

Metagenomic Study of Human Gastrointestinal Tracts in Health and Diseases

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ABSTRACT

Metagenomics is a new emerged technology used to investigate the microbial diversity in an environment and to study their function in a given environment. The Human Microbiome Project is a global project to research diversity of microbial communities associated with human body and to investigate their effects on physiology of organs. These studies also are interested to link between the existence of a microbial population and several inflammatory, physiological, metabolic and psychological disorders. Based on metagenomic studies, this paper aims to review the newest information regarding microbial diversity of the intestinal tracts and their physiologic and/or pathologic influences on human body.

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Key words: Metagenomics; Biodiversity; Intestinal flora; Malnutrition; Physiological disorders; Psychological disorders

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INTRODUCTION

Employment of new emerged detecting technologies, referred to as metagenomics, has changed our view of microbial diversity in different habitats, including the micro-flora of gastrointestinal

tracts. Metagenomics is an approach in which whole genome contents of a community of (micro) organisms in a niche of interest is investigated in order to detect its microbial diversity and also to study special trait(s) of the habitat^[1,2]. This technique employs the hyper-variable sections of special marker genes, especially the 16S ribosomal RNA (rRNA), to identify microbial diversity. Also, this technique is able to detect the functional ability of existing microorganisms in an environment based on the identified genes^[3]. The metagenomic approach can be used for any natural environment where microbial genomic sources are available. The Human Microbiome Project is an international study of the microbial communities associated with different parts of our body^[4]. Most of the existing information regarding the microbial diversity of human body and their role in different physiological functions of the gastrointestinal tract are based on *in vitro* or culture based studies. However, the new approaches have opened new windows for understanding these relationships between human cells and microbial strains.

The gastrointestinal tract consists of different parts, including mouth, esophagus, stomach, small intestine, colon and rectum. These tracts function as digestive and absorptive organ for our body and at the same time are known as a major exocrine and endocrine hormone producer and an important immune organs^[5,6]. Based on the conventional culture-based approaches, a few hundred microorganisms has been detected at different parts of gastrointestinal tracts and the population of obligate anaerobic bacteria, specially Bacteroidetes, are one thousands time more than the population of facultative organisms. However, based on culture-independent approaches 1,012 cells lives in 1g stool, consisting of over 13,000 microbial species that most of them belonged to the Firmicutes and Bacteroidetes (respectively, 75% and 16%)^[7,8]. This number in some papers is suggested more than 1,800 genera and 15,000 to 36,000 species, varying based on the bacterial classification system^[9]. Only 20% of these microorganisms are detectable by conventional culture-based methods^[7]. Although the diversity may differ in each individual person or society, the ecological niche of the microbial combination is similar^[10]. In this paper, diversity and function of normal flora of the gastrointestinal tracts is reviewed based on the newest information obtained from culture independent technologies.

THE NORMAL FLORA OF INTESTINAL TRACT AT BIRTH

While intestinal tract is sterile at the birth, it may be contaminated through birth^[11]. Although based on researches in 1990s, mode of delivery (normal vaginal birth or caesarean section) had no significant effect(s) on the normal flora of the neonatal intestinal tracts^[12,13], nowadays researchers believe in an entirely different way. In normal vaginal delivery, the neonate is affected by both mother's vaginal and intestinal flora, and the neonate's gut is colonized predominantly by *E. coli*, Enterococci, *Bifidobacterium*, *Lactobacillus* and *Prevotella*^[14-16]. However, since the neonates delivered via caesarean mode are not exposed to the mother's vaginal/intestinal flora, their intestinal microbial composition is similar to the microbial flora of skin, consisting of a lower microbial diversity in which *Staphylococcus* is a dominant population^[14,15].

In a culture based research, it has been shown that the mode of feeding has fundamental effects on the normal flora of the intestine at the first days of birth^[17]. Approximately 24 hours after a normal birth, the intestinal tract acquire some microorganisms originated from mother's vagina or her intestinal tracts through delivery or taking milk^[18]. Regardless of mode of feeding, the intestine is occupied mostly by *E. coli*, also some other enterobacteriaceae and gram-positive cocci by the end of first week^[12]. Microarray based investigations showed that the microbial population of intestine is formed after one week, but the flora will not reach to its equilibrium by the end of infancy^[18]. These flora constitutes mainly of *Bifidobacterium* sp., *E. coli*, *Enterococcus* sp., *Streptococcus* sp., *Staphylococcus* sp., *Actinomyces* sp., *Clostridium* sp. and *Bacteroides* sp.^[19,20]. It has been shown that roughly 6 day after birth the intestinal tracts of breastfed and bottle-fed infants were dominantly colonized by Bifidobacteria and enterobacteriaceae, respectively. These conditions continued by the end of one month when Bifidobacteria were the dominant bacteria in both groups. In this age, however, the number of Bifidobacteria in the stool of bottle-fed infants was 1/10 in comparison to breast-fed infants^[17]. In a study performed based on 16S rRNA amplification, *Bifidobacterium* sp. was dominant member of the fecal samples taken from infants below four month old, and the number of this bacteria raised from 45% in eight days old infants to 64% in 117 days old infants^[21]. It is important to be mentioned that since nutrition shortage affects directly mother's normal flora and her immune system^[22], it is suggested that malnutrition before and after delivery might change indirectly the neonate's microbiota. Furthermore, this condition affects milk components in terms of nutrients and different immunoactive compounds such as antimicrobial enzymes, Interleukin 10 (IL10) and several growth factors^[22].

