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The human microbiome: A hot spot of microbial horizontal gene transfer

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ABSTRACT

The human body harbors numerous microbes, and here exists a close relationship between microbes and human health. The Human Microbiome Project has generated whole genome sequences of several hundred human microbes. In this study, we identified horizontal gene transfer (HGT) events in human microbes and tried to elucidate the relationships between the gene-transferring microbes. A total of 13,514 high confidence HGT genes were identified in 308 human microbes. The horizontally transferred genes were enriched for Gene Ontology terms pertaining to catalytic functions and metabolic processes. Construction of an HGT event network suggested that the human microbes could be divided into specific communities which only partly overlap their distribution in human body. Our research suggests that human microbiome may facilitate frequent horizontal gene transfer among bacteria in human body. Awareness of HGT in human microbiome may aid our understanding of the relationship between the human microbiome and human health.

1. Introduction

The human body provides a habitat for a large number of different microorganisms, including bacteria, archaea and fungi. These microorganisms inhabit different parts of the body, including the skin, mouth, and intestinal tract. Collectively, all these different microbes make up the human microbiome. The total number of microbial cells hosted by the human body is huge, about 10 times the number of human cells in the body [1]. The gut harbors the majority of these microbes, comprising more than 1000 species and more than 100 billion microbes. The number of genes in the human microbiome is more than 100 times the number of genes in the human genome [2]. Moreover, the human microbiome is highly diverse, and the microbial community varies across different sites of the body, different people and over time [3]. Thus far, microbial community variation in the human gut has attracted far more research interest than variation at other body sites [3,4].

The human microbiome is closely related to human health. When a mixture of gut microbes sampled from normal mice was injected into the gut of germ-free mice, the body weight of the germ-free mice significantly increased under the same feeding conditions [5], suggesting that gut microbes contribute to the host's ability to digest and to absorb food and nutrition. The percentage of *Bacteroidetes* in gut microbes linearly correlates with the fat degree of mouse body weight [6]. Analysis of the gene composition of gut microbes revealed that the bile salt hydrolase (BSH) gene is enriched in the gut microbiome [7], indicating that gut microbes are well-adapted to the gut environment. Different types of food can affect the composition of gut microbes [8]. Traditional Chinese medical science has reported improvement in health through adjustment of the environmental balance of the human microbiome [9]. The human microbiome may also exert an important influence on some of the human body's physiological activities, such as increased nutrition quality, pathogen resistance, stimulation of angiogenesis, modulation of the intestinal immune system and regulation of host fat storage [4]. Recently, the human microbiome has attracted increasingly greater research interest. The Human Microbiome Project (HMP), supported by the NIH, is planned to conduct whole genome sequencing of human microbes from different body sites to understand the relationship between the human microbiome and human health [10]. Thus far, 308 human microbes have been sequenced and annotated (Mar 24, 2010 release). The genome sequence data and metagenome data are maintained and shared by the Data Analysis and Coordination Center (DACC) of the HMP [11].

A key characteristic of prokaryotes is their ability to exchange genes with each other through horizontal gene transfer (HGT) [12]. Horizontally transferred genes may have characters in the gene sequence composition that differ from the host (or receiving) genome, such as GC content, codon usage etc. [13]. A variety of methods for

