Chapter II

Literature Review

For large hindgut fermenters like elephants and rhinoceroses, the horse has been propagated as the appropriate model for the study of gastrointestinal health (Oftedal et al., 1996) as both are monogastric herbivores with fermentation occurring in the same position, the large intestine, and cecum.In the intestines the point at which the small intestine meets the large one is called the caecum, which is particularly rich in blood vessels. Here, bacteria aids in the fermentative digestion of the cellulose called hindgut fermentation. Hindgut fermenters such as proboscideans and large odd-toed ungulates such as horses and rhinos. In contrast,foregut fermentation is the form of cellulose digestion seen in ruminants, such as cattle,which have a four-chambered stomach that digests cellulose.Currently we knew very few facts about the elephant gastrointestinal tract, so the scientific data about the digestive system, bacteria or microorganisms, secretion and pH of the gut were largely not known (Ullrey et al., 1997). In addition to the primary digestive organs, the liver and the pancreas are key organs that aid in digestion; bile acid from the liver helps to digest and absorb lipid, while secretion from the pancreas plays role in carbohydrate and protein metabolism.

In horses, the caecum is the first section of the large intestine. It is also knownas the "water gut" or "hind gut". It contains anaerobic bacteria, fungi, and protozoa that digest plant fiber, through fermentation. These microorganisms have cellulolytic or hemi- cellulolytic properties, so they produce short-chain fatty acids through fermentation that can be directly absorb the host and utilized as energy. The bacteria in themicrobial ecosystem in cattle are known as rumen-cellulolytic Bacteria and include *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *R. albus*. There are some yeast strains and fungi also, such as *Saccharomyces cerevisiae* and *Aspergillus oryzae*, which have the same properties (Julliand et al.,1999. Herich and Levkut, 2002)

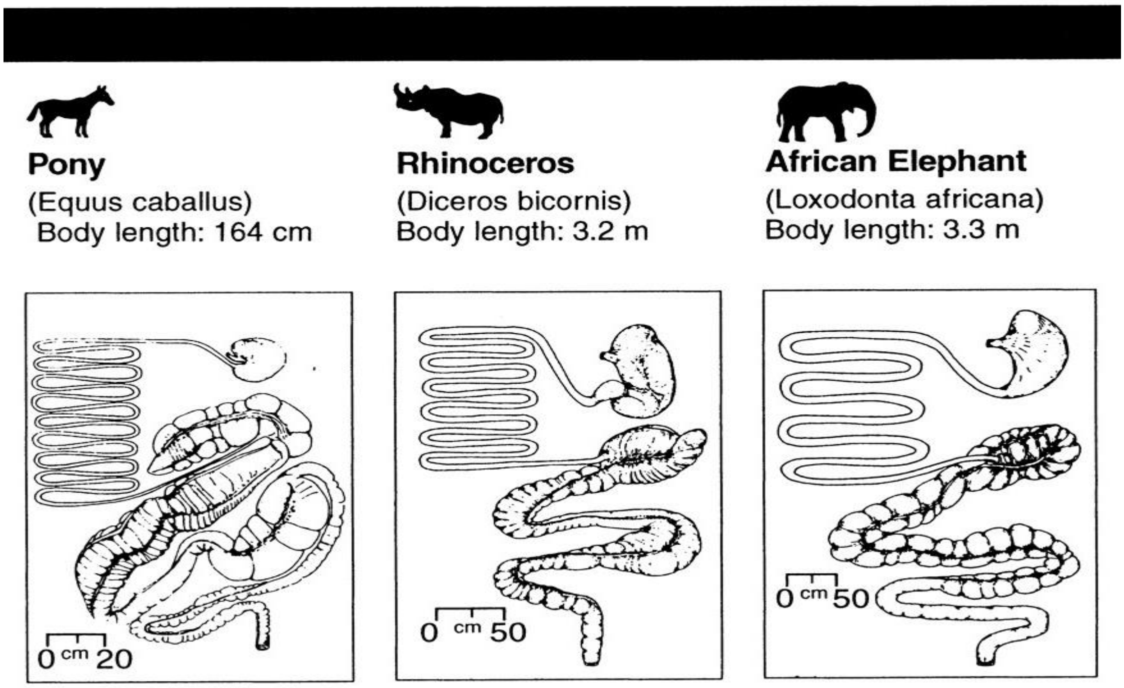

Figure 1 Gastrointestinal tracts of large hindgut-fermenting mammalian herbivores. The hindgut of these animals is dominated by a long and capacious colon. It is haustrated throughout its length inmost species and further compartmentalized in perissodactyls and elephants (adapted from Stevens and Hume, 1998).