THE ORAL CAVITY

The oral cavity provides different special habits where vast variety of microorganisms are colonized^[23]. Based on information in the Human Oral Microbiome Database (<http://www.homd.org/>) approximately 688 species of prokaryotes, including only one species of archaea (*Methanobrevibacter oralis*), have been characterized that only 49% of them are known microorganisms. These bacteria belong to different phyla, including Firmicutes (33.9%), Proteobacteria (16.57%), Bacteroidetes (17.73%), Actinobacteria (13.23%), Spirochaetes (7.12%), Fusobacteria (5.38%), TM7 (1.75%), Tenericutes (1.6%), Synergistetes (1.45%), SR1 (0.44%), GN02 (0.44%), Chloroflexi (0.15%) and Chlamydiae (0.15%) (Table 1)^[24]. The composition and abundance of microorganisms in different part of oral cavity is varied.

For instance, hard palate, keratinized gingiva and buccal mucosa are occupied with Firmicutes (mostly *Streptococcus* sp. and *Gemella* sp.) followed by Proteobacteria, Bacteroidetes, Actinobacteria and Fusobacteria in a diminishing order^[25]. Also, throat, tonsils, tongue and saliva are inhabited mostly by Firmicutes, especially *Streptococcus* sp., *Veillonella* sp. and Lachnospiraceae (*Oribacterium* sp. and *Catonella* sp.) followed by Bacteroidetes (*Prevotella* sp.), *Neisseria* sp., Fusobacteria (*Fusobacterium* sp. and *Leptotrichia* sp.), Actinobacteria (*Actinomyces* sp.) and TM7 in a diminishing order. However, the abundance of Firmicutes in the plaques formed on both supra-and sub-gingival habitats is decreased but the number of Actinobacteria shows a significant increase^[25]. In addition to the strains found in the throat and tongue, these last habitat are occupied by *Rothia* sp., *Corynebacterium* sp., *Porphyromonas* sp. and *Capnocytophaga* sp.^[25]. The pathogenic microorganisms involved in oral and systemic infections like *Treponema* sp., *Aggregatibacter* sp., *Megasphaera* sp., *S. pyogenes*, *S. pneumoniae*, *H. influenzae* and *N. meningitidis* are also found in those area in low abundance^[25].

ESOPHAGUS

The esophagus is a unique part of gastrointestinal tracts since foods are not depositing in the tract. Based on the culture dependent methods using the samples given from luminal washes of the tract, most researches showed a sterile environment where sometimes is contaminated by the transit microorganisms originating from upstairs or downstairs organs^[26] like Herpesvirus, *Cryptococcus* sp. and *Candida* sp.^[28-31]. However, based on 16SrRNA pyrosequencing, esophagus is occupied by a stable flora consisting of same series of microorganisms as the oral cavity and nasal swab and roughly 140 species from six phylum of the microorganisms Firmicutes (mostly *Streptococcus* sp. and Veillonellaceae), Bacteroidetes (mostly *Prevotella* sp.), Actinobacteria, Proteobacteria, Fusobacteria, and TM7^[27-29]. The habitat is dominantly occupied by Streptococci (78% of the microbial population)^[27]. The composition of esophageal flora change in different physiologic and pathologic conditions, such as gastro-esophageal reflux disease (GERD) and Barrett's esophagus. In these pathologic and inflammatory conditions, the tract is more hospitable for anaerobic and microaerophilic bacteria. For instance, it has been shown that the staphylococci population is declined to 29% in Barrett's esophagus^[27]. Also, 16sRNA assay showed that the normal flora of this area is changed from 10 to 5 species in the Barrett's esophagus and 17 species in the reflux esophagitis patients^[30].

THE STOMACH

The acid secretion into the stomach makes the tract as special micro-ecosystems, including the gastric mucosa-associated and the gastric juice-associated habitats, where many bacteria cannot survive. Although many microorganisms have been found in the habitats, many of them are transient and came by swallowed foods. Based on 16SrRNA sequencing, however, several acid resistant microorganisms like *Helicobacter* sp., *Prevotella* sp., *Lactobacillus* sp., *Streptococcus* sp., *Enterococcus* sp., *Staphylococcus* sp., *Stomatococcus* sp., *Pseudomonas* sp., *Rothia* sp. and *Neisseria* sp., originated from both upper and lower intestinal tracts, are normally isolated from these areas^[31]. The number of microorganisms inhabited in the stomach is increasing by using new metagenomic approaches, and up to now it is claimed that 13 classes of microorganisms, from 262 phylotypes, live in the stomach. Despite a relatively stable flora inhabited in the gastric mucosa-associated, micro-flora of gastric juice-associated environment is affected by the swallowed foods^[32].

