# Association of microbial flora with man and their maintenance through diet. Part 1*

**K.J. VENUGOPAL**

**AVT McCormick Ingredients (P) Ltd
South Vazhakkulam
Aluva-683107, Kerala State, India**
venugopal_kj@avtspice.com
venukj@yahoo.com

## INTRODUCTION

Microorganisms that reside on skin, mouth and in the gastrointestinal tracts of human being are generally referred to as human flora. They include bacteria, fungi and archaea (monocellular organisms having no nucleus or organelles). Some of these organisms are useful for the human host while the majority have no known effects on our body, and a few have detrimental effect.

The group of beneficial organisms which participate in maintaining health of their host are commonly referred as **microbiota** as the word 'flora' is technically a misnomer because it pertains to plants and microbes are not plants. This article reviews the present state of knowledge on association of microbes with our body and aims to emphasise the importance of maintaining a proper microbiotic population in the body by way of dietary supplements for a healthy and disease-resistant life. Especially when serious challenges are posed to the existence of microbiota in the body through antibiotics, medicines and other chemicals, it is important to understand why the symbiosis of man and microbes is essential for a normal and healthy life.

## HUMAN FLORA

Human flora is generally classified based on taxonomy or by host-substrate. Classification based on taxonomical group involves Archaean, fungal and bacterial flora.

### Archaean Flora

Archaea are present in the human gut, but their number is limited. The dominant group is the methanogens, particularly *Methanobrevibacter smithii* and *Methanosphaera stadtmanae*. Colonization of gut by methanogens varies and only about 50% of humans have easily-detectable populations of these organisms. Archaean flora has no apparent effect on health and no clear examples of archaeal pathogens are known till date. However, a relationship has been proposed between the presence of some methanogens and human periodontal disease. More research and scientific evidences are wanted to establish these facts, though.

### Fungal Flora

Fungi, specifically yeasts, are present in large numbers in the human gut. The best known amongst these are *Candida* species because of their ability to become pathogenic in immunocompromised hosts. Yeasts, particularly *Malassezia* species, are present on the skin where they consume oils secreted from the sebaceous glands.

### Bacterial Flora

Bacteria constitute the major portion of human flora. It is estimated that seizeably large numbers of bacterial flora are found on all surfaces exposed to the environment such as skin, eyes, mouth, nose and small intestine. Their classification, in general, is based on how these organisms grow, the type of substratum, color of the colony, staining characteristics and also on their DNA sequencing characteristics. It is now an established scientific fact that many bacteria are vital for maintaining health in human beings, while some pathogenic bacteria also pose a significant health threat by causing varioius diseases. A large number of bacteria live on the skin and in the digestive tract of man as their growth is supported by the warmth and sweat of our body. There are more than 500 bacterial species present in the human gut and most of them are able to break down nutrients such as carbohydrates that humans normally cannot digest. They

synthesize vitamins such as folic acid, vitamin K and biotin, and also ferment complex indigestible carbohydrates.

On the other hand, bacterial flora can also act as opportunistic pathogens at times of lowered immunity. For example, *Escherichia coli* are bacteria that normally lives in the colon, but certain mutated strains of these gut bacteria such as *E.coli O157:H7* cause disease. Similarly, *Actinomyces viscosus* and *A. naeslundii* which normally live in the mouth forming part of a sticky substance called plaque which, if not removed by brushing, hardens into calcus or tartar. These bacteria also secrete acids that dissolve tooth enamel, causing tooth decay.

Classsification of microbial flora based on anatomical area they inhabit involves skin flora, nasal flora, gut flora, vaginal flora, and oral flora has been studied in detail.

## Skin Flora

Around 200 microbial genera belonging to nineteen bacterial phyla have been so far identified in the skin. Majority of them belong to four phyla: Actinobacteria (50%), Firmicutes (25%), Proteobacteria (15%), and Bacteroidetes (5%).

## Gut Flora

The gut flora, widely known as the intestinal microflora, is the human microbial flora that lives in the digestive tract and performs a number of useful functions for their hosts. The average human body, which consists of about $10^{13}$ (ten trillion) cells, inhabits about 100 trillion of microorganisms in the gut alone. Around 500 different bacterial species have been estimated in the human intestine. Their effect is so enormous that metabolic activity performed by these bacterial cells is equal to that of an organ itself. Though fungi and protozoa also make up a part of the gut flora, little is known about their activities.

