



Antimicrobial Systems in Milk

The germicidal properties of milk and antimicrobial substances in milks of different species (cow and buffalo) have recently been characterized. Normal raw milk contains different levels of inhibitory substances that restrict the normal development of certain bacteria. The degree of inhibitory activity varies considerably in milks of different species viz., cow, buffalo, human (maximum activity present in human milk), in milks of same animal at different milking. Activity varies between milk samples collected from different quarters of udder of same animal. There are two groups of antimicrobial substances in milk:

- 1. Inherent or naturally occurring substances in milk**
- 2. Non inherent compounds secreted into milk from other sources**

Naturally Occurring Anti Microbial Substances

These compounds may be specific and non-specific in conferring protection to the new born. The specific factors are produced in response to specific antigen, whereas; non specific have less specific anti-microbial activity.

Specific factors

- A. Immunoglobulins
- B. Complement
- C. Macrophages
- D. Bifidus factor

Non specific factors

- A) Iron binding protein like lactoferrin
- B) Lysozyme
- C) Lactanins
- D) Lactoperoxidase
- E) Vitamin binding proteins and fatty acid

Specific factors:

Immunoglobulin (Ig)

- 1. These factors are responsible for immunological protection of newborns.**
- 2. In man, prenatal immunity is conferred primarily through the transmission of immunoglobulins from mother across the placenta to the foetal circulation.**
- 3. Buffalo and cow rely on the transfer of immunoglobulins from colostrum into new borne's circulation postnatally, when gastrointestinal tract is permeable to intact protein molecules.**
- 4. IgA is the chief immunoglobulin of milk and confers passive immunity. In human milk, the main Ig is called secretory IgA. It is present in colostrums in concentrations up to 50 mg per ml which drops to a level of 1mg/ml in mature milk. The sites of action of IgA are restricted, since Ig cannot be absorbed from infant gut in significant quantities.**

Functions of SIgA

- Neutralizes toxins and viruses
- Inhibits bacterial adhesions
- Prevents absorption of food antigens
- Suppresses bacterial growth
- Aids in phagocytosis by bringing about attachment of bacteria to macrophages.

In human milk anti *Escherichia coli* IgA has been identified that is active against infantile diarrhea.

Complement

1. Small amounts of nine components of complement have been detected in human milk and these are also associated with the bactericidal properties of milk.
2. Add to the defense mechanism due to lysis of a variety of potentially pathogens.

Macrophages

3. Viable leucocytes are present at a concentration of 2×10^6 cells/ml in human colostrums, falling to 2% of this value in normal milk.
4. About 80% of the cells in colostrums are macrophages that are associated with phagocytosis i.e. engulfing and subsequent killing of bacteria many of which are pathogenic in nature.

Bifidus Factor

- 1. Bifidus factor is a compound that specifically enhances the growth of bifidobacteria in either a product or in the intestines of humans. Products marketed as bifidogenic factors are several prebiotics and methyl-N-acetyl D-glucosamine in human milk.**
- 2. Bifidus factor supports the growth of *Lactobacillus*, beneficial bacteria that protect the baby against harmful bacteria by creating an acidic environment, where it cannot survive.**
- 3. In breast-fed infant mother's milk having bifidus factor leads to growth of *Bifidobacterium* having beneficial effects such as:**

- ❑ Inhibition of enteropathogenic *E. coli*, *Shigella* spp. and other pathogens due to production of lactic and acetic acid.
- ❑ Supports the growth of *Bifidobacterium* spp., *Lactobacillus* spp. and other probiotic is a beneficial bacteria that protects baby against harmful bacteria by creating an acidic environment.
- ❑ Acidic pH in stools that is necessary for absorption of calcium, Fe and phosphorus.
- ❑ Synthesis of Vitamin B-complex that helps in infant growth. In infants receiving baby foods, the following adverse effects can lead to over growth of *E. coli* in intestines, increase in alkaline pH of stools due to over growth of *E. coli*, decrease in synthesis of Vitamin B₁₂.

Non Specific factors:

Lactanins

1. The concentration of lactanin fractions viz lactanin 1 (L1) and lactanin 2 (L2) in milk varies. The bovine colostrums are rich in L1 and normal milk contains higher quantities of L2 fraction. A third fraction L3 has also been detected in raw milk. This differs from L1 and L2 on the basis of its source and inhibitory action.
2. There is a partial inactivation of L1 at 65°C for 30 mins. On heating raw milk to 70°C for 20 mins at pH 7.0, L1 is completely destroyed leaving behind L2 fraction in relatively higher concentration. It is proved that these two compounds L1 and L2, when interact are more bactericidal than either one alone.
3. Lactanin is mostly active against *Streptococcus pyogenes*. The ability of these microbes to grow in udder is due to the fact that lactanin has little activity under anaerobic conditions.
4. Addition of glutathione, cysteine and other reducing substances in milk reduces the anti microbial action of lactanins in milk. However, other factors in milk are stimulatory for the growth of *Streptococcus pyogenes* in udder that causes mastitis.