It is believed that the non-*H. pylori* microorganisms in the gastric environments function as antigenic stimulators and increase the abnormality caused by *H. pylori*^[33]. Furthermore, based on animal studies, it has been suggested that intragastric flora assist *H. pylori* to cause gastric cancer as a result of increase in repairing rate of mucosal injury, and therefore, due to increase in cellular replication^[34,35]. Also, activity of some microorganisms in the area, such as *Eubacterium limosum*, enhance colonization of *H. pylori* in the area^[36,37]. However, the relationship between *H. pylori* and other gastric microorganisms is not always friendly. For instance, several studies have shown that probiotic microorganisms, such as *Bifidobacterium*

sp., *Lactobacillus sp.* and *Saccharomyces sp.* are able to decline the inflammation caused by *H. pylori* through prevention of microbial colonization, eradication of *H. pylori*, decrease the side effects caused by administration of antibiotics and finally by reduction of relapse rates^[38-40]. On the other hand, stabilization of *H. pylori* in the gastric environments amends distribution and number of lactobacilli^[41].

INTESTINAL TRACTS

The small intestine consists histologically of three different parts, namely duodenum, jejunum and ileum. Due to inhibitory effects

Table 1 List of the normal flora of human oral cavity based on molecular approaches (the data have been taken from <http://www.homd.org/>).

Domain	Phylum	Class	Family	Most famous genera or species		
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriaceae	Methanobrevibacter oralis		
Bacteria	Actinobacteria	Actinobacteria	Actinomycetaceae, Dietziaceae, Bifidobacteriaceae, Corynebacteriaceae, Dermacoccaceae, Micrococcaceae, Mycobacteriaceae, Propionibacteriaceae, Sanguibacteraceae, Bifidobacteriaceae, Coriobacteriaceae, Microbacteriaceae	Actinomyces, Dietzia, Bifidobacterium, Scardovia sp., Parascardovia sp., Corynebacterium diphtheria, Rothia, Mycobacterium leprae, M. neoaurum, M. tuberculosis, Propionibacterium, Bifidobacterium, Gardnerella, Atopobium sp., Microbacterium sp., Olsenella sp., Slackia sp.,		
			Bacteroidetes	Bacteroidaceae, Bacteroidales [F-2], Bacteroidales [F-3], Porphyromonadaceae, Prevotellaceae, Bacteroidetes[F-1], Bacteroidetes[F-2]	Bacteroides, Porphyromonas, Prevotella	
	Chlamydiae	Chlamydiae	Chlamydiaceae	Chlamydiaceae	Chlamydomphila pneumoniae	
					Chloroflexi	Chloroflexi[C-1]
	Firmicutes	Bacilli	Bacilli	Bacillaceae, Listeriaceae, Paenibacillaceae, Staphylococcaceae, Aerococcaceae, Carnobacteriaceae, Enterococcaceae, Lactobacillaceae, Streptococcaceae	Bacillus anthracis, B. subtilis, Listeria monocytogenes, Paenibacillus sp., Gemella, Streptococcus epidermidis, S. aureus, Enterococcus durans, E. faecalis, lactobacillus acidophilus, L. salivarius, L. vaginalis, L. casei, L. lactis, Oribacterium sp., S. Pneumoniae, S. pyogenes, S. salivarius, S. sanguinis	
				Clostridia	Clostridiales[F-1], Clostridiales[F-2], Clostridiales[F-3], Eubacteriaceae[15], Lachnospiraceae [14], Peptococcaceae, Peptostreptococcaceae [11], Peptostreptococcaceae [13], Syntrophomonadaceae [8], Veillonellaceae	Clostridium sp., Eubacterium, Mogibacterium, Peptococcus, Johnsonella sp., Shuttleworthia sp., Peptostreptococcus, Peptoniphilus, Parvimonas, Filifactor, Veillonella, Anaerococcus sp
		Erysipelotrichi	Erysipelotrichi	Erysipelotrichaceae	Erysipelotrichaceae	Erysipelothrix, Solobacterium
						Negativicutes
		Fusobacteria	Fusobacteria	Fusobacteria	Veillonellaceae	Megasphaera sp., Selenomonas
					Fusobacteriaceae, Leptotrichiaceae	Fusobacterium, Leptotrichia
		GN02	GN02	GN02[C-1]	GN02[F-1]	
				GN02[C-2]	GN02[F-1]	
		Proteobacteria	Alphaproteobacteria	Alphaproteobacteria	Caulobacteraceae, Bartonellaceae, Bradyrhizobiaceae, Brucellaceae, Phyllobacteriaceae, Rhodobacteraceae, Rhizobiaceae, Sphingomonadaceae	Caulobacter, Bartonella, Bradyrhizobium, Agrobacterium, Rhizobium, Rhodobacter, Sphingomonas
					Betaproteobacteria	Alcaligenaceae, Burkholderiaceae, Comamonadaceae, Ralstoniaceae, Neisseriaceae, Rhodocyclaceae
	Deltaproteobacteria		Deltaproteobacteria	Bdellovibrionaceae, Desulfomicrobiaceae, Desulfovibrionaceae	Bdellovibrio, Desulfobulbus, Desulfomicrobium, Desulfobivrio	
	Epsilonproteobacteria		Epsilonproteobacteria	Campylobacteraceae, Helicobacteraceae	Bacteroides, Campylobacter, H. pylori	
	Gammaproteobacteria		Gammaproteobacteria	Cardiobacteriaceae, Enterobacteriaceae, Pasteurellaceae, Moraxellaceae, Xanthomonadaceae	Enterobacter, E. coli, P. mirabilis, Klebsiella sp., Yersinia pestis, Haemophilus aegyptius, H. ducreyi, H. haemolyticus, H. influenzae, H. parainfluenzae, Aggregatibacter actinomycetemcomitans, Acinetobacter, M. catarrhalis, Pseudomonas aeruginosa, P. fluorescens, Xanthomonas	
Spirochaetes	Spirochaetes	Spirochaetes	Spirochaetaceae	Treponema vincentii, T. denticola		
SR1	SR1	SR1	SR1[F-1]			
Synergistetes	Synergistetes	Synergistetes	Synergistetes[F-1], Synergistetes[F-2]	Synergistetes bacterium SGP1		
Tenericutes	Mollicutes	Mollicutes	Mollicutes[F-1], Mollicutes[F-2], Mycoplasmataceae	M. pneumoniae, M. salivarius, M. fermentans		
TM7	TM7	TM7	TM7[F-1], TM7[F-2]			