Research suggests that the relationship between gut flora and humans is not merely commensalic but is a symbiotic relationship. The microorganisms generally perform a host of useful functions, such as fermenting unused energy substrates, training the immune system, preventing growth of harmful species, regulating development of gut, producing vitamins for the host (such as biotin and vitamin K), and producing hormones to direct the host to store fats. The body maintains proper balance of microbial species by altering its pH, by the activity of immune
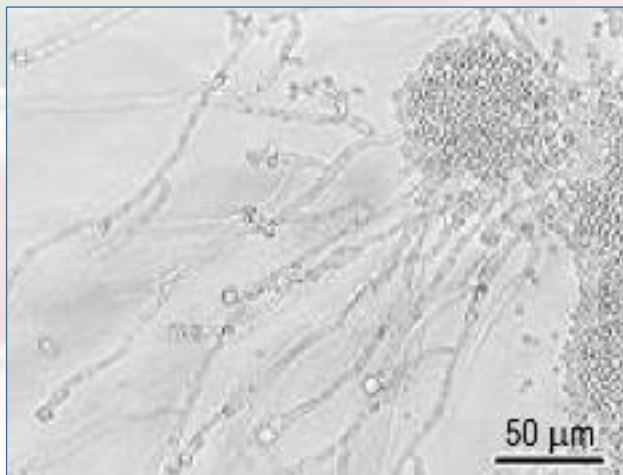


*Figure 1 – Candida albicans, a dimorphic fungus which grows as yeast in the human gut.*

system, and by peristalsis. However, under certain conditions, some species do cause disease by causing infection or increasing risk of cancer.

Of the gut flora, the colon has the greatest diversity and numbers of bacteria, and it is the activity of these bacteria that make the colon the most metabolically active organ of the human body. Bacteria make up majority of the flora of the colon. 60% of the dry mass of feces is bacterial mass, making feces an ideal source to test for gut flora for experimental purposes by generating bacterial 16S rRNA gene sequences using bacterial primers extracted from nucleic acid of fecal specimens. While the acid, bile and pancreatic secretions of the stomach hinder colonization of many bacteria in the stomach and the proximal small intestine, the colon provides ambient conditions for microbial growth and colonisation. Bacterial growth is rapid in the cecum and ascending colon, which has a low pH, and slow in the descending colon, which has an almost neutral pH.

Not all the species in the gut have been identified yet, because some cannot be cultured, making DNA isolation and identification difficult. It has been noted that populations of species vary widely among different individuals but stay fairly constant within an individual over time, even though some alterations may occur with changes in lifestyle, diet and age. Through the human microgenome project, an effort to better describe the microflora of the gut and other body locations has lately been initiated. In 2009, scientists from INRA, France highlighted the existence of a small number of species shared by all individuals that constitute the human intestinal microbiotal phylogenetic core. Most of these bacteria belong to the genera *Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus,* and *Bifidobacterium*.

Other genera such as *Escherichia* and *Lactobacillus* are also present but to a lesser extent. Of these, species from the genus *Bacteroides* alone constitute about 30% of all bacteria in the gut, suggesting that this genus is especially important in the functioning of the host. In case of fungi, the currently known genera in the gut flora include *Candida* (**Figure 1**), *Saccharomyces, Aspergillus,* and *Penicillium*.

*E. coli* is the most known bacterium that is associated with humans, being an invariable component of the human intestinal tract. Even though *E. coli* is the most studied of all bacteria, having the exact location and sequence of 4,288 genes on its chromosomes mapped, we do not fully understand its ecological relationship with human being. Both host and bacteria benefit from each other, and the associations are, for the most part, mutualistic. The normal flora derives a steady supply of nutrients, a stable environment, and protection and transport. The host obtains from the normal flora certain nutritional and digestive benefits, stimulation of the development and activity of immune system, and protection against colonization and infection by pathogenic microbes.While most of the activities of the normal flora benefit their host, some of the normal flora are parasitic (live at the expense of their host), and some are pathogenic (capable of producing disease). Diseases that are produced by the normal flora in their host are called endogenous diseases, which are actually opportunistic infections (organism need a special opportunity of weakness in the host defenses to infect). An example of opportunistic infection is chronic bronchitis in smokers wherein the normal flora invades the lung weakened by smoking. Sometimes the relationship between a member of the normal flora and its host is a commensal relationship. Many of the normal floras that are not predominant in their habitat, even though always present in low numbers, are thought of as commensal bacteria. However, if a presumed commensal relationship is studied in detail, parasitic or mutualistic characteristics often emerge.