The literature review showed that the horse gastrointestinal tract is the closest model for the elephant (**Figure 1, 2**); the highest amounts of *Ruminococcusspp.* were found in the rumen of both. A study by Mahatnirundkul and Kaewmong studied the *Ruminococcus* strains by polymerase chain reaction based approaches with specific primers and they found that about 64% of the 25 samples were *Ruminococcus obeum.*

The elephant diet contains large amounts of cellulose, hemicellulose, carbohydrates and sugar. The digestion of proteins, carbohydrates and lipids starts in the stomach and intestines. Elephants are monogastric herbivores, utilizing fermentation by bacteria, fungi, and protozoa at the large intestine and caecum, similar to cattle (Ullrey et al., 1997).

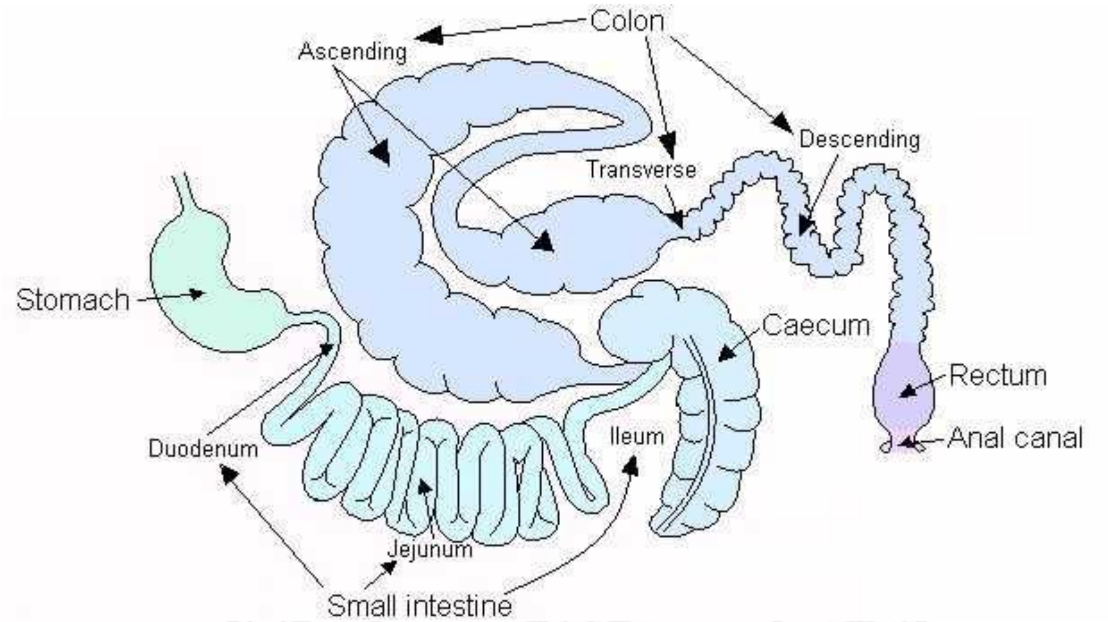

Figure 2 A typical illustration of horse gastrointestinal tract (Adapted from the web source: http://137.222.110.150/Calnet/vetab7/page2.htm)

The microorganisms that function in the fermentation process is diverse; there has been no detailed study about the ecology of elephants. Nevertheless, Ullrey pointed that the number of bacteria and protozoa present in elephant intestine, which increases from duodenum, jejunum and ileumin number; respectively. These bacteria have the ability to digest the plant fiber that enzymes cannot digest (cellulose and hemicelluloses) (Ullrey et al., 1997). The product from fermentation is a volatile fatty acid, which elephants can directly absorb and use as an energy source. If we know the specific type of fermentative bacteria, we may provide to elephant along with feed stuff, which will helps to reduce the digestive track problems and improves the total health. Elephant and buffalo are herbivorous animals that ingest the plant resources.