Lysozyme

- 1. It is present in different amounts in milks of different species.**
- 2. The average lysozyme content of human milk (30 mg per 100 ml) is 3000 times more than that of bovine milk. Such significant difference in the lysozyme content may have physiological or nutritional implications on infant feeding.**
- 3. Breast fed infants have greater resistance to enteric infections than those fed on artificial foods, and lysozyme is possibly associated with the mechanism of natural immunity and local resistance.**
- 4. Most of the Gram positive bacteria are very sensitive to the lytic action of lysozyme due to the hydrolysis of β 1-4 glycosidic linkage of the peptidoglycan component of their cell wall. However Gram negative bacteria are normally lysozyme resistant.**

Lactoferrin (Iron binding protein)

- 1. Lactoferrin (previously known as lactotransferrin or lactosiderophilin) is important, red glycoprotein that resembles blood serum transferrin and is found in varying concentrations in milks of different species.**
- 2. Human milk has a higher amount of this protein than cow or buffalo milk.**
- 3. The concentration of lactoferrin in human and buffalo colostrums is very high (1.2 g/100 ml and 100 mg/100 ml, respectively) and falls during lactation to 160 mg/100 ml and 50 mg/ml, respectively in mature milk.**
- 4. 1-4% of lactoferrin in human milk is saturated with iron. It is mainly a whey protein (70% found in whey and 30% bound to casein and fat).**
- 5. The inhibitory effect of lactoferrin is based on its ability to squeeze iron out from the medium, thereby making it unavailable to the microbes that compete with the lactoferrin for limited amount of iron as an essential growth factor.**
- 6. Antibacterial property against microorganisms (*S. aureus*, *Staphylococcus albus*, *P. aeruginosa*, *Vibrio cholerae*, *Clostridium tyrobutyricum*, *Bacillus stearothermophilus* and *B. subtilis*).**
- 7. Apolactoferrin (iron free lactoferrin) at 0.02 mg/ml concentration in milk**

Lactoperoxidase

1. Lactoperoxidase is present in cow milk in considerable amount (30 mg/ml) but is absent in human milk.
2. This enzyme has been found to be identical with L2 fraction of lactanin.
3. In bovine raw milk, the inhibitory action of this enzyme is due to formation of an antimicrobial system or LP system under normal conditions.
4. The secretion of thiocyanate component in milk is governed by nutrition of the animal.
5. Hydrogen peroxide can be contributed either by polymorpho-nuclear-leucocytes or by hydrogen peroxide producing udder micro flora, e.g. Streptococci
6. Lactoperoxidase system is bacteriostatic to Group B and Group N Streptococci, whereas; it is bactericidal for Group A Streptococci, *E. coli*, *Salmonella typhimurium* etc.
7. It protects calf against enteric problems and may have some role in providing defense mechanism to mammary gland against infections.
8. LP system is a method of preservation (i.e. cold sterilization) for raw milk. There is no inactivation of antimicrobial substances and other heat labile constituents of raw milk (like certain vitamins).
9. The two components of LP system viz. thiocyanate and hydrogen peroxide are added from outside to attain their balanced proportions.

Vitamin binding proteins

- 1. There are certain proteins present in milk of some species that bind with some essential vitamins firmly, thereby making bacteria starved. These proteins particularly bind with Vitamin B12 and Folate in milk.**
- 2. In milk of both cow and human, the binding capacity of folate binding protein is far in excess of natural folate content. The same is true for Vitamin B12 in human milk but in cow milk there is little or no excess of unsaturated binding protein. Hence, in presence of unsaturated binding proteins, the growth of microbes requiring vitamin B12 and folate is inhibited.**
- 3. Vitamin binding proteins have following functions such as**
 - Regulate bacterial growth in gastro intestinal tract of suckling animals by denying the vitamins to specific bacteria**
 - Aid in the absorption of vitamins in the gut**
 - Conserve vitamin B12 by aiding the re-absorption of vitamin B12 lost in bile**
 - Play a role in the mammary gland prior to milk secretion**

Fatty acids

- 1. Milks of different species also contain free fatty acids in appreciable concentrations.**
- 2. Generally, medium chain fatty acids, especially octanoic and decanoic acids and some polyunsaturated chains fatty acids are more active against Gram-positive microorganisms.**
- 3. Lower fatty acids liberated from the glycerides of milk fat are inhibitory to many bacteria including lactobacilli and lactic streptococci.**
- 4. Free fatty acids in milk result either from the action of lipase naturally present in milk or from the lipolytic activity of certain microbes. Although, many microorganisms require fatty acids, as nutrients for growth, but when their concentration exceed certain limits in medium, growth is strongly inhibited.**

Non Inherent Anti Microbial Substances

Antibiotics, sanitizers and other chemical substances used at some stage in dairy industry may gain access in to milk and exert antimicrobial effect.

Significance of Anti Microbial Substances in Milk

- **Health benefits:** **The primary significance of the antimicrobial substances detected in milk can be related to their possible role in conferring protection to mammary gland and neonates in mammals. The new borne should essentially be given the colostrums, which is very rich in most of these antimicrobial factors. ‘Breast feeding is the best for infants’ derives its substance from the abundance of antimicrobial factors in mother’s milk, as against animal milk based infant formulae.**

● **Preservation of milk:** Presence of antimicrobial factors cannot prevent or even retard significantly the development of mixed population of microbes in raw milk but some microbes get markedly affected. However, an artificial exploitation of milk natural antimicrobial system called lactoperoxidase system, for developing cold sterilization like process, can prove effective in preservation of raw milk. This process holds great promise under tropical farm conditions in countries like India.

● **Processing problems:** Raw milk is used for product making, e.g. in cheese making, the acid development in cheese vat may be retarded by inhibition of starter activity due to the presence of antimicrobial substances. In starter culture propagation, the heating of milk (80°C) is essential as it inactivates factors that may interfere with the normal starter growth.