounts and provide befits to the host health” (FAO/WHO 2002). Probiotic word was origin from Greek language in that ‘pro’ means ‘for’ and ‘bios’ means ‘Life’. Probiotics act through varied mechanisms that have an impact on the microbiota. This could cause the changes in either the populations of microorganism or bacterial metabolic activity. Probiotics were originally used to improve the health of both animals and humans through the modulation of the intestinal microbiota. At present, some most characterized and identified strains of Bifidobacteria and Lactobacilli are accessible for human practice to reduce the chance of gastrointestinal (GI) infections or to treat such infections (Salminen and Von Wright 2004). The health promoting benefits of probiotic consumption are: enhancement of intestinal health through the controlling of gut microflora, help to develop and stimulates the immune system, produce and improve the availability of nutrients, decreases the signs of lactose intolerance, and reducing the casual of some diseases (Kumar et al. 2011, Nagpal and Kaur 2011, Yadav et al. 2007, Yadav et al. 2008). The first clinical interest in the application of probiotics has been done for the prevention and treatment for GI infections and diseases (Parvez et al. 2006). Probiotics are usually isolated from the human and animal intestinal track. It’s been recorded that Greek and Romans made use of fermented food, indirectly using probiotics food in their day today life (Gismondo et al. 1999, Guarner et al. 2005). Russian researcher Ellie Metchnikoff, who was awarded Nobel Prize in 1908, was first to proposed the useful results of probiotic bacteria on human health. The term probiotic was first conveyed by Lilly and Stillwell in 1965 and in 1989, Roy Fuller first narrated the health benefits of probiotics. The term probiotics more precisely is symbolize “generally recognized as safe” (GRAS) organisms for human use by the US Food and Drug Administration. Lactobacillus and Bifidobacterium spp. are outstanding individuals of the intestinal flora and are the normally studied probiotic microorganism. LAB are amid the furthermost significant probiotic microbes usually related with the human GI tract. The screening criteria for probiotic bacteria comprises of resistance to acid and bile, antimicrobial activity, agreement with biosafety aspects, viability during storage, activity in delivery vehicles, adherence to epithelial layer of gut, ability to colonies the gastrointestinal tract, capability to stimulate immune response of host. Probiotic
bacteria may secreted numerous compounds like organic acids (lactic and acetic acids) and bacteriocin that has ability to inhibit the pathogen’s growth.

1.8. Taxonomic status and habitat of Probiotics.

LAB are main members of intestinal microflora and are generally measured as probiotics bacteria. LAB are member of Gram positive bacteria, catalase negative, non-spore forming, nutritional fastidious cocci/rods that produce lactic acid as main end product of carbohydrates fermentation. According to recent reports there are 11 genera of lactic acid bacteria including *Lactococcus*, *Lactobacillus*, *Bifidobacterium*, *Pediococcus* and *Leuconostoc*, found to be present in whole GI tract that possess potential beneficiary characteristics as probiotics. The activity such as antagonism which is acknowledged to inhibit a large number of urinary and enteric pathogenic bacteria is displayed by these probiotic bacteria (Hütt et al. 2006). LAB needs nutrient rich habitat that has simple sugars which includes meat, fruits, raw milk, and vegetables. Some of the species colonies in animal organs like mouth, intestine and vagina where their nutritional requirement is satisfied. LAB that are isolated from natural sources are generally reported to be physiologically different. Some species that are considered as probiotic are summarized in Table 1.2.

**Table 1.2: Probiotic microbes and its benefit to host (Hati et al. 2013)**

<table>
<thead>
<tr>
<th>Bacterial genus</th>
<th>Health promoting potential</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus</em></td>
<td>They produce large amount of lactic acid which inhibiting the growth of pathogenic bacteria e.g. <em>Helicobacter pylori</em></td>
</tr>
<tr>
<td><em>Bifidobacterium</em></td>
<td>Protect the body from the invasive pathogenic microbes which benefit to overwhelm tumors and decrease inflammation</td>
</tr>
<tr>
<td><em>Lactococcus</em></td>
<td>Produce antibiotics which diminishes the capability of pathogenic bacterial growth and causes infection</td>
</tr>
<tr>
<td><em>Enterococcus</em></td>
<td>They provide protection against <em>Listeria monocytogenes</em> and <em>vibro cholera</em></td>
</tr>
<tr>
<td><em>leuconostoc</em></td>
<td>They help to sustain gut health</td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td>They has antioxidant potential which scavenges free radicals and sustaining vaginal and gut health</td>
</tr>
<tr>
<td><em>Saccharomyces</em></td>
<td>They provide protection against Rotaviral and also for traveler’s diarrhea</td>
</tr>
</tbody>
</table>
1.9. Screening criteria of probiotics

Before using the probiotics, effective potential probiotic bacteria strain is likely to have several enviable properties. The screening criteria involves survive in acid condition, bile tolerance, ability to adhesion on mucosal surface, safe for clinical use and for food, clinically valid and recognized health effects, human origin is it’s for human usage (Ouwehand and Vesterlund 2004). Bacterial species that can fulfil all above criteria are used in order to get effects on health as functional probiotic strains. Some major selection criteria are as discussed below.