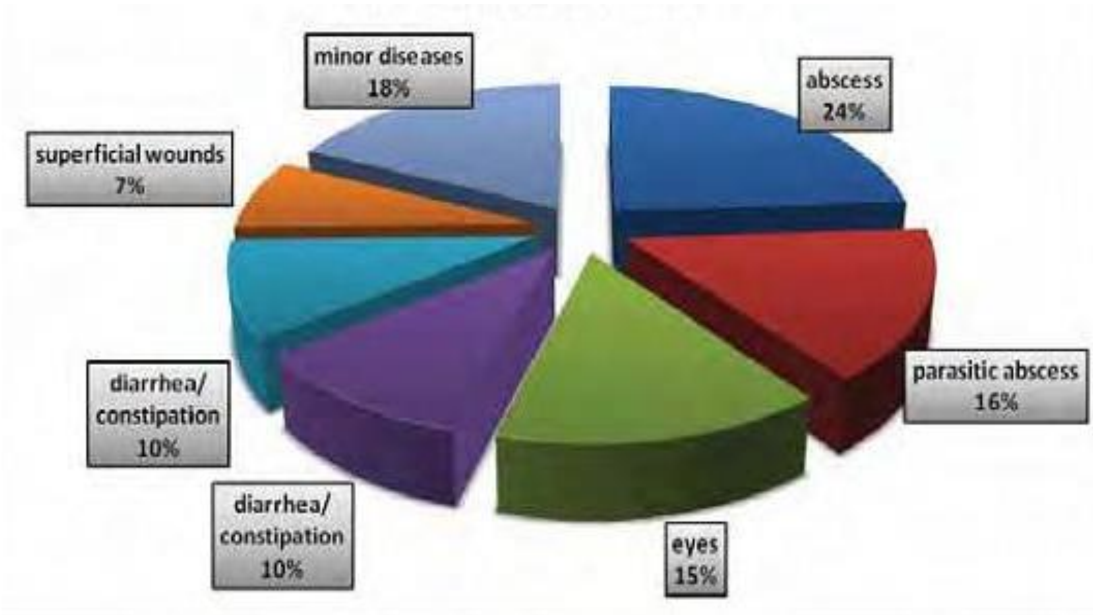

Figure 3 Representation of common disease/ infection among captive elephants (Source: http://www.elephantconservation.org/programs/archive-of-projects/lao-captive-elephant-care-management-programme/)

Their collective diets are grasses, leaves, vegetables and fruits. Many of the microbial species residing in the rumen or stomach, to digest the plant based compounds, which are obligate or facultative anaerobic bacteria. Many lactic acid bacteria are isolated from plant sources, fermented food and human GI tracts, to be used as potent probiotics (Thamacharoensuk, et al., 2013).

Elephant Anatomy and Biology:

Gastrointestinal system:

Elephants are herbivorous, single stomached animals. The main alimentary assemblies are similar to those of the horse. Elephants don't have gall bladder and depends on the hind gut fermentation of fecal matter in their large cecum with the help of symbiotic bacteria. The elephant digestive tract comprises of the mouth, pharynx, esophagus, stomach, small and large intestine, cecum, rectum and anus. The major auxiliary organs (molar teeth, tongue, salivary gland, liver and pancreas) are also involved in the proper digestion of the food.

