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ORIGINAL ARTICLE

Comparative gut microbiome analysis of the *Prakriti* and *Sasang* systems reveals functional level similarities in constitutionally similar classes

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Abstract

The traditional medicinal systems (TMS) of India (*Prakriti*) and Korea (*Sasang*) classify human individuals based on their constitution determined by the physiological and psychological traits of individuals. Similarities in the constitutions are already found between the classes of *Prakriti* (*Vata*, *Pitta*, and *Kapha*) and *Sasang* (*TE: Taeeumin*, *SE: Soeumin*, and *SY: Soyangin*) systems. Gut health is an important aspect of this constitution based classification in TMS. To determine the role of gut microbes in such classifications, we have analyzed the gut microbiome (taxa and imputed functions) in the constitutionally similar *Prakriti* and *Sasang* classes. An enrichment of *Bacteroides* and *Prevotella* enterotypes is observed in the *Sasang* and *Prakriti* samples, respectively. The impact of the constitution is found to be more prominent with respect to the taxa and predicted-functions within the *Prakri*ti classes. Gut microbiome functional-level similarities are found to correlate well with the host phenotypes of the constitutionally similar *Prakriti* and *Sasang* classes. An enrichment of carbohydrate and amino-acid metabolism is observed in the *Vata* and *SE* classes which may be responsible for meeting with their high energy demands and lean phenotype. The *Pitta* and *SY* classes exhibit the high capacity to metabolize toxins. An enrichment of functions responsible for predisposition to obesity and high drug metabolism is observed in the *Kapha* and *TE* classes. The contribution of gut adaptive functions is found to correlate with the constitution-based classification in both *Prakriti* and *Sasang* systems. The *TE* class harboured the highest number of biofilm-forming and stress-tolerant microbes thus exhibiting the maximum tolerance of environmental stress. Similarities in the gut microbiota and the resulting disease predisposition patterns are found to exist between the constitutionally matching *Prakriti* and *Sasang* classes.

Keywords Human gut microbiome · *Prakriti* · *Sasang* · Ayurveda · India · Korea · Traditional medicinal systems

Introduction

The traditional medicinal systems (TMS) have been existing across the globe from ancient times (Bodekar and Graz 2020). These systems stratify individuals into different classes based on their constitution as determined by their physical, psychological, social, and spiritual status (Kim et al. 2011). The TMS diagnose the health status of individuals on the basis of their constitutionally determined pathological conditions which ultimately serves as the foundation for the treatment regime to be adopted for a medical condition. The Asian region is particularly known to be enriched in the application of these TMS, including Indian TMS, viz., *Ayurveda*, Korean TMS, viz., *Sasang*, and Chinese TMS, viz., traditional Chinese medicine (TCM) (Kim et al. 2011). Although the underlying fundamental principles to stratify people for diagnosis and cure are different among all these

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systems, yet the *Ayurveda* and *Sasang* systems share some commonality with respect to the underlying convention for the determination of constitution.

According to the Ayurvedic system, healthy individuals are classified into their dominant *Prakriti* or constitution based on the proportion of different *doshas* viz., *Vata*, *Pitta*, and *Kapha* (Kim et al. 2011). Similarly, the Korean *Sasang* system classifies healthy individuals into mainly three classes, viz., *Soeumin* (*SE*), *Soyangin* (*SY*), and *Taeeumin* (*TE*). Both the *Prakriti* and *Sasang* constitutional systems differ in their classification approaches, but they put a priority on a human being itself. In fact, the *TE* class is found to be similar to the *Kapha* class, *SY* class to *Pitta* class, and *SE* class to *Vata* class on comparing the *Ayurveda* and *Sasang* medicinal systems within the framework of disease prevention and quality-of-life evaluation (Lee et al. 2009). The disease predisposition and development patterns of the *Kapha* class of *Prakriti* and the *TE* class of *Sasang* are found to be similar. For example, they both have revealed predisposition towards the development of obesity (Jang et al. 2013; Pallavi et al. 2018). In addition, both classes have also found to possess a high-fat mass (Pallavi et al. 2018; Chae et al. 2003) and a high cholesterol level (Kim et al. 2018; Prasher et al. 2008). Moreover, cardiovascular risk factors are found to be highly associated with the *Kapha* class (Prasher et al. 2008), whereas the *TE* class is found to be an independent risk factor for the development of metabolic syndrome, which can lead to the development of cardiovascular diseases (Jang et al. 2013a, b). Also, both classes have been found to possess a higher tendency towards diabetes (Cho et al. 2014; Sitara et al. 2015).