caused by acidic pH (of gastric juices), bile salts (released from gallbladder), IgA (immunoglobulin A), the antimicrobial compounds (released from small intestine like C-type lectins, cathelicidins and defensins), little amounts of microbes (less than 10³) have been isolated from duodenum^[42-44]. In addition, several physiological conditions, such as peristalsis movement of the tract, desquamation of epithelial cells and mucosal flow speed up the transient period of foods through the tract (between 30 minute to 3.5 hours) and thus wash the microbial cells up from this tracts^[45]. The number of microorganisms raises through the small intestine and reach to 10⁸ microorganisms per milliliter intestinal juice due to decrease in the level of the inhibitory factors^[46]. For instance, alkaline secretions from pancreas lead to decrease of pH profile in a healthy person from 5.5-7.0 in the duodenum to 6.5-7.5 in the ileum^[47]. Furthermore, metagenomic studies showed that the bile salt hydrolysis (BSH) activities enhance bacterial resistance to the Conjugated bile acids (CBAs), facilitating their colonization in the gut^[48].

Based on the NIH human microbiome project (HMP), the microbiota in oral cavity is more diverse than small and large intestines. In the other word, no members of Chlamydiae, Chloroflexi, GN02, Spirochaetes, SR1, Tenericutes and TM7 as

well as the members of Alpha proteobacteria have been found in the healthy human gut (Table 2) (<http://www.hmpdacc.org/catalog/>). In upper parts of the small intestine, most habitats are occupied by acid tolerant streptococci and lactobacilli^[49]. Due to the physical, chemical and physiological changes through the small intestine, the dominant members of microbial community in jejunum shifts to class bacilli, mostly Streptococci, and Proteobacteria^[46]. Normal flora of the ileum is similar to colon and contains more than 1,013 microbes, consisting of 500 different species^[50]. While the Enterobacteriaceae are dominant microorganisms within the lumen of small intestine, the majority of microorganisms identified in this intestinal mucosa of this area consist of the Clostridiales clusters XIV, XI and IV and Bacteroidetes^[51-53].

Due to low concentration of the inhibitory factors, lack of Peyer's patches and lower peristalsis of the large intestinal tract (and longer retention time of food in the tract), the large intestine (colon) contains higher microbial population than small intestine^[54]. The colon is populated with a big community (1×10¹³-1×10¹⁴ cells per gram stool) of microorganisms (approximately 10-30 percentage of stool weight), consisting of more than 500 species and around 2×10⁶ genes (100 times more than the human genome)^[55,56]. Normal flora

Table 2 List of the normal flora of the human gut based on molecular approaches (the data have been taken from <http://www.hmpdacc.org/catalog/>).