1.9.1 Resistance to acid and tolerance against bile juice

Bacteria which consider as probiotic strains are combined within the human diet with a journey to the lower intestinal tract via the mouth. During their passage along with food, probiotic bacteria should show resistant against the enzymes like lysozyme within the oral cavity. Only then they could continue towards the stomach and reach the upper intestinal tract that consist of bile. During the entire processes it been observed that the bacteria are resistant to the process of digestion. Finding says that, it takes three hours for the release of the digestive components right from the primary entrance till the end of the journey into stomach. It is not enough but the strains also show resistant against the harsh environment of the stomach with pH 1.5-3.0 and upper intestine which consist of bile (Cakir 2010, Chou and Weimer 1999). For demonstration of probiotic sufficiency, they must reach up to lower intestine and sustain themselves over there. For the fascinating purpose, initial criteria are to seem probiotic strains tolerance against bile and acid. Bile acids are produced from cholesterol within the liver and directed to the gall bladder afterward secreted to the small intestine within the conjugated type (500-700 ml/day). Within the large intestine these acids undergo more or less chemical changes (deconjugation, deglucuronidation, dehydrogenation and dehydroxylation) due to the bacterial activity. In vitro study reveals that conjugated and deconjugated bile juice shows antagonistic activity particularly on *E.coli* spp., *Enterococcus* spp. and *klebsiella* spp. The deconjugated acid are greater effective on gram positive bacteria (Dunne 2001).
1.9.2. Antimicrobial activity

Antimicrobial activity is foremost necessary screening criteria for probiotics bacteria. For confirming the probiotics nature of bacteria, their antimicrobial activity is checked against the enteric undesirables and pathogens (Klaenhammer and Kullen 1999). Antagonistic potential of probiotics was found due to organic component i.e. acetic, lactic, propionic acids, diacetyl, hydrogen peroxide, carbon dioxide, low molecular weight protein stretch along with antimicrobial activity and bacteriocins (Ouwehand and Vesterlund 2004). Till date research shows that a specific antimicrobial material is produced by the bacteria includes: *Lactobacillus reuterii*, that is a member of common microbiota of animals and human which secreted a low molecular weight bacteriocin i.e. reuterin. Similarly other species of *Lactococcus lactis* secretes *Nisin A* which belongs to category I bacteriocin; *Enterococcus feacalis* DS16 secretes a category I bacteriocin cytolysis; *lactobacillus acidophilus* produces *Acidophilucin A* which belongs to a class III bacteriocin; *Lactobacillus plantarum* produces a category II. Factors which effects extremely on the bacteriocin production are bacterial species along with its constituents, incubation temperature, time and pH of medium. Lactis is mostly characterized bacteriocin which is mainly used in food packaging and preparations (Cakir 2010). *Bifidobacteria* and *Lactobacilli* were isolated from small intestine of human have examined and reported to have antagonistic activity towards a variety of indicator pathogen, *Bacillus, staphylococcus, Listeria, Enterococcus, Lactobacillus, Lactococcus, Bifidobacterium, Streptococcus, E. coli, Pseudomonas* and *clostridium*. When Yakult and D’light probiotics were studied for their antimicrobial activity, it has ability to inhibit growth of most human pathogens, Nestle yogurt probiotics shows bactericidal effects against *S. aureus* and *P. aeruginosa* however it was repressive for *S. typhi*. Nestle probiotics kills *E. coli* and *S. typhi* where as they were suppressed *S. aureus* and *C. albicans* (Chuayana Jr et al. 2003). It is currently well established that a number of the infections and disorders within the human body, like irritable inflammatory bowel disease, bowel syndrome and diarrhea which are induced by antibiotic might be because of deficient or compromised intestinal micro flora - probiotics can be considered to be one among the disease control strategies to beat such disorders. Lactic acid bacteria, particularly *Lactobacillus spp.* are the most widely used microorganisms as probiotics because of the perception that they are common resident of the intestinal microbiota and due to this microorganism are “Generally recognized as safe” (GRAS) standing.
1.9.3. Gut adhesion properties