## Other Flora

Microbes also colonise various other parts of the human body. Vaginal flora, respiratory flora and oral flora are important microbial colonies hosted by the human body. Since they do not form part of this review, they are not discussed in great length in this article.

## ASSOCIATION BETWEEN MAN AND THE MICROFLORA

How do these microbes colonise human body? Are they present right from the foetus stage? This aspect has been well studied. The gastrointestinal tract of a normal fetus is sterile, but during birth and thereafter, bacteria from the mother and the surrounding environment colonizes the infant's gut, and immediately after birth babies acquire bacterial strains in their upper gastrointestinal tract derived from the mothers' faeces. Infants born by caesarean procedure may also be exposed to their mothers' microflora, but the initial exposure is mostly from the environment (air, other infants, nursing staffs etc. which serve as vectors for transfer). Also, the primary gut flora in infants born by cesarean delivery are disturbed for up to 6 months after birth while infants born by normal delivery take only 1 month for their intestinal micro flora to get established. After birth, environmental, oral and cutaneous bacteria are transferred from the mother to the infant through suckling, kissing, and caressing. Initially, infants are colonized by *E. coli* and *Streptococci* and within a few days, bacterial numbers reach upto 1010 per gram of feces according to research studies. During the first week of life, these bacteria create a reducing environment in the gut, thereby making a favourable climate for the subsequent bacterial succession. These successors are anaerobic species belonging to the genera *Bifidobacterium, Bacteroides, Clostridium*, and *Ruminococcus.* Breast-fed babies become dominated by *Bifidobacteria*, possibly due to the contents of bifidobacterial growth factors in breast milk. In contrast, the microbiota of formula-fed infants is more diverse with high numbers of *Enterobacteriaceae, Enterococci, Bifidobacteria, Bacteroides,* and *Clostridia*. However, once solid food has been introduced, the microflora of breast-fed infants becomes similar to that of formula-fed infants. After the initial stabilisation of microbial flora in the body,

by the second year of life the fecal microflora of the child resembles that of adults.

### Tissue Specificity

The human flora prefers to colonize certain tissues and not others. This tissue specificity is usually due to properties of both the host and the bacterium. Tissue tropism (bacterial preference for certain tissues for growth) is one of the mechanisms by which tissue specificity is achieved. One explanation for tissue tropism is that the host provides essential nutrients and growth factors for the bacterium, in addition to suitable oxygen, pH, and temperature for growth.

| Bacterium | Bacterial adhesin | Attachment site |
|---|---|---|
| Streptococcus pyogenes | Cell-bound protein (M-protein) | Pharyngeal epithelium |
| Streptococcus mutans | Cell-bound protein (Glycosyl transferase) | Pellicle of tooth |
| Streptococcus salivarius | Lipoteichoic acid | Buccal epithelium of tongue |
| Streptococcus pneumoniae | Cell-bound protein (choline-binding protein) | Mucosal epithelium |
| Staphylococcus aureus | Cell-bound protein | Mucosal epithelium |
| Neisseria gonorrhoeae | N-methylphenylalanine pili | Urethral/cervical epithelium |
| Enterotoxigenic E. coli | Type-1 fimbriae | Intestinal epithelium |
| Uropathogenic E. coli | P-pili (pap) | Upper urinary tract |
| Bordetella pertussis | Fimbriae (filamentous hemagglutinin) | Respiratory epithelium |
| Vibrio cholera | N-methylphenylalanine pili | Intestinal epithelium |
| Treponema pallidum | Peptide in outer membrane | Mucosal epithelium |
| Mycoplasma | Membrane protein | Respiratory epithelium |
| Chlamydia | Unknown | Conjunctival or urethral epithelium |

*Table I*

| Anatomical location | Predominant bacteria |
|---|---|
| Skin | Staphylococci and corynebacteria |
| Conjunctiva | Sparse, Gram-positive cocci and Gram-negative rods |
| Oral cavity, Teeth | Streptococci, lactobacilli |
| Mucous membranes | Streptococci and lactic acid bacteria |
| Upper respiratory tract, Nasal membranes | Staphylococci and corynebacteria |
| Pharynx (throat) | Streptococci, Neisseria, Gram-negative rods and cocci |
| Lower respiratory tract | None |
| Gastrointestinal tract - Stomach | Helicobacter pylori (up to 50%) |
| Small intestine | lactics, enterics, enterococci, bifidobacteria |
| Colon | bacteroides, lactics, enterics, enterococci, clostridia, methanogens |
| Urogenital tract - Anterior urethra | sparse, staphylococci, corynebacteria, enteric |
| Vagina | lactic acid bacteria during child-bearing years, otherwise mixed |

*Table II*

For example, *Lactobacillus acidophilus,* otherwise known as "Doderlein's bacillus" colonizes the vagina because glycogen produced there provides the bacteria with a source of sugar which they ferment to lactic acid. Specific adherence is the other means of tissue specificity. Most bacteria

can colonize a specific tissue or site because they can adhere to that tissue or site in a specific manner that involves complementary chemical interactions between the two surfaces. Specific adherence involves biochemical interactions between bacterial surface components (ligands or adhesins) and host cell molecular receptors. Some established examples of bacterial adhesins and their attachment sites to human tissues are given in Table I.