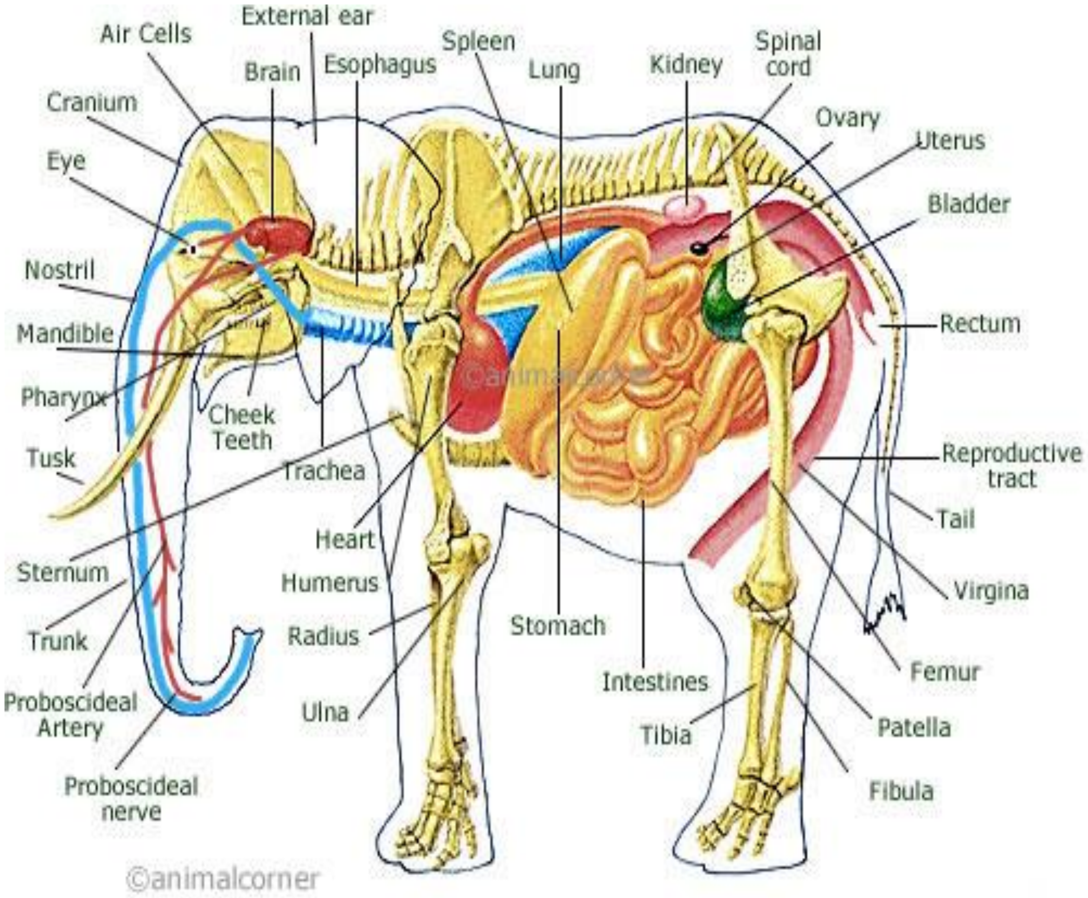

Figure 4 A Typical anatomy of an elephant

The elephant digestive system is not very effective with respect to the absorption of nutrients. About only 44% of the food materials will be absorbed by the elephant. Moreover, an adult Asian elephant can eat 150-200 kg of food and 200 litters of water per day, and the amount of food may differ based on the circumstances.

Mouth:

The elephant mouth opening is very small relative to body dimension compared to several other animals. The oral opening has molar teeth, a tongue, and cavity for salivary ducts and glands. The pharynx and the upper respiratory tract are connected to the mouth of the elephant. The muscular mandibular action and synchronization of teeth and tongue role are significant for the generation of the horizontal crushing action of mastication of elephants.

Molar teeth:

The dental arrangement formula of an adult elephants is normally I 1/0 C0/0 PM 3/3 M 3/3, with a sum of 26 teeth, and the two superior incisors are named as tusks. The typical weight of elephant's teeth is about 5 kg. In each jaw quadrant, a new born elephants have 2-3 teethes, They will develop in the fetus and will be observed in the jaw after a few months after the birth. Elephants may have 6 sets of molar teeth through their life period but they may not grip all the 6 sets at a particular time. The molar teeth will lean-to sporadically. They move forward in the jaw to relocate the old and damaged teeth that piece and frequently drop out on their own and are consumed. The elephants have complex structure of the teeth and it is closely similar to the structure of the teeth of other mammals. They are consisting of cementum, a pulp cavity, enamel, dentin, and pulp tissue which includes odontoblasts, vessels, odontocytes, and nerves. All teeth of elephant are detained together by cementum which shapes the base for the outcrops on the occlusal exteriors of the molars which are enclosed with light or white enamel. This edge dissimilarity with the channels where the murky colored dentin is observed. The shape of shelf on molar occulsal plane can be used to recognize the species of the elephant. In African elephants, molar bulges are pastille shaped and where as loop shaped molar bulges are there in Asian elephants.

Esophagus:

The esophagus expands from the pharynx to the stomach organization in close relative to the trachea. This will form the structure of musculomembranous tube, shaped principally by the tracheoesorphagial muscle. The esophageal mucosa has several mucous glands which ooze mucus to grease the food bolus as it exceed during the esophagus.

Stomach:

The stomach is of the elephant is cylindrical and it is roughly 75-90 cm in long in adult elephants. An adult elephant stomach has the capability to anything between 30 to 70 liters.

Intestine, cecum and rectum:

An adult elephants have the small intestine about the length of 66 to74 feet. The intestines are alienated into three rooms such as the duodenum (roughly 1.5 feet long), jejunum (around 11 feet long) and the ileum. The approximate total length of the intestine of a well grown adult elephant is just about 38-43 feet long which is further separated into a 20 to 22 feet long colon and 12 to 14 foot rectum the finished at a muscular anus below the tail. The cecum is about 5 to 7 feet long and situated near the junction of the ileum and the colon. It is a main location of fermentation in the elephant. The ability of the small intestines is roughly 135 liters and the large intestines and caecum grip about 480 liters of food stuffs.