A recent study based on the application of the Forest Therapy Programs in elderly healthy people revealed a constitution specific effect measured through the electroencephalogram (EEG) and heart rate variability (HRV) biomarkers (Yi et al. 2019). The progress in the genomic studies has unravelled the existing genomic differences among the *Sasang* constitution classes (Won et al. 2009; Kim et al. 2012). In addition, the serum and urine associated metabolic markers are also different among the *Sasang* classes (Kim et al. 2019a). Another recent study revealed the presence of a lower level of high-density lipoprotein cholesterol (HDL-C) in the blood which is also found to be a risk factor for the cardiovascular disease which might be one of the factor for the highest occurrence of this disease in *SY* type (Kim et al. 2017). Yet another recent study based on the temporal and network analysis of the published literature on the latest trends in the Korean medicine found a clustering of the *Sasang* constitution, obesity, and herbal medicine (Kim et al. 2019b). This study clearly provided clear insight towards the well-known fact about the higher propensity of the *TE* group individuals of the *Sasang* constitution to obesity. The *Sasang* constitution-based stratification of individuals has been found to be helpful in the identification, diagnosis, treatment regimes and management of the disease. For example, a herbal drug, Chowisengcheong-tang, has been found to exert an anti-obesity effect in the *TE* groups leading to the induction of weight loss and may be considered as the personalized medicinal therapy in stratified individuals (Kim et al. 2020). In addition, a recent exploratory study revealed that a majority of Hemophilia patients are of the *TE* type (Lee et al. 2020) which can be used for better predictive, preventive, personalized management of this disease. Moreover, the inclusion of traditional medicinal treatment with western medicine is also found to be effective. For example, the traditional medicines given to cancer patients who were undergoing chemotherapy revealed a highly beneficial effect to treat the side effects in a patient of *SE* group suffering from primary central nervous system lymphoma (Lee et al. 2019).

The very popular TMS of India viz., *Ayurveda*, has been utilized to shield the well being of healthy individuals and to cure the illness of unhealthy individuals from ancient times. It offers preventive measures, herbal remedies, and nutritional interventions to restore the health or cure the disease based on an individual's *Prakriti*. The *Prakriti* is inferred from the physiological and psychological traits present within an individual. Recently, the well-anticipated assumption of the genetic basis of the *Prakriti* has been established through modern scientific approaches (Govindaraj et al. 2015). The three classes of *Prakriti*, viz., *Vata*, *Pitta*, and *Kapha,* not only differ from each other based on the underlying constitutions but also in several other aspects. For example, a difference in drug-metabolizing capacity of the three *Prakriti* classes has been observed (Ghodke et al. 2011). Further, a lower platelet aggregation capacity was observed in *Kapha* whereas the highest activity was registered in the *Vata–Pitta* individuals. This is in agreement with the highest and lowest metabolism capacities of the *Pitta* and *Kapha Prakriti* classes, respectively (Bhalerao et al. 2012). The level of the immune response of individuals has also been found to be linked with the *Prakriti* classes. For example, the *Kapha* class was found to harbor enriched levels of CD25 and CD56 indicating a better immune response capacity which also correlates with the *Kapha Prakriti* phenotype (Rotti et al. 2014). Interestingly, the biochemical entities of blood of individuals have been found to vary among the *Prakriti* classes and correlates well with the various phenotypic characteristics (Prasher et al. 2008). Moreover, the epigenetic signatures have been found to be enriched in the *Prakriti* classes (Rotti et al. 2015). The identification of enrichment of methylation of *CDH22* 5′-UTR CpG region in individuals of the *Kapha* class has revealed a good corroboration with its phenotypic traits viz., elevated level of body mass index (BMI). Besides, the analysis of plasma metabolome revealed an enrichment of various metabolic pathways across the *Prakriti* classes which explain

the existing differences in the physiological and psychological traits (Shirolkar et al. 2018). Interestingly, most of the molecular, biochemical, immune response, genetic, epigenetic, and metabolic determinants which are found to vary among the *Prakriti* classes have been found to be contributed or regulated by the human gut microbiome (Miro-Blanch and Yanes 2019; Goodrich et al. 2014; Mezouar et al. 2018; Visconti et al. 2019).