Formation of Biofilm is another mechanism of tissue specificity. Some of the indigenous bacteria are able to construct biofilms on a tissue surface, or they are able to colonize a biofilm built by another bacterial species. Biofilms are usually formed when one bacterial species attaches specifically or non-specifically to a surface, and then secretes carbohydrate slime (exopolymer) that embed the bacteria and attracts other microbes to the biofilm for protection. An example of biofilm is dental plaque on the teeth, which is a naturally-constructed biofilm with thickness of 300-500 bacterial cells on the surfaces of the teeth. These accumulations subject the teeth and gingival tissues to high concentrations of bacterial metabolites, which result in dental disease.

### Composition of the Normal Flora

The normal flora of humans is exceedingly complex and consists of more than 200 species of bacteria and their makeup may be influenced by various factors, including genetics, age, sex, stress, nutrition and diet of the individual.

Three important developmental changes in humans, namely weaning, eruption of teeth, and onset and cessation of ovarian functions, affect the composition of the normal flora in the intestinal tract, the oral cavity, and the vagina, respectively. However, within the limits of these fluctuations, the bacterial flora of humans is sufficiently constant.

As explained earlier, a human first

becomes colonized by a normal flora at the time of birth and passage through the birth canal. Birth care activities such as handling and feeding of the infant leads to establishment of a stable normal flora on the skin, oral cavity and intestinal tract in about 48 hours.

It has been calculated that a human adult houses about $10^{12}$ bacteria on the skin, $10^{10}$ in the mouth, and $10^{14}$ in the gastrointestinal tract. It is noteworthy that the latter number is far in excess of the number of eucaryotic cells in all the tissues and organs of a human.

The predominant bacteria on the surfaces of the human body are listed in Table II.

## Normal Flora of the Skin

Adult human body is covered with approximately 2 m² of skin. The density and composition of the normal flora of the skin varies with anatomical locale. The high moisture areas such as the axilla, groin, and areas between the toes support the activity and growth of bacterial cells, but the density of bacterial populations at most other sites is fairly low, generally in 100s or 1000s per cm². Most bacteria on the skin are sequestered in sweat glands.

Skin microbes found in the most superficial layers of the epidermis and the upper parts of the hair follicles are Gram-positive cocci (*Staphylococcus epidermidis* and *Micrococcus* spp.) and *Corynebacteria* such as *Propionibacterium* spp (Figure 2). These are generally nonpathogenic and commensal, although mutualistic and parasitic roles have been assigned to them.

Sometimes potentially pathogenic *Staphylococcus aureus* is found on the face and hands in individuals who are nasal carriers. This is because the face and hands are likely to become inoculated with the bacteria on the nasal membranes. Such individuals may autoinoculate themselves with the pathogen or spread it to other individuals or foods.

## Normal Flora of the Conjunctiva

A variety of bacteria inhabit the normal conjunctiva, but their number is usually small. *Staphylococcus epidermidis* and certain *Coryneforms* (*Propionibacterium acnes*) are dominant. *Staphylococcus aureus*, some *Streptococci*, *Haemophilus* spp. and *Neisseria* spp. are occasionally found. The conjunctiva is kept moist and healthy by the continuous secretions from the lachrymal glands; and, blinking wipes the conjunctiva every few seconds mechanically washing away foreign objects including bacteria. Lachrymal secretions (tears) also contain bactericidal substances including lysozyme. Hence, there is little or no opportunity for microorganisms to colonize the conjunctiva without special mechanisms to attach to the epithelial surfaces and some ability to withstand lysozymal effects.