(http://www.asianelephantresearch.com/about-elephant-anatomy-and-biology-p3.php#Gastrointestinal)

The term probiotic is derived from Greek word, meaning "for life" and originated to describe the substances produced by one microorganism which stimulate the growth of others (Lilly and Stillwell, 1965). Nowadays an expert panel commissioned by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization defined probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (Food and Agriculture Organization of the United Nations and the World Health Organization, 2001).

For humansand livestock, probiotics have been used for a long time. Probiotics are microorganisms that afford the health benefits when consumed.Fermented food and intestinal lactic acid bacterial species with alleged health beneficial properties have been familiarized as probiotics, including *Lactobacillus, Enterococcus,* and *Bifidobacterium*. Genus *Lactobacillus* has several strains that are categorized as probiotics such as L. *acidophilus, L. crispatus, L. amylovorus, L. gallinarum, L. johnsonii, L. casei, L. paracasei, L. ramnosus, L. reteri* and *L. Fermentum*(Ibid).

Genus *Enterococcus* strains that are categorized as probiotics are *E. faecium* and *E. faecalis. Bifidobacterium* strains are considered as a significant probiotics and used in the several food industry. Different species and strains of bifidobacteria may employ a wide range of advantageous health effects.Strains widely used for livestock and humans are *B. animalis, B. bifidum,* and *B. infantis*. Another useful microorganism with cellulolitic and hemi-cellulolytic properties include *Fibrobacter succinogene, Ruminococcus flavefaciens, R. albus, Saccharomyces cerevisiae,* and *Aspergillu soryzae* (Musa, 2009).

Example of some useful probiotics (Suyanee, 2006)

Probiotics have effectively used to maintain intestinal homeostasis effectively since 1900. In particular, *L. acidophilus* and *B. bifidum* has an impact on the colonic microflora, both *in vitro* and *in vivo,* showing antagonism against pathogenic bacteriaby producing bacteriocins.It was shown that *L. acidophilus,* grown in suitable conditions (anaerobic conditions and bile salt) showed cholesterol acclimatization properties.

Table 1 Commonly used probiotics

Lactobacillus	*Bifidobacterium*	*Lactococcus lactis*subsp.	*Streptococcus*	*Enterococcus*	*Saccharomyces*
L. acidophilus	*B. adolescentis*	*Lct. lactis* subsp.	*S. thermophilus*	*Ent. faecium*	Sc. *cerevisiae*
L. brevis	*B. animalis/lactis*[a]	cremoris			(Sc. *boulardii*)[b]
L. delbuekii	*B. bifidum*	*Lct. lactis* subsp. lactis			
L. fermentum	*B. breve*				
L. gasseri	*B. infantis*				
L. johnsonii	*B. lactis*				
L. lactis	*B. longum*				
L. paracasei	*B. thermophilus*				
L. plantarum					

L. hamnosus

L. reuteri

[a] The current taxonomic status of *B. animalis* and *B. lactis* is unclear.

[b]*Sc. Boulardii* is likely to be identical to *Sc.cerevisiae*

This strain was proved as a known probiotic bacterium have the ability to improve the lactose malabsorption in lactose intolerance or lactose maldigestion condition. It also reported for the production of β-galactosidase in the same way as *Bifidobacteria*. The latest study has tried to improve the galactose digestion in humans by adding *L. acidophilus* to unfermented milk.

The anti-mutagenic and anti-carcinogenic properties of bacteria ingested via food and representing the microflora of the gastrointestinal tract have been widely studied. In human trials, Probiotic strains have been associated with the reduction of fecal mutagenicity or fecal enzymatic activities involved in mutagen or carcinogen activation.Reduction of fecal enzyme activities has also been shown after the consumption of milk fermented with the help of L. *acidophilus* LA-2.Gut-associated lymphoid tissue may have contact with adhesive probiotic strains and their components. Therefore, adhesion is one way of provoking immune effects. Human studies have shown that probiotic bacteria have positive impact on the immune system of their host.