Various mechanisms like biofilm formation, cell surface hydrophobicity, auto-aggregation and co-aggregation enhance the gut adherence, which in turn makes stronger probiotics properties. Moreover, for colonization of probiotic bacteria in GI track, adhesion is the most important selection criteria (Ali 2000). Successful probiotics bacteria are generally able to colonize the intestine by adhering to the intestinal mucosa this can be temporary. Adhered probiotics bacteria could prevent the attachment of pathogens which may include *Coliform* bacteria and *Clostridia* in the intestinal tract (Lee et al. 2000). Research shows interaction of *Lactobacillus* species with pathogens occurs using different mechanism such as production of antimicrobial compound i.e. lactic acid, bacteriocin and hydrogen peroxide (Kos et al. 2003). *L. reuteri* create biofilm which secrete certain compound like reuterin and is responsible for immune modulation and pathogens inhibition (Perdigón and de Ruiz Holgado 2013). A significant link between the presence of Enterococcal surface proteins (Esp) and the ability of an *Enterococci* strain to form biofilm *in vitro* has been reported. Esp dependent biofilm formation is influenced by the growth medium and conditions used, Esp expression at the cell surface significantly increases the cell surface hydrophobicity. Cell surface hydrophobicity has play vital role in the preliminary stages of biofilm development by promoting cell substrate interactions. For colonization, organisms have to display significant level of aggregation and surface hydrophobicity properties (Del Re et al. 2000). After adhesion, the probiotic bacteria have ability to auto-aggregates as well as co-aggregates and also colonize in the epithelial layer of gut for nourishing health promoting consequence (Kaushik et al. 2009). *In vitro* study for cell surface hydrophobicity using xylene, ethyl acetate and chloroform, as hydrocarbon source showed gut adherence potential of probiotic organism.

1.10. Mode of action of probiotics

Processed food removes beneficial bacteria normally ingested from fruits, milk products and vegetables. At the same time, use of antibiotics kills pathogens and destroy the beneficial microbes. Probiotics to a certain extent helps to regain microflora and maintain their population by producing antimicrobial compound like toxins and organic compounds that inhibit the growth of pathogenic bacteria at the same time also stimulate immune response to
counter attack pathogens. Thus, every species have different mode of action to act against invading pathogens (Figure 1.4).

**Figure 1.4: Schematic diagram of postulated action of probiotic bacterial towards gastrointestinal infection (Thirabunyanon 2011)**

These mechanism are as listed below (Vanderpool et al. 2008)

1) By producing inhibitory substances like bacteriocin, organic acid and hydrogen peroxide that are bactericidal to foremost bacteria species.

2) By blocking the adhesion site i.e. probiotics bacteria inhibit the binding of pathogenic bacteria, they adhere to intestinal epithelial layer and block the adhesion site.

3) Competition for nutrients: it’s been anticipated that probiotics consuming the nutrients which in turn pathogens need to survive and multiply, thus suppressing the need and inhibiting the growth of pathogens.
4) Simulating of immune system: One of the conceivable mechanism of probiotic bacteria is that probiotics might be enhanced nonspecific and specific immunity to protect the host against infection which is not well documented. However, it is expected that specific cell layers or cell wall components might be work as an adjuvants and also increases humoral immune response.

5) Degradation of toxin receptor: probiotics destroy the toxin receptor present on the intestinal mucosa, thus in turn protect the host from intestinal disease. Beside this there are certain other mechanisms like repression of toxin secreted, decrease in gut pH and diminution of virulence which play important role in decaying receptors.