Pathogens which infect the conjunctiva (e.g. *Neisseria gonorrhoeae* and *Chlamydia trachomatis*) are able to
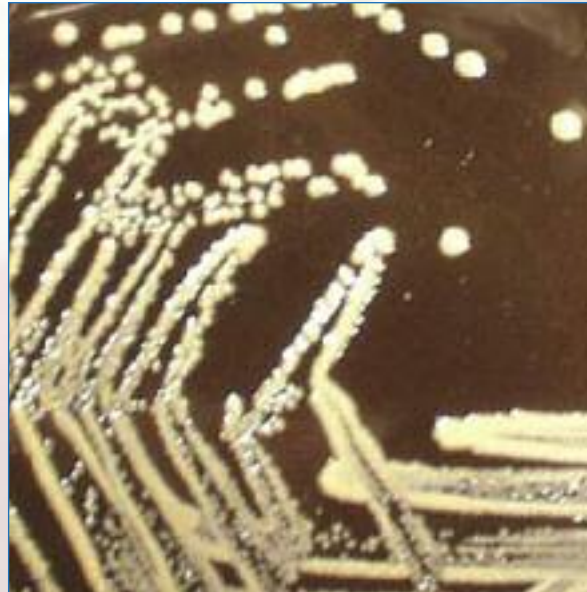
*Figure 2 – Colonies of* Propionibacterium acnes, *found on skin and the conjunctiva.*
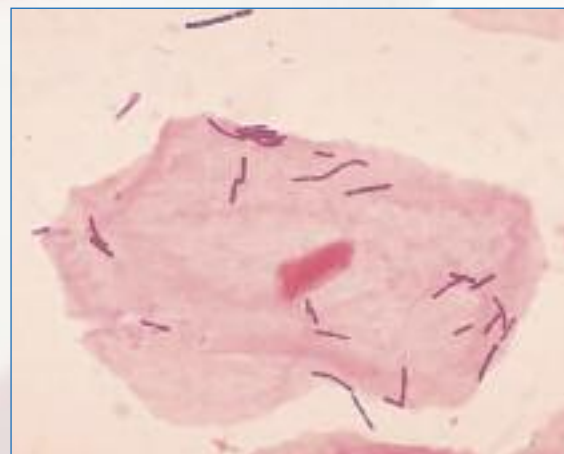
*Figure 3 – A* Lactobacillus *species in association with a vaginal epithelial cell.*

specifically attach to the conjunctival epithelium. Newborn infants may be especially prone to bacterial attachment. Since Chlamydia and Neisseria might be present on the cervical and vaginal epithelium of an infected mother, silver nitrate or an antibiotic are generally applied into the newborn's eyes to avoid infection after passage through the birth canal.

## Normal Flora of the Respiratory Tract

A large number of bacteria colonize the upper respiratory tract (nasopharynx). The nostrils are always heavily colonized, predominantly with *Staphylococcus epidermidis* and *Corynebacteria*, about 20% of the general population is colonised with *Staphylococcus aureus*, this being the main carrier site of this important pathogen. The healthy sinuses, in contrast are sterile. The pharynx is normally colonized by *Streptococci* and various Gram-negative cocci. Sometimes pathogens such as *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Haemophilus influenzae* and *Neisseria meningitidis* are also found in the pharynx.

The lower respiratory tract (trachea, bronchi, and pulmonary tissues) is virtually free of microorganisms, mainly because of the efficient cleansing action of the ciliated epithelium lining the tract. Any bacteria reaching the lower respiratory tract are swept upward by the action of the mucociliary blanket that lines the bronchi, to be removed subsequently by coughing, sneezing, swallowing, etc. If the respiratory tract epithelium becomes damaged, as in bronchitis or viral pneumonia, the individual may become susceptible to infection by pathogens such as *Haemophilus influenzae* or *Streptococcus pneumoniae* descending from the nasopharynx.

## Normal Flora of the Urogenital Tract

Urine is normally sterile, and since the urinary tract is flushed with urine every few hours, microorganisms do not generally gain access and establish here. The flora of the anterior urethra, as indicated by urine cultures, does consist of *Staphylococcus epidermidis*, *Enterococcus faecalis* and some alpha-hemolytic *Streptococci* though their numbers are few. In addition, some enteric bacteria (e.g. *E. coli*, *Proteus*) and corynebacteria, which are probably contaminants from the skin, vulva or rectum, may occasionally be found at the anterior urethra.

The vagina becomes colonized soon after birth with *Corynebacteria*, *Staphylococci*, *Streptococci*, *E. coli*, and a lactic acid bacterium historically named "Doderlein's bacillus" (*Lactobacillus acidophilus*). During reproductive life, from puberty to menopause, the vaginal epithelium contains glycogen due to the actions of circulating estrogens. Doderlein's bacillus predominates, being able to metabolize the glycogen to lactic

acid (Figure 3). The resulting low pH of the vaginal epithelium prevents colonisation of other bacteria as well as the potentially-pathogenic yeast, *Candida albicans*. This is a striking example of the protective effect of the normal bacterial flora for their human host.