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predicting HGT candidates in prokaryotic genomes have been reported. Generally, methods for prediction of HGT candidates in bacterial genomes can be divided into two categories; composition-based methods, which screen for atypical characters in gene sequence composition [14], and the phylogenetic methods which distinguish incongruences between the species tree of a considered species and the orthologous gene tree of the transferred gene [15,16]. Composition-based methods test the similarity in sequence composition between the host genome that tend to share greater similarity and recently acquired genes. As recently acquired genes generally will have more atypical sequence compositions, such genes are regarded as HGT candidates. However, horizontally transferred genes can ameliorate to their host genome over time [17], and also genes in the same genome may have some dissimilarity. Thus, distinguishing host genes and acquired genes is not straightforward, and different composition-based methods show variation in their performance [14]. Phylogenetic methods look for incongruence between gene trees and associated species tree. Such methods highly depend on related orthologous genes. Because of the rapid accumulation of genome sequence data, these methods can produce accurate result, but on the other hand, these methods are more time consuming [18]. Current methods for predicting HGT events are far from perfect. Due to different underlying assumptions, individual methods detect HGT events at different phylogenetic distances and of different ages. It is therefore not very surprising that different methods return disparate sets of HGT candidates in a genome [19–21]. Continual efforts are being made to verify which individual genes have actually been horizontally transferred, and systematic analyses of the metabolic functions and evolutionary impact of transferred genes [22,23] suggest that bacterial HGT events have important effects on evolution and adaptation to the environment [24]. Systematic analysis of the HGT events in groups of prokaryotic genomes suggests that prokaryotes may have a number of acquired genes in their genomes [25,26]. Several works have also investigated the mechanisms and barriers of horizontal gene transfer, suggesting that a variety of factors, including restricted recombination, the function of the acquired genes, and similarity of tRNA pools between recipient and donor organisms, may affect the successful integration of a horizontally transferred gene [27–29].

Prior to this study, a few reports have discussed HGT events in human microbes. A digestive gene of marine bacteria was found in the gut microbiome of a Japanese population. This gene was regarded as an HGT candidate and it was proposed that its presence in the human gut microbiome is related to aspects of the Japanese diet [30,31]. Conserved genes and transposon families among human microbes suggest that HGT events occur in the human microbiome [32,33]. A DNA segment of the human genome was shown to have been transferred into the human pathogen *Neisseria gonorrhoeae* [34]. More HGT takes place between human-associated bacteria than among ecologically diverse non-human isolates [35]. However, few analyses of HGT events within a community of microorganisms that live in human body have been performed. Studying the HGT events in the human microbiome may help to understand the relationship between human microbes, their adaptation to the environment and their influence on human health [36]. In this paper, we identified and analyzed HGT candidates in 308 human microbes to gain a more in-depth understanding of these gene transfer events. We then built an interaction network of human microbes based on HGT events among them to elucidate the relationships between human microbes.

2. Results and discussion

2.1. Frequent horizontal gene transfer has occurred in the human microbiome

Horizontal gene transfer is common in prokaryotes and has attracted considerable research effort. Following publication of the human microbiome genome sequences by the Data Analysis and Coordination Center (DACC) of the HMP [11], whole genome sequences were available for investigation of HGT events in microbial communities in which microbes are in more or less intimate contacts. We predicted HGT events in 308 human microbes for which whole genome sequences and annotations were available (DACC). First, the codon usage of all genes in each human microbe were analyzed, and genes with atypical codon usage were picked out using the CU.KL method, which used the Kullback–Leibler matrix for codon usage analysis and showed good performance in identifying HGT candidates [14]. Using the CU.KL method, genes with atypical codon usage were picked out. Second, the genes with atypical codon usage were further analyzed for phylogeny incongruences using DarkHorse procedure [16,26]. In DarkHorse procedure, a query gene was BLASTed against the NR database of GenBank (NCBI BLAST Database ftp://ftp.ncbi.nih.gov/blast/db/) to search for similar sequences. Then, the results of BLAST were analyzed using DarkHorse analysis platform with default model and parameters, which looked for phylogenetically atypical genes.

The CU.KL approach identified 225,779 genes with atypical codon usage in the 308 microbes. These were further analyzed for phylogeny incongruences to identify probable HGT events and the hypothetical HGT donors from which the candidate genes were transferred into the human microbes using DarkHorse procedure, which took about 8600 CPU hours when executed on Mac Pro Server with two 2.26 GHz 4-Core Intel Xeon processors. Possible eukaryotic HGT donors and related HGT events were omitted because HGT gene transfer from eukaryotes to microorganisms is rare and difficult to verify, since the appearance of this type of HGT event may be due to pseudosequences arising from experimental contamination or other unknown sources. A total of 13,514 HGT genes were identified in the 308 human microbes examined (Supplemental file 1). The 308 human microbes were found in or on different parts in the body, including the gut, mouth, skin and so on. We identified on average 43.9 HGT candidates per microbe. The number of HGT candidates varied considerably among the different parts of the body, the gut microbiome harboring most HGT candidates (Table 1).