Domain	Phylum	Class	Family	Genus			
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriaceae	M. smithii			
	Bacteria	Actinobacteria	Bifidobacteriaceae	Bifidobacterium sp.			
Coriobacteriaceae			Collinsella sp., Eggerthella sp.,				
Corynebacteriaceae			Corynebacterium ammoniagenes				
Propionibacteriaceae			Propionibacterium sp., Gordonibacter sp., Slackia sp.				
Bacteroidetes		Bacteroidia	Prevotellaceae	Prevotella sp., Paraprevotella sp.			
			Bacteroidaceae	Bacteroides sp., Parabacteroides sp.			
			Porphyromonadaceae	Porphyromonas sp., Dysgonomonas sp., Tannerella sp., Barnesiella sp.			
			Rikenellaceae	Alistipes sp.			
			Bacillaceae	Bacillus sp.			
			Streptococcaceae	Streptococcus sp.			
Bacteria	Firmicutes	Bacilli	Enterococcaceae	Enterococcus sp., E. saccharolyticus			
			Lactobacillaceae	Lactobacillus sp., Pediococcus acidilactici			
			Leuconostocaceae	Leuconostoc mesenteroides, Weissella sp.			
			Listeriaceae	Listeria grayi			
			Paenibacillaceae	Paenibacillus sp.			
			Lachnospiraceae	Coprococcus sp., Blautia sp., Roseburia sp., Dorea sp., Butyrivibrio sp., Anaerostipes sp., Marvinbryantia sp.			
			Clostridiaceae	Clostridium sp.,			
			Unclassified Clostridia	Flavonifractor sp.,			
			Ruminococcaceae	Eubacterium sp., Cyndroides sp., Anaerofustis sp.			
			Eubacteriaceae	Peptococcus, Desulfotobacterium			
	Firmicutes	Clostridia	Peptococcaceae	Ruminococcus sp., Faecalibacterium sp., Anaerotruncus sp., Subdoligranulum sp.			
			Peptostreptococcaceae				
			Clostridiales Family XI	Anaerococcus sp., Parvimonas sp., Finegoldia sp.			
			Incertae Sedis,				
			Erysipelotrichi	Erysipelotrichaceae	Turicibacter sp., Coprobacillus sp., Catenibacterium sp., Holdemania sp.		
			Negativicutes	Veillonellaceae	Veillonella sp., Megamonas sp., Mitsuokella sp., Dialister sp.		
				Acidaminococcaceae	Acidaminococcus sp., Phascolarctobacterium sp.		
			Fusobacteria	Fusobacteriia	Fusobacteriaceae	Fusobacterium sp.	
			Bacteria	Proteobacteria	Betaproteobacteria	Sutterellaceae	Sutterella parvirubra
						Oxalobacteraceae	Oxalobacter sp.
Burkholderiaceae	Neisseriaceae	Neisseria macacae					
Sutterellaceae	Burkholderiaceae	Ralstonia sp.					
Deltaproteobacteria	Sutterellaceae	Parasutterella excrementihominis					
Epsilonproteobacteria	Desulfobivriaceae	Desulfobivrio piger, Bilophila wadsworthia					
	Helicobacteraceae	H. pylori, H. cinaedi, H. pullorum, H. winhamensis					
Gammaproteobacteria	Campylobacteraceae	Campylobacter sp., Arcobacter butzleri					
	Moraxellaceae	Acinetobacter radioresistens					
	Enterobacteriaceae	E.coli, Providencia sp., Citrobacter sp., Hafnia, Klebsiella sp., Edwardsiella, Proteus sp., Enterobacter sp., Shigella sp, Cedecea sp., Raoultella					
	Succinivibrionaceae	Succinatimonas sp.					
	Desulfobivriaceae	Desulfobivrio sp., Bilophila sp.					
	Pseudomonadaceae	Pseudomonas sp.					
Synergistetes	Synergistia	Synergistaceae	Synergistes sp., Anaerobaculum hydrogeniformans				

of stool consists frequently of Bacteroidetes (especially *Bacteroides* sp.), Firmicutes, Proteobacteria, Actinobacteria and Fusobacteria in decreasing order^[25]. However, 30-40 species of microbial population in stools constitute 99% of the population. Although the habitat is occupied mainly by Firmicutes (clusters IX, XIV, and XVI) and Bacteroidetes (35%) (especially, *Bacteroides*, *Prevotella* and *Faecalibacterium*), other bacterial phyla, including Proteobacteria (13-15%) and Fusobacteria (7-8%), Actinobacteria, Verrucomicrobia as well as few archaea have been also found in this tract^[7,57,58].

Furthermore, the habitat is occupied by approximately 50 fungal phylogroups (like *Saccharomyces* sp., *Galactomyces* sp., *Penicillium* sp., *Candida* sp., *Gloeotinia* sp. and *Paecilomyces* sp.)^[59,60]. Based on 18S rRNA studies, *Blastocystis* sp subtypes II, III and IV are dominant fungi in the distal intestinal tract^[60]. While few numbers of protozoans have been adapted themselves to live in host associated niches, these microorganisms can be found in a vast variety of vertebrate hosts. By now, several types of parasite or commensal protozoa like *Endolimax* sp., *Entamoeba histolytica*, *Entamoeba coli*, *Entamoeba invadens*, *Iodamoeba* sp., *Trichomonas hominis*, *Chilomastix* sp., *Pentatrichomonas* sp. and *Giardia intestinalis* have been isolated in human gut^[61,62]. Although these protozoa are distributed worldwide, their prevalence in a geographic area is mostly dependent to poor sanitation of inhabitants^[63]. Also, while existence of Cyanobacteria in human gut has been proven by molecular studies, no cyanobacterial strain has been isolated yet^[64]. Methanobrevibacter smithii and Methanosphaera stadtmanae are the only confirmed archaea isolated from human gut. However, some other archaea like Methanobrevibacter oralis (the only isolated archaea from saliva), Methanosarcina, Stadtmanae and Methanosphaera have been occasionally isolated from these area^[65]. Also, presence of low abundant of some pathogenic microorganisms, such as *H. pylori* in the lower intestinal tracts has been proven^[25]. Despite the homology between bacterial population of the intestine, intestinal virome is unique to each person and 95% of this viral diversity is retained in individuals over times^[61].