## Normal Flora of the Oral Cavity

Presence of nutrients, epithelial debris, and secretions makes the mouth a favorable habitat for a variety of bacteria. Oral bacteria include streptococci, lactobacilli, staphylococci and corynebacteria, with a great number of anaerobes, especially bacteroides (Figure 4).

At birth, the oral cavity is composed solely of the soft tissues of the lips, cheeks, tongue and palate, which are kept moist by the secretions of the salivary glands. At birth the oral cavity is sterile but rapidly becomes colonized from the environment, particularly from the mother in the first feeding. *Streptococcus salivarius* is dominant and make up 98% of the total oral flora until the appearance of the teeth. The eruption of the teeth during the first year leads to colonization by *S. mutans* and *S. sanguis*. These bacteria require a nonepithelial surface in order to colonize. They will persist as long as teeth remain. Other strains of streptococci adhere strongly to the gums and cheeks but not to the teeth. The creation of the gingival crevice area (supporting structures of the teeth) increases the habitat for a variety of anaerobic species. The complexity of the oral flora continues to increase in due course, and bacteroides and spirochetes colonize around puberty.

The normal flora of the oral cavity clearly benefit from their host who provides nutrients and habitat. At the same time, the oral flora contributes to host nutrition via synthesis of vitamins, and to immunity by inducing low levels of antibodies that cross react with pathogens. The oral bacteria also help the host by exerting microbial antagonism by production of inhibitory substances such as fatty acids, peroxides and bacteriocins. But, in general, the oral flora are the usual cause of various oral diseases in humans, including abscesses, dental caries, gingivitis, and periodontal disease. If oral bacteria gain entrance into deeper tissues, they cause abscesses of alveolar bone, lung, brain, or the extremities. Such infections usually contain mixtures of bacteria with Bacteroides, *Melaninogenicus* being a dominant genus of them.

## Normal Flora of the Gastrointestinal Tract

The bacterial flora of the gastrointestinal (GI) tract of animals has been studied more extensively than that of any other site. The composition differs between various animal species, and also within an



*Figure 4 – Various* Streptococci *in a biofilm in the oral cavity.*



*Figure 5 – Colonies of* E. coli *growing on EMB agar.*

animal species. In humans, composition of the flora differs having been influenced by age, diet, cultural conditions, and the use of antibiotics. The last greatly perturbs the composition of the intestinal flora.

In the upper gastro intestinal tract of adult humans, the esophagus contains only those bacteria that are swallowed with saliva and food. Because of the high acidity of the gastric juice, very few bacteria (mainly acid-tolerant lactobacilli) can be cultured from the normal stomach. However, according to statistical surveys, at least half the population in the United States has been colonized by a pathogenic bacterium, *Helicobacter pylori* which is now known to be the major cause of gastric ulcers, and is probably a cause of gastric and duodenal cancer as well. The Australian microbiologist, Barry Marshall, received the Nobel Prize in Physiology and Medicine in 2005, for demonstrating the relationship between Helicobacter and gastric ulcers.

The proximal small intestine has a relatively sparse Gram-positive flora, consisting mainly of lactobacilli and *Enterococcus faecalis*. The distal part of the small intestine contains greater numbers of bacteria and additional species, including coliforms (*E. coli* and relatives) and bacteroides, in addition to lactobacilli and enterococci (Figure 5).

The composition of flora of the gastrointestinal tract varies along the tract and across the tract (at horizontal levels), where certain bacteria attach to the gastrointestinal epithelium and others occur in the lumen. There is a very close association between specific bacteria in the intestinal ecosystem and specific gut tissues. Gram-positive bacteria, such as the *Streptococci* and *Lactobacilli*, are thought to adhere to the gastrointestinal epithelium using polysaccharide capsules or cell wall teichoic acids to attach to specific receptors on the epithelial cells. Gram-negative bacteria, such as the enteric, attach by specific fimbriae which bind to glycoproteins on the epithelial cell surface.

Interactions of bacterial flora with the human body are exhibited the most in case of the intestinal due to their large mass and numbers. Hence the dietary habits of a person become very important for the upkeep of normal flora in the gut. Bacteria in the human GI tract have been shown to produce vitamins and may contribute to nutrition and digestion as well. But their most important effects are in their ability to protect their host from establishment and infection by foreign microbes and their ability to stimulate the development and the activity of the immunological tissues.