According to the modern medicinal practices, the human gut microbiome has been found to play an important role in the overall health and disease status of individuals (Kho and Lal 2018; Jackson et al. 2018; Sharma et al. 2018). Although, the healthy human gut microbiome is dominated by two major phyla, viz., Bacteroidetes and Firmicutes, minor inter-individual variations are found to exist across individuals (Arumugam et al. 2011). Multiple factors affecting human gut microbiome variations have been identified which include age, geography, genetics, environment, and birth-mode (Dominguez-Bello et al. 2019; Odamaki et al. 2016; Mobeen et al. 2018; Voreades et al. 2014). In fact, probiotics have been found to exert beneficial effects on human health through the action of favourable human gut microbiome (Yadav et al. 2018, 2019). Thus, a proper understanding of the functional potential of the human gut microbiome may also contribute to understanding the potential contribution of probiotics in the constitutional classes of the *Prakriti* and *Sasang* systems. It is interesting to note that several phenotypic attributes that are being associated with the microbiome differences are also found to be different between the constitution types. These include desire and suitability for different diets, metabolic and digestive patterns, weight-gain tendencies, gut-motility, and excretory patterns (Prasher et al. 2008, 2016). The microbiome variations can be correlated to the constitutional variations observed in the *Prakriti* and *Sasang* systems. Towards this, two previous independent studies have assessed the role of gut microbiome in healthy individuals classified based on the *Prakriti* (Chauhan et al. 2018) and *Sasang* (Kim et al. 2013) constitutional systems.

The human gut microbiome is found to be an important forgotten organ which plays a significant role in an individual's health as well as in several diseases. Further, gut health plays an important role in the classification of individuals by both the *Prakriti* and *Sasang* constitutional systems (Chauhan et al. 2018; Kim et al. 2013; Chaudhari et al. 2019). Though studies have revealed an association of human gut microbiome with the constitution in both the *Prakriti*- and *Sasang*-classified individuals independently, but a comparative analysis towards analyzing the similarity and dissimilarity between the classes of these systems based on the gut microbiome is still lacking. Such comparative study becomes more important due to the presence of constitutional level similarities (*Kapha* and *TE*, *Pitta* and *SY*, and *Vata* and *SE* classes), based on their disease prevention patterns (Lee et al. 2009). Besides, the disease predisposition tendency of the *Prakriti* and *Sasang* classes are also found to be similar for the *Kapha* and *TE* classes (Cho et al. 2014; Sitara et al. 2015). The underlying similarities in gut microbiomes of the similar classes of the *Prakriti* and *Sasang* will help in the prediction of disease predispositions and the translation of treatment regimes between these two traditional constitutional systems of medicine. Towards this, in the present work, we have carried out an extensive comparative gut microbiome analysis using the taxonomic and imputed-functional profiles of the similar classes of the *Prakriti* and *Sasang* medicinal systems, viz., *Kapha–TE*, *Pitta–SY*, and *Vata–SE*.

Materials and methods

Description of datasets used in this analysis

The main aim of this analysis is to compare the gut microbiomes of healthy individuals classified into different categories according to the two independent TMS, viz., Indian Ayurveda and Korean Sasang. Towards this, the following inclusion criteria were used for the datasets: (1) human gut microbiome datasets derived from individuals categorized into different constitution types by Indian and Korean TMS, (2) feces was used as the source of metagenomic DNA isolation, (3) datasets derived from similar next-generation sequencing technologies to maintain confounding factors, (4) datasets derived from only healthy subjects, and (5) datasets derived from subjects from same geographical location for each traditional medicinal systems which may reduce the effect of factors which influence the human gut microbiome like genetics, diet, environment etc. and may highlight the effect caused by the inherent constitution. Based on these criteria, we have selected the datasets used by Chauhan et al (2018), Kim et al. (2013) for our analysis. The dataset for the *Prakriti* and *Sasang* samples were downloaded from Figshare repository (https://figshare.com/s/e981faa54cc3347999d9) and European Nucleotide Archive (ENA) repository (https://www.ebi.ac.uk/ena/data/view/ERP002551). The present analysis includes publically available 16S rRNA gene amplicon sequence data of fecal samples from 153 healthy adult individuals, which include 113 *Prakriti* and 40 *Sasang* samples. The *Prakriti* samples include *Vata* (42 samples), *Pitta* (29 samples), and *Kapha* (42 samples) classes, whereas the *Sasang* samples include *SE* (13 samples), *TE* (14 samples), and *SY* (13 samples) classes. The distribution of the number of samples in the *Sasang* and *Prakriti* classes is given in Table S1.