Based on the experiments conducted with new emerged techniques, the intestinal microbiome participate in several activities, including normal cell differentiation and development of the intestinal tracts, tissue homeostasis, natural defence system against pathogenic microorganisms of the tract, host energy metabolism and as an effective facilitating factor for metabolism of different swallowed foods, complex hydrocarbons and fibres^[6,66,67]. These flora assist the body to keep its health as a result of production of short chain alcohols and acids, production of vitamin K and B family, alteration in bile salt composition, degradation of food and preparation of a better condition for adsorption of nutrients by intestinal cells^[11,66]. Although each society may show a special microbial diversity profile in their intestinal tracts, there is a core gut microbiome in all investigated societies which totally harbour a similar collection of genes. Indeed, as far as a diversity of microorganisms is able to provide the special niche required in a healthy intestinal tracts, it can efficiently work for the body. However, the slight alterations in the phylotype composition of a person, due to excitement, hunger, nutrition, antibiotic treatment and infection, leads to an unique ecological condition that may intensify susceptibility of a person to infectious or physiological disorders^[10,20,57].

By now, several physiological features like energy metabolism^[11,68,69], degradation of xenobiotics and drugs^[70,71], cell differentiation and development^[6,72], maturation and activity of immune system^[73,74] and the host response to damages in the intestinal epithelial cells have been attributed to the gut microbiome^[75,76]. For

instance, the intestinal tracts of germ-free mice showed abnormal long intestinal villi^[6,72], abnormal enlarged cecum and altered gastrointestinal motion^[6]. In this hypothesis, the SCFAs (short chain fatty acids) produced by commensal bacteria play as an immune system modular through two different mechanism: activation of cellular GPRs (G-coupled-protein-receptor) and inactivation of histone deacetylase (HDAC), thus leading to the immunological responses seen in autoimmune disorders such as atopy and asthma^[77,78]. Based on studies on germ free rats, the commensal bacteria are essential for development of gastrointestinal-associated lymphoid tissue (GALT) and IECs (intestinal epithelial cells) in the small intestine^[73,74]. The IECs use TLRs (Toll Like Receptors), PRPs (Pattern Recognition Receptors) and NLRs (Nod Like Receptors) to differentiate pathogens from commensal microorganism^[76]. Since the constitution of microbial society in the intestinal tracts of preterm neonates who are kept in incubator is delayed in compare to the healthy neonates, the intestinal tract of these infants show slow peristalsis and the neonates are severely susceptible to different intestinal infectious disorders^[79]. Also, it has been shown that the intestine of germ free mice do not produce angiogenin 4, delaying the formation of villus capillaries in their small intestine^[68,80]. Furthermore, it has been shown that the intestinal flora are able to induce production of the transcription factor NF- κ B^[81]. The NF- κ B released into the intestinal tracts induces expression of a variety of genes involved in cell proliferation, cell differentiation and pro-inflammatory responses through infection. Overexpression or deficiency of these factors may lead to occurrence of several pathogenic conditions like obesity and chronic IBD (inflammatory bowel disease)^[82-84].

Since hosts live with this flora for millions of years and different types of ecological relationship have been established between host and the microorganisms, it is suggested that any factor that affects these relationships might lead to pathologic conditions. Overall, the balance in population of microbial flora is critical for our health and according to hygiene hypothesis, it is believed that imbalanced microbial population in our body due to improvement in public health is a potential etiological factor for several intestinal pathogenic situations like inflammatory bowel diseases (IBDs), circulatory disease, obesity and autism to the microbiota, atopy and asthma^[85-92]. Studies on several intestinal associated diseases or even many systemic disorders have indicated intensive changes in microbial diversity and their composition. For instance, studies on CD (Coeliac disease) showed that the microbial diversity, especially in the proportion of members of Firmicutes decreased significantly while there were highly increases in the number of bacterial population, particularly in Gram negative bacteria. At the same time, the ratio of *Bacteroides-E. coli* (shigella) to *Lactobacillus-Bifidobacterium* was higher in these patients^[90-92]. It has been shown that overgrowth of *E. coli* in the gut lead to LPS-TLR4 (Toll like receptor 4) signalling in the gut and therefore to trigger an inflammatory response^[93]. Antibiotic associated disorders are well-known clinical problems in which oral administration of some broad-spectrum antibiotic, such as clindamycin, ampicillin, neomycin, erythromycin, metronidazole and cephalosporins alter temporarily the aerobic flora of intestinal tract mostly to Clostridiales order like *Subdoligranulum*, *Acetivibrio*, *Butyricoccus*, *Dorea*, *Collinsella*, leading to severe intestinal diseases such as antibiotic-associated diarrhea and pseudomembranous colitis^[94].

Although it has not been clearly proven, intestinal microorganisms can function as a potent etiology of colorectal cancer (CRC) due to release of free radicals and genotoxins and also as a result of

induction of Th dependent cell proliferation and TLR dependent procarcinogenic mechanisms^[95]. Based on 16S rDNA DGGE analysis, a significant increase in diversity of microorganisms, especially in *Clostridium coccooides* and *Clostridium leptum*, was recorded in the patients with polyposis and CRC (colorectal cancer). Increase in the number of sulphate reducing bacteria and production of H₂S (a cytotoxic factor) and at the same time, decrease in the number of sulphide oxidizing bacteria, such as *Desulfovibrio*, has been suggested as a probable etiological factor of CRD and polyposis^[96,97]. In a separate research, increases in the levels of some probiotic bacteria, like *Faecalibacterium*, *Fusobacterium*, *Roseburia* and *Coriobacteridae* and a decrease in the level of enterobacteriaceae were observed within the gut of CRC afflicted patients^[98].