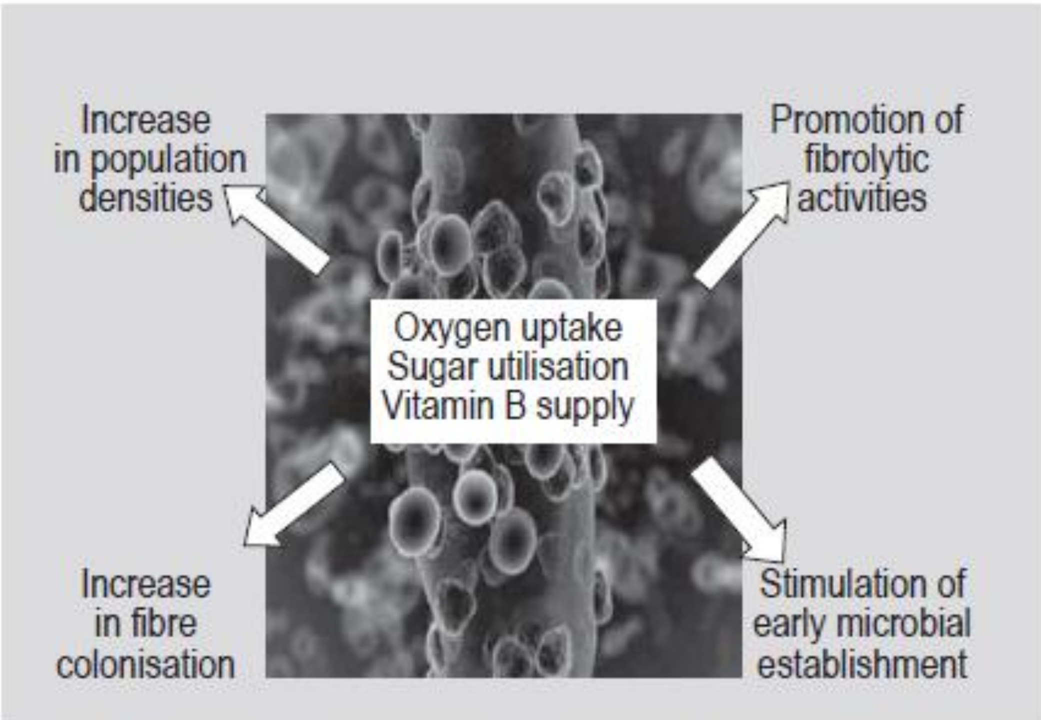

Figure 5 The mechanisms of action of probiotics on ruminal fiber-degrading communities (adapted from Durand and Durand, 2010)

There are some key aspects of the mechanism of action of probiotics. For adhesion of the probiotic microorganisms to the intestinal, upon reaching the intestine, the probiotics must attach to the brush border of microvilli or adhere to the mucus layer in order not to be swept from the colon by peristalsis. They prevent the adherence of the pathogenic bacteria to the host cells and release of gut-protective metabolites (arginine, glutamine, short-chain fatty acids and conjugated linoleic acids) (Figure: 2).

The mechanism of action of probiotic microbes inside the host system have been studied for several years using many kind of animal model. They provided some distinct mechanism of action. In deed all the probiotic can works on any one of the described mechanism or combination of several.The survival of probiotic microbes inside the gut region and other GI track region is the major step which determine the efficacy of a probiotic preparation and it is depends on what kind of strain we are used for the specific host. The release of organicacids (lactic or acetic acid) by bacterial probiotics will helps todecrease the pHof the gut, create more promisingenvironmentalconditions for the inhabitant microbial population and decrease thehazard of colonization by pathogenic microbes.

The dischargeof antimicrobial peptides, for example bacteriocins, whichprevents the growth of pathogens, or suppress the productionof microbial enzymes which will helps to hydrolyze the bacterial toxins.Some strains of probiotic have the ability to remove the pathogenic bacterium completely from the intestinal track of the host by competing them for the nutrient and adhesion. In other hand, some the reported probiotic bacteria can produce or release some metabolites which will further induces the growth of beneficial microbes inside the host system and also stimulates the defense system of the host thereby it helps the host to eradicate the infection.

Some of the probiotics can metabolize or support in the detoxificationof some of the inhibitory compounds likely amines or nitratesor free radicals, which is of an abundantsignificance in gutanaerobic environments (Durand and Durand, 2010).

Probiotic acts as antimicrobials by secreting metabolites called bacteriocins and substances such as organic acids (lactic, acetic and butyric acid), and H_2O_2. The microorganisms are present in the gastrointestinal tract interact with the epithelial and immune cells. The cytokine response is initially manifested by the release of macrophage, known as the immunomodulatory effect.