The number of HGT genes obtained with the combined approach employed in this study is relatively low (43.9 HGT genes per microbe). Popa and colleagues [37] found that about 20% of the genes had atypical GC contents. These atypical genes were then analyzed using phylogenetic approach, yielding about 32,028 HGT genes among 657 sequenced prokaryote genomes, yielding an average 48.7 HGT candidates in each genome [37]. Using a composition-based approach only, Yoji and colleagues [38,39] predicted 46,759 HGT genes in 116 prokaryotic complete genomes. This corresponded to an average 403 HGT genes in each genome and suggested that about 14% of all genes were horizontally acquired [38]. Composition-based methods are biased towards recently transferred genes which have not ameliorated to the host genome [17], while phylogenetic analyses are able to identify both recent and ancient horizontally transferred genes with sufficient reference sequences [18,40]. Combinations of composition-based and phylogenetic approaches for predicting HGT events will thus generate fewer, but more confident HGT candidates than when only one approach is applied. Combined approaches have been used

Table 1
HGT candidates in microbes from different sites of the human body.

Body site	No. of species	No. of HGT candidates	Average
Airways	10	352	35.2
Gastrointestinal	165	8022	48.6
Oral	46	1702	37.0
Skin	31	1286	41.5
Urogenital	48	1914	39.9
Others	8	238	29.6
Total	308	13,514	43.9

together in previous studies aiming at identifying HGT events in bacteria, fungi and insects [41–43]. On the other hand it is reasonable to assume that the sensitivity is lower by a combined approached, since amelioration or absence of homolog sequences in existing reference databases will cause a number of likely HGT candidates to be excluded in the final result.

2.2. The human microbiome facilitates microbial horizontal gene transfer

HGT candidates in a human microbe may originally come from either external microorganisms or from other microbes within the human body. To obtain a more detailed picture of the HGT events occurring in human microbes, we divided the HGT events in the human microbiome into two categories: 1) Internal HGT (HGTi) events occurring between two species within the human microbiome. The microorganism receiving a horizontally transferred gene is the receptor (HGTi receptor), and the source organism is the donor (HGTi donor). 2) External HGT (HGTe) events occurring from an external microorganism (HGTe donor) to a human microbe (HGTe receptor). Among the 13,514 HGT genes were identified in the human microbes 3978 (29.4%) genes occurred among the 308 human microbes, while in 9536 (70.6%) genes the donor was one of 1399 different external microorganisms. On average, an HGTi donor transferred 12.91 genes to another member of the human microbiome, whereas for the identified HGTe donors an average of 6.81 genes was transferred into the human microbiome. Thus, the average number of HGT candidate genes transferred from an HGTi donor was much higher than for an HGTe donor (t-test, P value $= 9.30 \times 10^{-14}$). These results suggested that the chance of a horizontal gene transfer between two members of the human microbiome were much higher than the chance of a transfer from an external microorganism to a human microbe. Human microbes might preferentially obtain genes from internal human microbes rather than external microorganisms.

The human microbiome consists of a rich diversity and abundance of microbe species that are in intimate contact in the human body, especially in the gut, and this is likely to facilitate the exchange of genetic material among the microorganisms. As for external microorganisms which occasionally may enter the human microbiome with the diet, their number is generally much smaller than the number of microbes already present in the gut. There is thus less chance of communication between human microbes and external microorganisms and less possibility for horizontal gene transfer between human microbes and external microorganisms. In addition, the human body provides similar niche for the bacteria living in it, and organisms that share a common ecological niche tend to have similar tRNA pools, which also appear to facilitate horizontal gene transfer events [27]. Taken together, this suggests that the human body provides favorable conditions for frequent horizontal gene transfer among bacteria residing in the body. We guess that other animal bodies or other enclosed environments may similar facilitate gene transfer among its resident bacteria.

2.3. Horizontal gene transfer occurs between human microbes from different sites of human body

To analyze the relationship between human microbes from different human body sites, a human microbe interaction network was constructed based on the HGTi events among human microbes. Members of the human microbes were taken as network nodes and horizontal gene transfers between microbes were taken as directed edges, which were weighted by the number of transferred genes between related microbes. To improve the reliability of the edges, only edges with weights over 3 in the network were considered. After removal of a few isolated nodes, the resulting directed and weighted network contained 165 connected nodes and was used to analyze the relationships among microbes from different body sites.