Nowadays, researches regarding the effect(s) of gut microbial composition on psychology (mental health and mood) of an individual are progressively reported. The intestinal flora can influence development of brain cells and personal behaviors^[99]. It has been proven that any alteration in the probiotic bacterial composition can directly affect the neurochemical secretions outside the gut. An intensified response in the hypothalamic-pituitary-adrenal axis to stresses, and at the same time, meaningful reductions in the production of brain-derived neurotrophic factors by the hippocampus and the cortex are proven through investigation on experimental animals^[100,101]. Furthermore, a study on GF mice showed that the gut microbial flora affect the post-natal development of brain, control of brain motor and occurrence of an anxiety-like behavior by modulation of synaptophysin and PSD-95 (two critical glycoproteins involved in maturation of neuronal synapses)^[102,103]. The gut microbial community might also affect the neuronal development and person's behaviour by modulation neuronal transmitters such as acetylcholine, GABA (gamma-aminobutyric acid), melatonin, serotonin and histamines) within the intestinal tracts^[102,103]. Based on the gut-brain communication hypothesis, it appears that gut microbial composition is closely associated with the psychiatric disorders such as depression^[6]. Autism is the best known neural disorder associated with alteration of gut microbiota where the number of (spore forming) clostridia, specially *Clostridium bolteae* is meaningfully increase^[104,105]. Based on this theory, it is easy to describe the reason of family involvement to the autism and the cause of its relapse after the treatment is cut^[105]. Furthermore, it has been indicated that roughly 30% of the patients suffering of Major depressive disorder (MDD) are afflicted by irritable bowel syndrome (IBS)^[106]. As it has been mentioned, IBS itself is caused partly by increase in the number of aerobic bacteria. It appears that the number of aerobic bacteria in the intestinal tract is critical and increase in the number of these bacteria can be associated with different physiological and psychological disorders. In addition to the case of MDD, it has been shown that the severity of neurological signs in fibromyalgia (FM) and Chronic Fatigue Syndrome (CFS) is directly associated with the abundance of these group of bacteria in the gut^[107-110]. However, it is important to be clarified whether the psychological disorders are caused as a result of alteration in the composition of bacteria or inversely, the changes are a side effect of the diseases due to change in dietary regimen, physical inactivity or any changes in the level of secretion of digestive factors such as acids, bile salts and enzymes. It has been proven that the cytokines involved in depressive symptoms, like TNF α (tumour necrosis factor alpha)^[111,112] and IL-1 β (interleukin1-beta)^[113] decrease the secretion of HCl in the stomach. On the other hand, it is necessary to be mentioned that the signs of fatigue were reduced in the patients suffering from CFS who took a tablet containing *Lactobacillus casei* strain Shirota (LcS)^[100].

It also has been shown that composition and distribution of microbial flora in gastrointestinal tracts is directly associated with absorption of nutrients and establishment of malnutrition^[11,68,69]. For instance, extra-proliferation of some bacteria, such as *Bacteroides*, *Clostridia* and *Enterococci* are attributed to several disorders such as megaloblastic anemia (due to absorption of vitamin B12)^[114], steatorrhea (due to imbalance absorption of fatty acids and monoglycerides)^[115], reduction of the gastric acidity and deficiency in digestive enzymes^[116], obesity^[117-119], bowel cancer^[120] and allergic disease^[121]. studies on malnourished children showed significant increases in the population of *Campylobacteraceae*, *Helicobacteraceae*, *Bacteroidaceae* and *Porphyromonadaceae*^[122].

In contrast, the gut of healthy children was mostly enriched by *Actinomycetales*, *Burkholderiales*, *Halobacteriales*, *Planctomycetales*, *Bifidobacteriales*, *Pseudomonadales*, *Enterobacteriales*, *Chloroflexales*, *Desulfovibrionales*, *Xanthomonadales*, *Lactobacillales*, *Rhizobiales*, *Planctomycetales* and *Clostridiales*, in a diminishing order^[122]. These changes are accomplished with several relapsing gastrointestinal infections, weight loss and growth impairment in the malnourished children^[123-127]. Also, it has been indicated that majority of the microbial genes involved in obesity were derived from Firmicutes (25%) and Actinobacteria (75%), while the majority of genes involved in leans was mainly derived from Bacteroidetes (42%)^[10]. Such these changes have been demonstrated in the microbiome of obese and lean twins^[128] and researchers were able to transfer the obesity phenotype from obese mouse to lean animals^[117].

A direct correlation between the resident microbial diversity of HBF (human baby flora) and host metabolic profiles has also indicated in several studies. For instance, it has been indicated that colonization of microorganism in the intestine leads to increase in the level of phenyl-containing amino acids. Colonization of *Clostridium sporogenes* in the gut is also associated with increase in the level of indole-3-propionic acid in host serum^[102,129]. Furthermore, based on investigations on HBF inoculated mice, higher levels of myo-inositol and ethanolamine in the duodenum, higher concentration of betaine and taurine in the ileum, higher glutathione level in the jejunum and lower concentrations of myo-inositol and taurine in the colon were measured in compare to other regions of their gut^[130]. The metabolic ability of microorganisms can affect the bioavailability of several medicines like simvastatin (used for decreasing the serum levels of cholesterol), salicylazosulfapyridine (administrated for ulcerative colitis), l-Dopa (for treatment of Parkinson) and digoxin (administrated for treatment of congestive heart failure)^[131]. Simvastatin, for instance, down-regulate production of hepatic cholesterol through inhibition of the activity of HMG-COA (3-hydroxy-3-methylglutaryl coenzyme A). A Hydroxylation/dehydroxylation, methylation and beta-oxidative activity applied by intestinal microorganisms convert simvastatin into 2-hydroxyisovaleric acid and some other simple organic acids, leading to decrease the drug bioavailability^[132].

