

Abstract

Microbial masses that reside on and inside human beings are collectively referred to as human microbiota or human microflora. Human beings act as heavily colonized ‘microbial depots’ right from birth. Microbial population of human microflora outnumbers human somatic cells and inhabit virtually all parts of human body. Prokaryotic and eukaryotic organisms, constituting human microbiome, possess a rich stock of genes that by far exceeds the gene pool found in the human genome. Gastrointestinal tract (GIT) acts as the most heavily colonized site of human body. Large surface area and availability of diverse nutrients makes human gut an ideal niche for the microbes to thrive and live in intestinal environment. Human gut microflora, on full development, serves as an ‘essential’ acquired organ and executes several vital functions associated with the ‘well-being’ of human host.

Firmicutes and Bacteroidetes are the major bacterial phyla that occur in human gut. Certain members representing Actinobacteria,

Proteobacteria, Verrucomicrobia and Fusobacteria also reside in GIT, but in lower proportions. Members of gut microbiota bestow several beneficial, protective and ameliorating effects on human health. Microbial members of human gut derive essential nutrients from its host and in lieu facilitate digestion and help in maintenance of intestinal equilibrium. However, gut microbiome has also been associated with certain pathogenic manifestations on disruption of intestinal balance and disturbance in gut microbial composition. Some of the major diseases associated with perturbation of gut balance (dysbiosis) include inflammatory bowel diseases (IBD), irritable bowel syndrome (IBS), colorectal cancer (CRC) and gastric cancer. Thus, human beings can be considered as ‘superorganisms’ that carry numerous microbes associated with both beneficial activities and adverse health effects.

The main purpose of this work has been to investigate the genomic and proteomic traits of human gut-associated microbes and untangle the

mysteries of complex interactions that the bacterial members exhibit with human host.

Extensive codon and amino acid usage analysis in several crucial bacterial genera like *Bifidobacterium*, *Ruminococcus* and *Helicobacter* revealed some interesting facts. Codon usage behavior of *Bifidobacterium* and *Ruminococcus* was found to be an upshot of several vital factors like compositional bias, natural selection for efficient translation, gene expression level and length of the coding sequences. Interestingly, in *Helicobacter pylori* strains AT compositional constraint appeared to be the most convincing factor contributing to observed codon usage variations. However, subtle impact of gene expression level and length of protein coding sequences was evident on the mode of codon usage in *H. pylori*. Investigation pertaining to arrangement patterns of successive synonymous codon pairs revealed a distinct tendency of employing identical codon pairs and non-identical co-tRNA codon pairs and avoiding the usage of codon pairs for non-isoaccepting tRNAs among the members of *Bifidobacterium*, *Ruminococcus* and *H. pylori*. Such a tendency was a

reflection of the strategy of the bacterial masses to enhance translation precision.

Members of *Bifidobacterium*, *Ruminococcus* and *H. pylori* were found to resemble the codon usage signatures of human host. Comparative codon usage profiling of the concerned bacterial members with human host revealed such a tendency of the gut-associated microbes. Such resemblance in codon usage patterns seem justified from the fact that these microbes co-evolve and co-exist with human host and acclimatize in human gut niche.

Amino acid usage in the members of *Bifidobacterium*, *Ruminococcus* and *H. pylori* was found to be governed by crucial determinants like hydrophobic and aromatic character of the encoded proteins and biosynthetic cost (protein energetic cost) of the encoded proteins. The fact that highly expressed gene products were found to employ less costly (in terms of protein energetic cost) amino acids, invariably in all concerned genera, might be a simple reflection of cost-minimization strategy in which a bacterium stringently minimizes the biosynthetic cost of highly expressed protein sets.

Comparative genomics and proteomics based analysis was performed on the

bacterial members of various important genera like *Ruminococcus*, *Bacteroides* and *Eubacterium*. It was markedly evident from our analysis that the bacterial masses have been well-equipped with metabolically sophisticated proteomic machineries that provide them an advantage to adapt and sustain in human gut. Microbial residents of human gut were also found to possess diverse metabolic pathways associated with carbohydrate degradation that are absent in human host. Furthermore, the bacterial masses were also found to be enriched with large array of diverse Carbohydrate-Active enZymes (CAZymes) that efficiently degrade undigested carbohydrate components of human diet. Human genome has been found to encode few CAZymes and thus, rely heavily on its gut microbial pool for proper and proficient digestion. Presence of a rich stock of carbohydrate degradation pathways and CAZymes among the gut-associated microbes paves way for successful microbial residence in human gut niche by proper utilization of the nutrient resources. Simultaneously, they also facilitate and enhance digestive abilities of human beings.

Extensive analysis of secretomes, the complete set of secretory proteins, among various bacterial members of gut microflora proved instrumental in elucidating the complex behavior of the secretory components. The secreted proteins were found to employ less costly (in terms of biosynthetic cost) amino acids and avoid the practice of aromatic and bulky amino acids unanimously among all concerned bacterial members. Such an observation could be well explained in light of the fact that protein secretion is often a 'one-way' street as the loss of secretory proteins is virtually irreparable. It would be pragmatic for the microbial systems to employ cheaper amino acids which are metabolically less taxing for the cell to produce, especially in case of secretomes that are lost permanently. Secretomes of gut-associated bacteria were found to execute several crucial biological phenomena like transport of carbohydrates and amino acids, cell motility, biogenesis of cell membranes and efficient cell signaling.

Human gastrointestinal tract provides shelter to numerous microbes. Some microorganisms bestow beneficial effects whereas, others exhibit pathogenic behavior resulting in severe

critical infections. Several strains of *Helicobacter pylori* constitute a significant part of the human intestinal microflora. Disruption of gut microbial balance results in proliferation of *H. pylori* strains that exert severe degrees of pathogenesis causing active gastritis, duodenal ulcers, and gastric cancer in human. *H. pylori* has been a subject of extensive biomedical research. Potential therapeutic targets in various *H. pylori* strains like HpB38, HpP12, HpG27, etc., have recently been accomplished. However, potential ‘druggable’ targets in *H. pylori* 35A still demands to be identified with

finesse. In this present work apt identification of drug targets in *H. pylori* 35A has been executed and has been further validated by molecular docking investigations. Proper screening methodologies resulted in a set of 9 tentative ‘druggable’ targets and a set of 95 potential ‘novel’ therapeutic targets. Druggability and molecular docking analysis of the putative targets revealed that chemical Nitrofurantoin and phytochemical D-Limonene might serve as potential lead molecules for successful drug discovery against *H. pylori* associated infections.