Application of the Blondel Community Detection algorithm [44] showed that, similar to other biological networks, the human microbe interaction network was composed of modules. The 165 members of the human microbes could be divided into 12 communities (C1–C12) with relative high intra-community HGT frequencies (Table 2, Fig. 1A). Several of these communities contained two or more sub-communities. Further analysis showed that edges within communities (C1–C12) had higher weights than edges between communities (BC; Fig. 1B). Communities were composed by microbes from different body sites, indicating that HGT events also frequently took place between microbes from different body sites.

Communities C4, C7, C8 and C11 were mainly composed of gastrointestinal microbes (>80%). Also communities C2, C3, C6 and C9 contained high fractions of gastrointestinal microbes, but these communities also contained many microbes from the airways, skin and urogenital system. Community C12 was mainly composed of microbes from the skin and urogenital system (83.3%). Oral microbes mainly appeared in communities C1 and C5, whereas urogenital microbes were predominantly found in C10. Thus, microbes belonging to the same community did not always reside in or on the same parts of the body, and most communities were composed of microbes from two or more body sites. Therefore, microbes from the same sites of the human body frequently exchanged genetic information; however, exchange also occurred between human microbes from different parts of the body. Human microbes from the same part of the body did not necessarily gather in the same community or distribute randomly but rather tended to gather in 2–3 communities. As a contrast, some human microbes occupied the same part of the body but apparently engaged in few HGT events between each other.

In the human microbe interaction network, gastrointestinal microbes can largely be divided into two types, one type that mainly transfers genes to other gastrointestinal microbes, e.g., the gastrointestinal microbes in C4, C7, C8 and C11, and a second type transfer genes to a wider range of microbes, including both other gastrointestinal microbes and microbes from other parts of the body (e.g., the microbes in C2 and C10 that mainly interact with urogenital and oral microbes). Urogenital microbes are concentrated in community C10, and prefer to exchange genes with gastrointestinal and oral microbes. Oral microbes are mainly distributed on communities C1, C2 and C5, and frequently transfer and receive genes among themselves and to and from gastrointestinal and urogenital microbes. Skin microbes were mainly found in communities C10 and C12, and interact with gastrointestinal, oral and urogenital microbes. Communities C1, C2, C3 and C5 are mainly composed of oral and gastrointestinal microbes suggesting more frequent contacts occur between microbes from these two parts of the body than between microbes in other body parts.

2.4. Transferred genes showed preference in special gene functions

Gene Ontology (GO) annotation analysis was carried on the 13,514 predicted HGT genes. As some genes occurred in multiple HGT events, this number of HGT events involved 8986 genes, of which 5338 genes were annotated with a GO term in the AgBase [45]. GO annotations of all the HGT events in the human microbiome were analyzed using WEGO [46]. The analysis showed that HGT events in the human microbiome mainly involved genes with similar GO terms, specifically *cell*, *cell part*, *binding*, *catalytic*, *cellular*, *localization* and *metabolic processes* (Fig. 2). GO annotation of HGT candidates suggested that transferred genes showed preference in special gene functions.

Kanhere and Vingron [47] analyzed the functions of HGT candidates in prokaryotes, and found that genes with functions related to metabolism and translation were enriched among the transferred genes. The transferred genes in the human microbiome were preferentially metabolic and catalytic genes. Early research revealed that bacteria can obtain antibiotic resistance through HGT [48]. Recently,

Table 2
Community components of the human microbe interaction network.