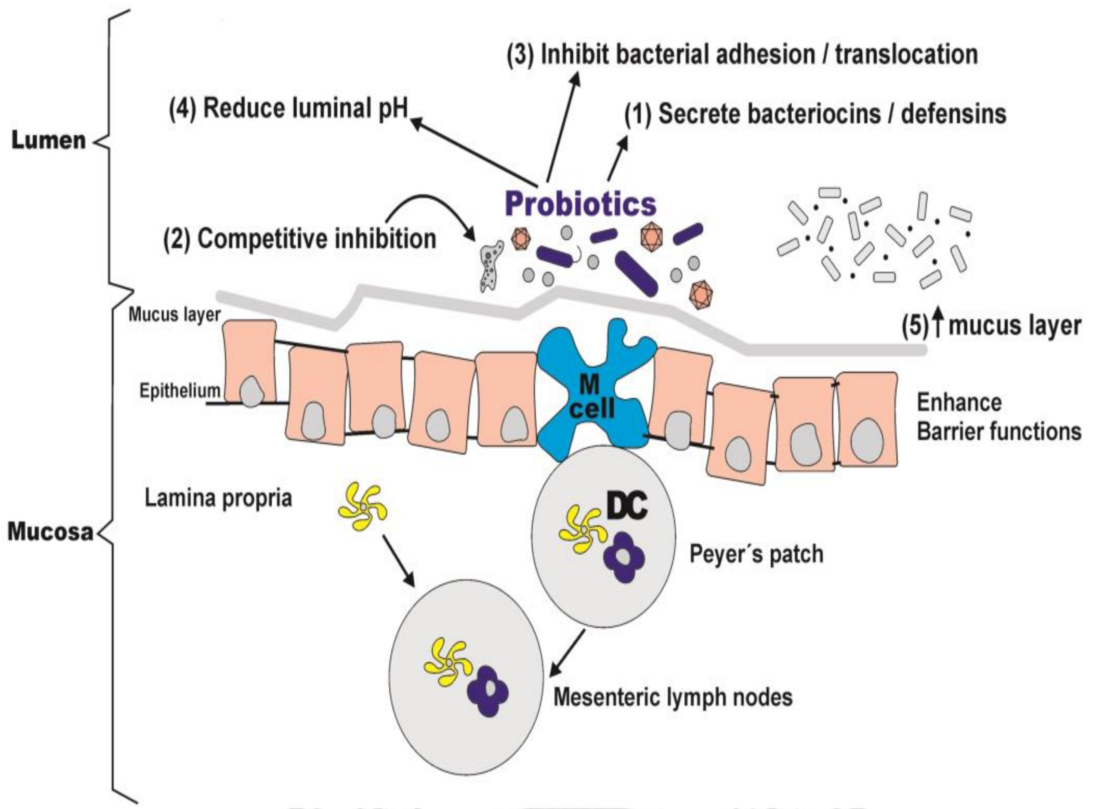

Figure 6 Schematic representations of inhibition of enteric bacteria and enhancement of barrier function by probiotic bacteria. The crosstalk between probiotic bacteria and the intestinal mucosa. Antimicrobial activities of probiotics include the (1) Secretion of bacteriocins or defensins, (2) Inhibition of pathogenic bacteria, (3) Inhibition of microbial adherence or translocation, and (4) reduction of luminal pH. Probiotic bacteria can also enhance intestinal barrier function by (5) increasing mucus production (Adapted from Veterinary Medicine and Science. "Probiotic in Animals", book edited by Everlon Cid Rigobelo, ISBN 978-953-51-0777-4, October 3, 2012).

The probiotic bacteria should have the ability to colonize and compet pathogenic bacteria.The clinical data show that the intake of probiotics significantly reduces the incidence or severity of diarrhea of different origins and reduces the incidence or severity of gastrointestinal illness or infection.

Some digestive disorders are the indicator of disturbance in the balance of intestinal bacteria. It can occur after an infection, food, stress or after taking antibiotics. Intestinal problems can also arise when the lining of the intestineis damaged. Consumption of probiotics may help to recover from these problems by the following mechanisms:

- Producing organic acids such as lactic acid that destroy pathogens
- Secreting antimicrobial products called bacteriocins
- Adhesion of the probiotic microorganisms to the intestinal lining to prevent the adherence of the pathogenic bacteria to the host cells
- Stimulating the human antibodies especially local antibodies
- Providing useful metabolites such as arginine, glutamine, short-chain fatty acids and vitamins
- Activating macrophage to destroy pathogen