CONCLUSION

Before application of metagenomic approaches for environmental samples, our knowledge was limited to some effects of microbial communities on that environment. However, metagenomic approaches and bioinformatic technologies enable researchers to find the exact microbial diversity and gene composition of an environment and to investigate effects of the genes on the environment. The outcomes of these researches, based on modulation of gut microbiome, are potentially applicable for therapeutic

approaches, like antibiotic therapy, bacteriophage therapy and probiotic methods used not only for intestinal disorder also for some physiological abnormalities caused directly or indirectly by the gut microbiota^[133-135]. Probiotic therapies, for instance, are nowadays proposed for several gut-related illnesses within gut or outside of the tracts.

For the first time, when Metchnikoff (1970) could link between the longevity of Bulgarian peasants with normal flora of their colon, he called these organisms as probiotics. It has been proved that the intestinal microbiota consisting of saccharolytic microorganisms, including *Lactobacillus* sp., *Bifidobacteria* sp., *E. coli*, *Enterococci*, *Bacillus* sp., *Bacteroides*, *Faecalibacterium*, *Propionibacterium* and *Saccharomyces boulardii*, increase our resistance against opportunistic infections due to alteration in the balance between microbial communities of intestinal tracts^[136-138]. These microorganisms apply their probiotic activity based on metabolic excretion, immunomodulation and epigenetic modifications^[139,140]. Some of the effects exerted by probiotic microorganisms include: (a) occupation of different parts of intestine and thus, prevention of colonization and stabilization of pathogenic microorganisms^[141]; (b) production of antimicrobial compounds (such as bacteriocins, antibiotics, H₂O₂)^[142,143]; (c) detoxification of many poisons and carcinogens^[144]; (d) Regulation of immune responses to pathogens via up-regulation of anti-inflammatory modules and suppression of proinflammatory modules^[145]; (e) stimulation of local immune system (cellular immune system or or sIgA)^[146]; (f) production of degrading enzymes^[147]; (g) attenuating the activity of many microbial enzymes involved in their metabolism (like nitroreductase, azoreductase, beta-glucosidase, beta-glucuronidase, ornithine decarboxylase and tryptophanase^[148-150]; (h) attenuating the activity of many microbial enzyme involved in their virulence (eg, neuraminidase and mucinase)^[151,152]; (i) reducing the level of polyamines, indol, ammonia, nitrate and nitrite in intestinal tracts^[153-155]; (j) improvement the function of liver via stimulating secretion of bile salts^[119]; (k) adjustment the blood pressure^[156,157]; (l) increase in metabolism of cholesterol^[158,159]; (m) positive impacts on proliferation and differentiation of epithelial cells in the intestine^[160]; and (n) improvement of intestinal barrier function^[138].

It has been shown that *Lactobacillus* sp. and *Bifidobacterium* sp. play as anticancer factors in the gut. The anticancer activities are applied by stimulation of the immune system^[161], inactivation of mutagenic agents (such as nitrosamine) inhibition of the activity of microbial enzymes present in feces (such as nitroreductase) and reduction in tumor cell proliferation (such as the inhibitory role of the cell wall of lactobacilli on cellular proliferation)^[162,163]. Also, it has been indicated that probiotics improve digestive activity of intestine in several ways like production of the degrading enzymes for proteins, carbohydrates and fiber^[147], improving the permeability of intestinal cells^[164,165], reduction of the intolerance to foods, such as lactose^[166], reducing of intestinal inflammation^[167,168], treatment of intestinal disorders such as diarrhea and constipation^[169,170], treatment of malnutrition via increase in absorption of minerals and production of different vitamins (such as family B, biotin, A, E, K and folic acid)^[171,172].

Overall, presence and activity of probiotics in intestine is closely associated with human health and longevity. Consumption of certain foods such as yogurt and alcohol affect positively and negatively (respectively) on the intestinal flora bacteria. Consumption of special strains of *Lactobacillus* (or fermented milk) adjusts blood pressure via production of a tripeptide that inhibits the activity of Angiotensin; this enzyme functions in hypertension^[156,157]. Also, it has been shown that consumption of yogurt for a week can reduce the level of cholesterol

in blood via absorption of cholesterol as well as production and accumulation of hydroxyl-methyl-glutarate-coenzyme A (HMG-CoA) (an inhibitor of accumulation of HMG-CoA reductase)^[158,159]. Furthermore, several non-digestible food referred to as prebiotics such as inulin-containing oligosaccharides and fructooligosaccharides pass through the small intestine without any digestion and are able to activate selectively the bacterial duplication of bifidobacteria and lactobacilli in the large intestine^[173,174].

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