Community	Body site	Quantity*	Total	Community	Body site	Quantity*	Total
C1	Oral	9(47.4%)	19	C6	Gastrointestinal	2(66.7%)	3
	Gastrointestinal	4(21.1%)			Urogenital	1(33.3%)	
	Skin	2(10.5%)		C7	Gastrointestinal	13(81.3%)	16
	Airways	2(10.5%)			Urogenital	2(12.5%)	
	Blood	1(5.3%)			Oral	1(6.3%)	
	Urogenital	1(5.3%)		C8	Gastrointestinal	3(100%)	3
C2	Gastrointestinal	11(45.8%)	24	C9	Gastrointestinal	3(60.0%)	5
	Oral	8(33.3%)			Skin	2(40.0%)	
	Urogenital	4(16.7%)		C10	Urogenital	10(47.6%)	21
	Airways	1(4.2%)			Gastrointestinal	4(19.0%)	
C3	Gastrointestinal	2(66.7%)	3		Oral	4(19.0%)	
	Oral	1(33.3%)			Skin	3(14.3%)	
C4	Gastrointestinal	24(92.3%)	26	C11	Gastrointestinal	28(96.6%)	29
	Urogenital	2(7.7%)			Oral	1(3.4%)	
C5	Oral	5(50.0%)	10	C12	Skin	3(50.0%)	6
	Gastrointestinal	2(20.0%)			Urogenital	2(33.3%)	
	Urogenital	1(10.0%)			Gastrointestinal_skin	1(16.7%)	
	Skin	1(10.0%)					
	Airways	1(10.0%)					

* Number of human microbes. The percentage is the rate of number of microbes from a body site to the total number of microbes in a community.

genes functioning in food digestion and nutrition metabolism were found to be transferred between bacteria [30]. Some transferred genes might help human microbes to adapt to different environments and gain a competitive advantage in the environment. Microbes with such HGT candidates might survive and reproduce, and the HGT candidates might be maintained and delivered throughout the population.

3. Conclusions

Analysis of horizontal gene transfer in the human microbiome identified 13,514 high confidence HGT genes in 308 prokaryotes. The horizontally transferred genes were enriched for Gene Ontology terms pertaining to catalytic functions and metabolic processes. Construction of an HGT event network suggested that the human microbiome could be divided into specific communities which only partly overlap the spatial distribution of the microbiome on the human body. Our research suggests that human microbiome may facilitate frequent horizontal gene transfer among bacteria in human body, and awareness of the distribution and frequency of HGT events in human microbiome may further our understanding of the relationships between the human microbiome and human health.

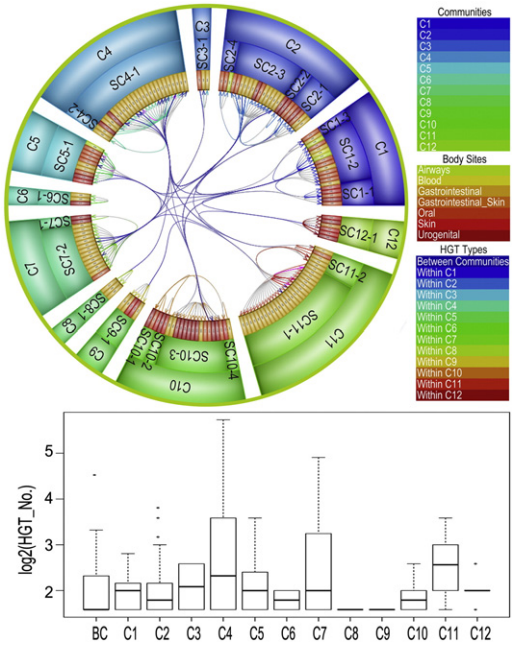

Fig. 1. Horizontal gene transfer network of the human microbiome. A. The panel shows the hierarchical structure of the HGT network composed of 165 reference genomes. The 12 parts represent microbial communities with their respective sub-communities. The 165 reference microbes are indicated on the inner ring with a color representing their location in or on the human body. The edges are bundled to reduce visual clutter in the center of the ring [50]. Edges between different communities are indicated in blue color, while intra-community edges follow the color representing each respective community. B. The panel shows the weight distribution of the edges. BC denotes "between communities" edges, C1–12 denotes community 1 to 12.

4. Materials and methods

4.1. Data and software

Sequences and annotations of the human microbiome were downloaded from the DACC (Data Analysis and Coordination Center) of the HMP (Human Microbiome Project) (http://www.hmpdacc.org/). The CU.KL program [14] for HGT candidate prediction was written using Perl script. The DarkHorse resource codes used to predict HGT candidates were obtained from the website of DarkHorse HGT Candidates Resource (http://darkhorse.ucsd.edu), and the DarkHorse analysis platform was set up on our local servers according to the instruction.