1.11. Prebiotics

‘A prebiotic may be a non-digestible food element that has beneficial effects on host by allowing the growth of only health promoting bacteria i.e. probiotics in colon’. Prebiotics are important because of: (i) A study reveals that prebiotics are balanced gut microbiota, (ii) prebiotics may change the composition of the microbiota and developed healthy environment for the growth of probiotics, (iii) prebiotics are valuable ingredients in diets, which improved organoleptic properties of many dairy products. The consumption of prebiotics as food elements has various benefit but if it consumed with a well-balanced nutritional composition (Coussement 1997). The majority of oligosaccharides are confirmed to improve Bifidobacteria numbers inside the colon (Playne and Crittenden 1998). Many authors have recommended that the intake of 10 g/day of galacto-oligosaccharides is sufficient to enhance the growth Bifidobacteria bacteria. Daily ingesting of 2.5 g prebiotics/day is sufficient to increase growth of Bifidobacteria (Gibson 1999, Sako et al. 1999). The quantities of different non-digestible oligosaccharides (NDOs) crucial for bifidogenic effects are almost similar to that of galacto-oligosaccharides. For xylo-oligosaccharides, 2 g/day is to be taken into account sufficient to acquire bifidogenic impact (Sako et al. 1999). Rivero-Urgell and Santamaria- Orleans (2001) reported that the fructo-oligosaccharide (FOS) ingestion essential to perform as a bifidogenic stimulation is between 2 and 10 g/day in adults. But, a minimum of 4 g FOS/day would be required to boost the Bifidobacteria levels inside the human gut (Gibson et al. 2004). For isomalto oligosaccharides, the daily amount necessary is 8–10 g (Rastall 2004). Adding inulin to food products as a prebiotic may improve the viability and
action of probiotic bacteria (Nazzaro et al. 2009) for example *L. casei* LC-01 (Paseephol and Sherkat 2009). Populations of *Bifidobacterium* genus, *Lactobacillus* and *Enterococcus* are increased inulin fermentation (Connolly et al. 2010). Cellulose and inulin present in Kiwifruit was found to enhance the adhesion of *L. rhamnosus* and decrease the adhesion of *Salmonella typhimurium* to Caco-2 cells. Inulin and citrus pectin significantly improve the adhesion of *Bifidobacterium bifidum* to Caco-2 cells (Parkar et al. 2010). Prebiotics are substrates that may only be taken in little by specific bacteria and they are encourage probiotic growth because of its chemical structure (Bielecka et al. 2002).

1.12. Bacteriocin produced by probiotics

A pronounced number of Gram positive and negative microbes have potential to produce substances which are protein in nature that possess antagonistic activities, named bacteriocins. Though bacteriocins might be considered as antibiotics but they are not, a key variance between antibiotics and bacteriocins is that bacteriocins are ribosomally produced and synthesized for the period of the initial phase of growth (lag or log phase), however antibiotics are commonly secondary metabolites (Beasley and Saris 2004). Generally, bacteriocins are low molecular weight (hardly over 10 kDa) and go through the posttranslational modification and also easily degraded via proteolytic enzymes mainly through the proteases of the human gastrointestinal tract that makes bacteriocin safe for human consumption. Bacteriocins contain a high amount of arginyl and lysyl residues which make them cationic nature and amphipathic molecules (Martí et al. 2003, Rodríguez et al. 2000). They are mainly amorphous when they are unified in aqueous medium but once react to structure promoting solvents like mixed with anionic phospholipids membranes or triolfluroethanol, they transform to helical structure (Moll et al. 1999). Among the Gram positive microbes, LAB have secured precise attention in current time, because of the bacteriocins production (Ross et al. 2002, Todorov SD and Dicks 2005a). Bacteriocin from LAB can be used as natural preservatives in the food industry. The LAB and their metabolic products application is accounted to be safe for human consumption (GRAS, Grade One).
1.12.1. Classification of bacteriocins

Mostly bacteriocins are small about 10 kDa in size, cationic, heat-stable, membrane permeabilizing peptides and amphiphilic. They are mainly classified into three major classes (Martí et al. 2003, Rodríguez et al. 2000). Numerous bacteriocins seem to display relatively less adsorption capacity. The cell wall of Gram positive microbes permits only relatively large molecules to cross cell wall. A lipoteichoic and teichoic acids is an anionic cell surface polymers, found in cellular wall are significant for initial reaction of anionic bacteriocins.

1.12.1.1. Class I bacteriocin: The Lantibiotics

Class I bacteriocin also known as lantibiotics, are a class of peptide substances that are mainly heat-stable, small and membrane-active peptides which consist thioether amino acids, like B-methyllanthione and lanthionine. Class I bacteriocin are again divided in two different form according to structural similarities. Mainly, Type A bacteriocin have elongated, positively charged, screw shaped, flexible molecules amphipatic. They have molecular weight between 2 to 4 kDa and they are commonly act using membrane depolarization or via pore formation. Lacticin and Nisin are the main instances which belongs to this group. Type B bacteriocin, have globular structure and restrain through cellular enzymatic reaction. Their molecular weight ranged between 2 to 3 kDa and moreover they have net negative charge or no net charge (Cleveland et al. 2001).

1.12.1.2. Class II bacteriocin: the Non-Lantibiotics

Class II bacteriocins has appeared in current years as the utmost potential bacteriocin used in food preservation because it has superior physiochemical properties and biological activity, than other bacteriocins classes (Klaenhammer 1993, Nes et al. 1996).