Properties of a potent probiotic bacteria

1. A strain must survive the conditions of the host gastrointestinal tract, mouth, stomach, and intestines to act as a probiotic.
2. Adhesion of the probiotic microorganism to the intestinal mucosa is a prerequisite for the colonization and antagonistic activity against enteropathogens.
3. Must not have pathogenic activity
4. Produce antimicrobial substances (bacteriocins) and able to activate local immunity
5. Should have the ability to grow in starving condition
6. Produce lactic acid
7. Viable in dry conditions in an economical process for preparing industrial scale quantities
8. Able to survive in a wide range of temperature, 20°C – 60°C.
9. Short generation time, quick growth

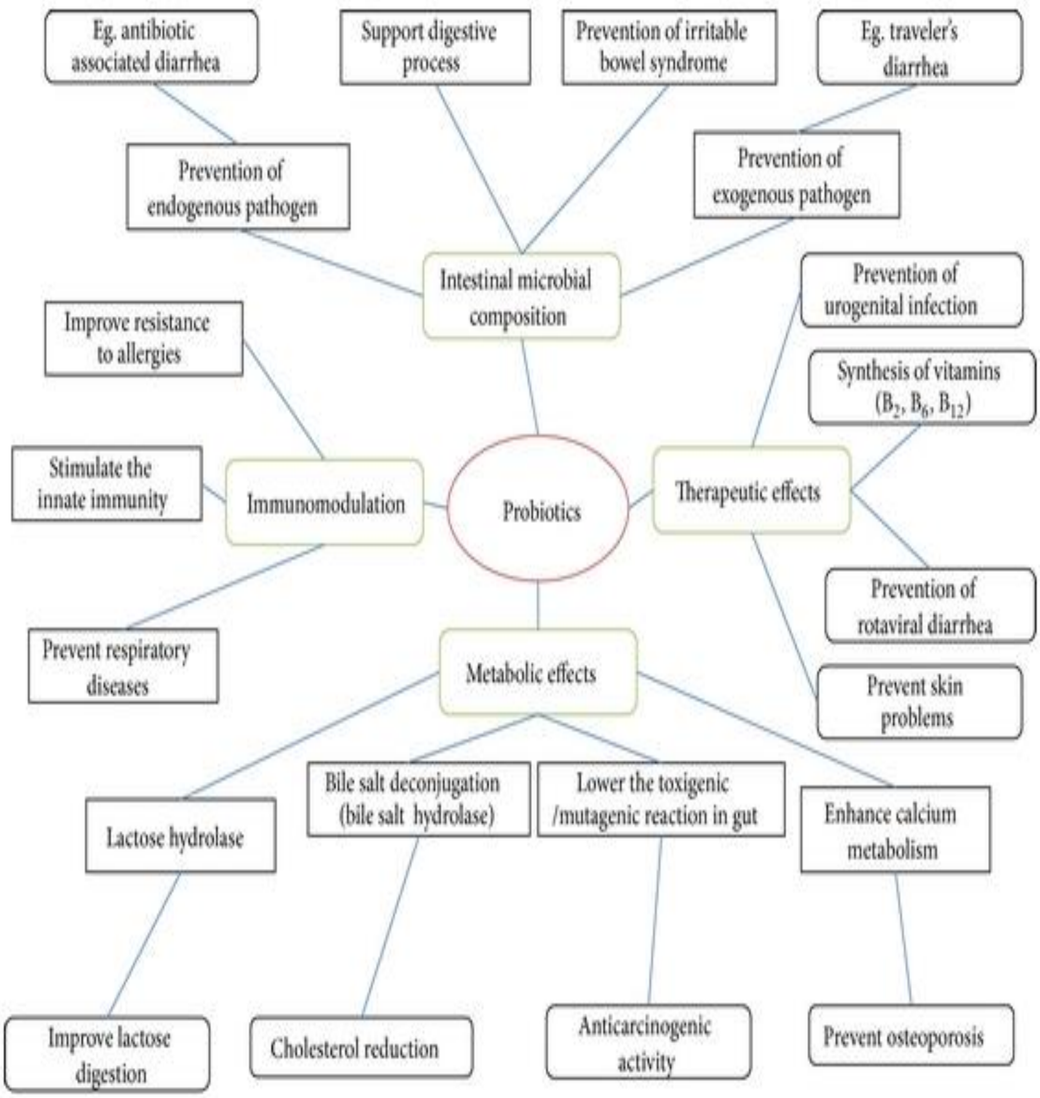

Figure 7 Schematic representaion of benefits of probiotics (adapted from Anandharaj M et al., 2014)

10. Acquired resistance derives either from genetic mutations or acquisition of foreign DNA from other bacteria.
11. Do not produce toxins that can accumulate
12. Provid useful products from bacterial digestion such as amino acids, fatty acids, and vitamins.