4.2. Predicting HGT events in the Human Microbiome

CU.KL, a composition-based method using atypical codon usage to predict HGT candidates, was written using Perl script and applied to HGT candidate prediction. Protein sequences of 308 human microbes were screened using the CU.KL method to identify genes with atypical codon usage, which were then analyzed using a phylogenetic method named DarkHorse to confirm the predicted HGT candidates with atypical codon usage. First, BLASTp was used to align the protein sequences of atypical genes to the NR dataset to find similar sequences (local BLAST, e-value lower than 10^{-5}, over 70% sequence length coverage of both query and subject sequence). Then, the results of BLASTp were analyzed using the DarkHorse analysis platform with default model and parameters to find phylogenetically atypical genes automatically. The candidate genes with such phylogenetically atypical characters were regarded as real HGT genes.

4.3. GO annotation and analysis of horizontally transferred genes

We employed the GORetriever tool in the AgBase [45] to directly obtain the GO items and annotations with GI numbers of the

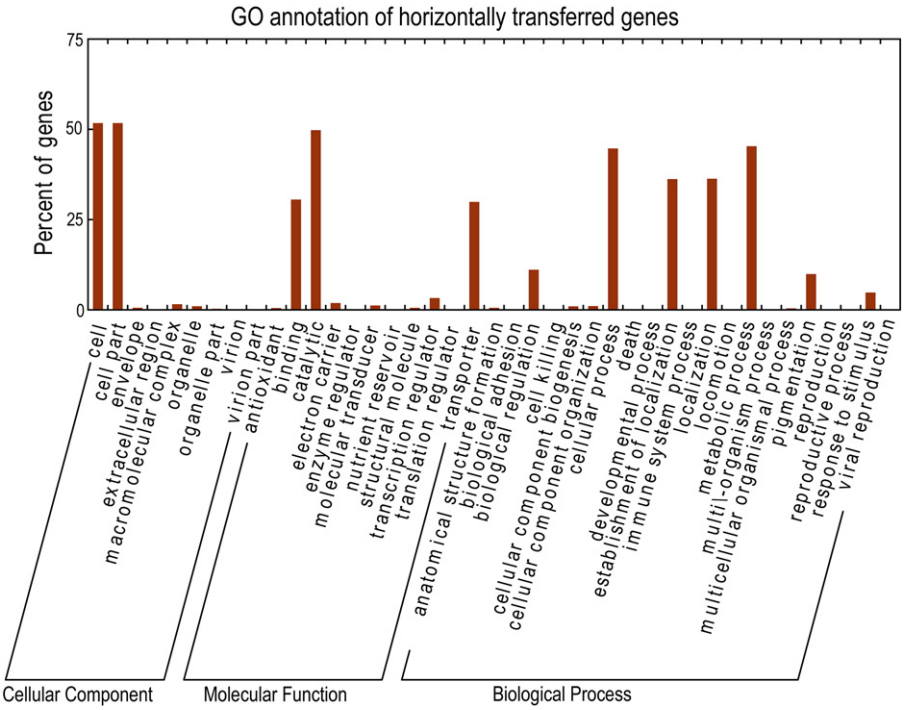

Fig. 2. Gene Ontology analysis of horizontally transferred genes. The HGT candidates from all human microbes showed preference in special gene functions. The components of cell and cell part, the functions of binding, catalytic and transporter, and the biological processes of cellular, localization and metabolic processes showed the greatest enrichment.

corresponding HGT candidates. We then used WEGO [46] to analyze the GO annotations.

4.4. Network construction and modularity analysis

We defined the members of the human microbiome as network nodes and HGT events between two microbial genomes as directed edges with weights corresponding to the number of transferred genes.

We employed the Blondel Community Detection algorithm implemented in the network workbench [49] to detect communities and sub-communities in the network. We employed the Hierarchical Edge Bundles algorithm [50] implemented in SolidSX (educational/OSS license) (http://www.solidsourceit.com) to display the hierarchical layout.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ygeno.2012.07.012.

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