On the other hand, some of the bacteria in the colon (e.g. Bacteroides) have been shown to produce metabolites that are carcinogenic, and there may be an increased incidence of colon cancer associated with these bacteria. Alterations in the GI flora brought on by poor nutrition or in take of antibiotics can cause shifts in
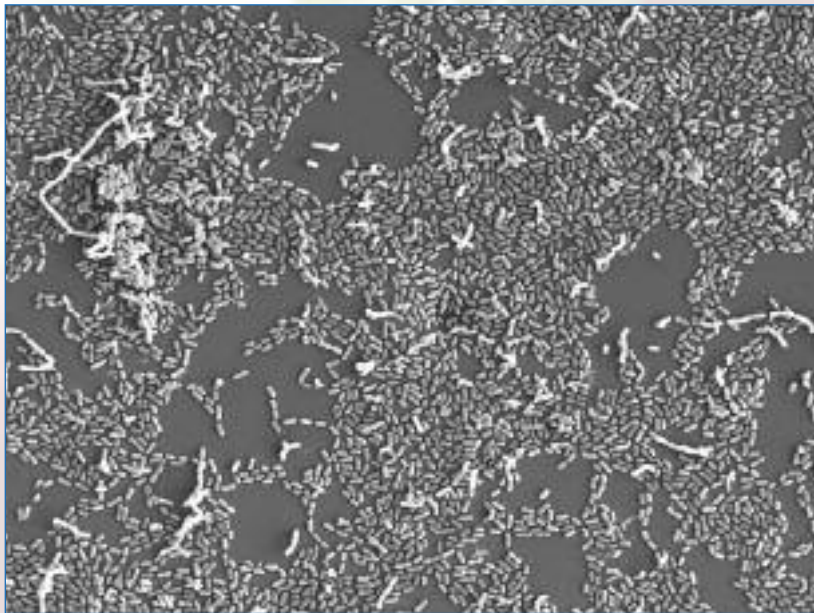
populations and colonization by non-resident population that leads to gastrointestinal disease.

## Beneficial Effects of the Normal Gut Flora

From our earlier discussions, it becomes clear that bacteria associated with our body perform many beneficial functions for the host, and such functions include digestion of unutilized energy substrates, stimulation of cell growth, repression of growth of harmful microorganisms, training the immune system to respond only to pathogens, and defence against some diseases.

While taking part in carbohydrate metabolism, the gut flora performs the function of utilizing those carbohydrates which the human body is not normally equipped to break down. The gut flora has enzymes that our cells lack for breaking down certain polysaccharides, fibre, oligosaccharides and sugars, and turn them to short chain fatty acids such as acetic acid, propionic acid, butyric acid and gases and organic acids like lactic acid, which are then converted to energy and nutrients for the host cells. The resultant fatty acids increase absorption of water by the gut, reduce population of harmful bacteria, increase growth of human gut cells, and also facilitate growth of indigenous bacteria.

The gut flora takes part in proteolytic fermentation also, by breaking down proteins such as enzymes, dead host and bacterial cells, collagen and elastin from food. In this process, however, toxins and carcinogens are also produced, so a diet lower in protein should be preferred to lower the exposure to these toxins. Scientific evidences also suggest that bacteria enhance the absorption and storage of lipids. Other beneficial metabolic effects of the gut flora include helping absorption of vitamins like vitamin K, altering intestinal growth by changing the expression of cell surface proteins such as sodium/glucose transporters and aiding cells in preventing injury to the gut mucosa.

Gut flora also plays a vital role in preventing harmful species from colonizing the gut, a role generally termed as the "barrier effect". Yeasts and harmful bacterial species such as *Clostridium difficile* (overgrowth of which can cause *Pseudomembranous colitis*) are unable to grow beyond a level due to competition from helpful gut flora to adhere to the mucosal lining of the intestine. Thus, animals without gut flora are infected very easily by pathogens. The barrier effect protects humans from both invading species as well as species normally present in the gut in low numbers, whose growth remian inhibited by the gut flora. Normally, the helpful bacteria prevent the growth of pathogenic species by competing for nutrition and binding sites to the epithelium of the colon. The process of fermentation, since it produces lactic acid and different fatty acids, also serves to lower the pH in the colon, preventing the proliferation of harmful species of bacteria and facilitating that of helpful species.