Class II bacteriocins are small molecular weight near to 10 kDa, heat stable and also have membrane active peptides like non-lanthionine. Class II bacteriocin are classified into two subclasses. Subclass II a is listeria active or pediocin-like bacteriocins that are characterized by a highly conserved Tyr-Gly-Asn-Gly-Val-Xaa-Cys consensus motif at N-terminal ends (Patton and Van Der Donk 2005). Subclass II b is a two–peptide bacteriocins and for its antimicrobial activity, they needs two peptides. Lactococcin G and lactacin F belongs to this group (Daw and Falkiner 1996).
1.12.1.3. Class III bacteriocins

This class contains of heat labile proteins which have large molecular weight (>30 kDa). This class has not been widely explored. Bacteriocins presents in this group are helveticin I which produce by *Lactobacillus helveticus* and enterolysin secreted by *Enterococcus faecium* (Ross et al. 2002, Todorov Svetoslav Dimitrov and Dicks 2005b).

1.12.2. Applications of bacteriocins

At the present time, bacteriocins have been broadly utilized specially in the area of food preservation mainly in food industry for product related to dairy, egg, vegetable and meat. Amid the other bacteriocins nisin A and its variant nisin Z has been verified as a most effective against food poisoning and spoilage causing microbial agents. Moreover, nisin is the merely bacteriocin which has been formally used in the food industry worldwide (Deegan et al. 2006, Moll et al. 1999). Several preservation techniques have been applied for prevention of food spoilage and food poisoning which in account are thermal process by pasteurization or sterilization via heating, diminish pH and also water activity by acidification or dehydration and adding of preservatives like organic compounds (propionate, sorbate, benzoate, lactate, and acetate) and antibiotics. Though these techniques have been confirmed to be very successful but there is an increasing a demand for microbiologically safe and natural products which gives health benefits to consumers (Deegan et al. 2006). Bacteriocins can be used in purified or crude form or direct incorporation of bacteriocin producing strain during food processing (Ross et al. 2002). Likewise, bacteriocins are combined with supplementary antimicrobial components like sodium acetate and sodium lactate which enhanced inactivation of microbes. Bacteriocins are also applied for improving sensory properties and food quality, as an example rising the rate of proteolysis or avoid defect found in cheese due to gas blowing. Bacteriocins is also used for bioactive packaging, is a process which may defend the food against exterior contaminants. For example, refrigerated food start to spoil as bacteria grow on the surface food which force the use of bacteriocins in combination with packaging to improve self-life and food safety (Ross et al. 2002). Bioactive packaging use either bacteriocin directly immobilizing in the food packaging or by adding bacteriocin in the packaged food that slowly diffuse during food product storage. Numerous approaches are used to make packaging films along with bacteriocins. One of the technique is, bacteriocin are directly incorporate on
polymers. For instance, nisin are directly incorporated on biodegradable protein films. This type of incorporation are done by heat press and protein film are used like soy proteins or corn zein. (Deegan et al. 2006).

1.13. Hypothesis

In the present study effort are made to confer relationship between somatic cell count and breast health status and also to explore microbiome of breast milk collected from healthy lactating mother using culture dependent method and metagenomic approach. Study objectives is also extended to identified prevalent probiotics bacteria in the breast milk and factor which enhancing its potential as a probiotics. Moreover, a potential bacteriocin is purified from most efficient probiotic bacteria. With improved understanding of the impact of breast milk microbiota, it may be possible to manipulate these microbial communities to improve the health and development of mothers and their neonates.

1.14. Relevance of the work

In last many decay, bacteriological analysis of breast milk only focused on the infections condition of mother and there was no idea about the occurrence of non-pathogenic bacteria in healthy human milk that may be health promoting. Use of more sophisticated culture-dependent and metagenomics techniques like pyrosequencing are continuously developing through which significance of the milk microbiome can be discovered. An improved understanding of the relation among the human milk microbiome and its health promoting effect can open new avenues in the field of pregnancy and lactation. Breastfeeding which is important for the infant and protect them against many diseases during the early stage of development. This may be with the support of health promoting probiotic bacteria which are transferred from mother to child during feeding. Also the same health promoting bacteria from the gut microflora of the infants. The use of culture independent technique can in depth provide insight to the microorganisms that are present in the milk and further using them to improve the health of the infant and modified baby food quality.
1.15. Objectives

1. Exploring the microbiota of human milk using the culture-dependent method.
2. Exploring the microbiota of human milk by metagenomic approach.
4. Extraction and purification of bacteriocin from potential probiotic bacteria.

1.16. References