Gut flora has a continuous and dynamic effect on the host's gut and systemic immune systems. The first bacteria to settle in the gut as soon as birth are able to affect the immune response, making it more favorable to their own survival and less so to competing species. Thus, the first bacteria to colonize the gut are important in determining the person's lifelong gut flora makeup. Recent findings have shown that gut bacteria play a role in the expression of Toll-Like Receptors (TLRs) in the intestines – molecules that help the host repair damage due to injury. TLRs cause parts of the immune system to repair injury caused by agents such as radiation. Bacteria can also influence the phenomenon known as Oral Tolerance, in which the immune system is less sensitive to an antigen (including those produced by gut bacteria) once it has been ingested. Some species of gut flora, such as those in the Bacteroides genus, are able to change their surface receptors to mimic those of host cells in order to evade immune response. Bacteria with neutral and harmful effects on the host also use these types of strategies. The host immune system has also adapted to this activity, preventing overgrowth of harmful species.

The resident gut microflora positively controls the intestinal epithelial cell differentiation and proliferation through the production of short-chain fatty acids. They also mediate other metabolic effects such as the syntheses of vitamins like biotin and folate as well as absorption of ions including Magnesium, Calcium and Iron.

Bacteria are also important in preventing allergies, an overreaction of the immune system to non-harmful antigens. Studies on the gut flora of infants and young children have shown that those who have or later develop allergies have different compositions of gut flora from those without allergies. These allergenic populations have higher chances of having the harmful species *C. difficile* and *S. aureus* and lower prevalence of *Bacteroides* and *Bifidobacteria* in their gut. One explanation is that since helpful gut flora stimulates the immune system and "trains" it to respond to antigens, a lack of these bacteria in early life leads to an inadequately trained immune system which overreacts to antigens. On the other hand, the differences in flora could also be a result, not a cause, of the allergies.

The effects of the normal flora are generally derived from experimental comparisons between "germ-free" animals (which are not colonized by any microbes) and conventional animals (which are colonized with a typical normal flora). Some of the characteristics of germ-free animals that are thought to be due to lack of exposure to a normal flora are vitamin deficiency (especially Vitamin K and B12), increased susceptibility to infectious disease, poorly developed immune system (especially in the gastrointestinal tract), lack of "natural antibody" or natural immunity to bacterial infection. These conditions in germ-free experimental animals do not occur in conventional population because of natural introduction of a bacterial flora at appropriate times of development.

## Harmful Effects of the Normal Flora

Bacteria residing in the digestive tract can also have pathogenic effects in addition to their health-inducing ones. They can produce toxins and carcinogens and have been implicated in such conditions as multisystem organ failure, sepsis and colon cancer. So, an important factor in maintaining health is the balance of bacterial numbers; if the numbers grow too high or low, it will result in harm to the host. The host has also developed enzymes to regulate this balance.

It is important to review the harmful effects under different categories. Bacterial synergism between a member of the normal flora and a potential pathogen is the foremost category. Here, one organism is helping another to grow or survive. There are examples of a member of the normal flora supplying a vitamin or some other growth factor that a pathogen needs in order to grow. This is called cross-feeding between microbes. A typical example of this kind of synergism is seen during treatment of "staph-protected infections" when a penicillin-resistant staphylococcus that is a component of the normal flora shares its drug resistance with pathogens that are otherwise susceptible to the drug. Competition for nutrients is another harmful effect of the human flora. Bacteria in the gastrointestinal tract do absorb some of the host's nutrients for their own needs and transform them into other metabolic compounds, so some nutrient(s) may be lost to the host. Germ-free animals are known to grow more rapidly and efficiently than conventional animals. The practice of incorporating antibiotics into farm animals for faster growth contributes to the development and spread of bacterial antibiotic

resistance within the farm animals, as well as humans. Another established effect is the induction of low grade bacterial toxins (e.g. endotoxin) in the circulation as result of floral population. However, the effects of these toxigenic antigens are beneficial as it is these small amounts of bacterial antigen that stimulate the formation of natural antibodies.

Members of the normal flora may cause endogenous disease if they reach a site or tissue where they cannot be restricted or tolerated by the host defenses. Many of the members are potential pathogens, and if they gain access to an imuno-compromised tissue from which they can invade, disease may result. Some pathogens of humans that are members of the normal flora may also rely on their host for transfer to other individuals where they can produce disease. This includes the pathogens that colonize the upper respiratory tract such as *Neisseria meningitidis, Streptococcus pneumoniae, Haemophilus influenzae* and *Staphylococcus aureus*, and potential pathogens such as *E. coli, Salmonella* or *Clostridium* in the gastrointestinal tract.

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