Pre-processing and taxonomic analysis of the human gut microbiome samples

The pre-processing of raw sequences of the *Sasang* samples is carried out in the QIIME package (Caporaso et al. 2010) using previously defined criteria for the *Prakriti* samples (Chauhan et al. 2018) (Fig. S1). Further, the chimeric sequences are removed from quality-filtered dataset using USEARCH v6.1 (Edgar 2010). In addition, the taxonomic profiling of dataset is performed in the automated pipeline of amplicon analysis of the Parallel-Meta 3.4.3 software (Jing et al. 2017). This automated pipeline uses Bowtie 2 (Langmead and Salzberg 2012) to align sequences to Parallel-Meta reference database (integrated database of gg_13_8 (DeSantis et al. 2006), RDP (Cole et al. 2013), and SILVA (Quast et al. 2012)) using 97% pair-wise identity threshold to calculate operational taxonomic unit (OTU), assign consensus taxonomy, and build phylogeny. The phylogeny is created using FastTree software (Price et al. 2010). In addition, Parallel-Meta 3.4.3 software (Jing et al. 2017) also performs copy number normalization using the Integrated Microbial Genomes and Microbiomes (IMG) database (Markowitz et al. 2011) for the calculation of accurate relative abundance of each taxon. The microbial diversity present within samples is calculated using the Shannon Index-based diversity matrix (Jing et al. 2017). Further, beta diversity between samples is calculated using the unweighted and weighted Meta-storm scoring function which reveals similarity/dissimilarity across samples (Su et al. 2012). The weighted meta-storm is a phylogeny-based algorithm which utilizes the information of relative abundance of taxa to calculate the similarity score. Further, principal component analysis (PCA) is performed using the meta-storm distance to visualize the similarity and dissimilarity across samples.

Enterotype analysis

Enterotype clusters present in the dataset are analyzed using the BiotypeR package (https://github.com/tapj/biotyper) by providing relative taxonomic abundance at the genus level. The distance between the samples is calculated using the Jensen–Shannon distance (Jing et al. 2017). The number of optimum clusters in the *Prakriti* and *Sasang* human gut microbiomes is identified using genus-level composition data by the Calinski and Harabasz (CH) index. The optimality of samples grouped in these two clusters is assessed using the Silhouette score. The PCA and double principal coordinate analysis (dPCOA) are used to visualize human gut microbiome predicted within these two clusters.

Identification of taxa to classify the constitutions

The selbal method is used to identify the taxa associated with constitutions which can discriminate between any two given constitution types. A high area under the curve (AUC) value shows a higher accurate prediction of the constitution (Rivera-Pinto et al. 2018).

Predictive functional profiling

The predictive functional profiling of human gut microbiome is performed with the Phylogenetic Investigation of Communities by Reconstruction of Unobserved STates (PICRUST) software (Langille et al. 2013) using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa et al. 2013). The taxonomic and functional differentiating biomarkers present within the classes of *Prakriti* and within the classes of *Sasang* systems are calculated using two approaches viz., determination of statistically significant abundance and identification of important functional features using the randomForest method (Breiman 2001). The statistical tests on relative functional abundance are used in this study for the identification of differentiating functional features. The pair-wise Wilcoxon test (Jing et al. 2017) is used for the analysis between two classes and the Kruskal–Wallis rank-sum test (Jing et al. 2017) is used for comparison among multiple classes. Further, the *t* test and *F* test are also used to find statistical significance using the taxonomic and functional relative abundance. The variables with $p \leq 0.01$ are considered as statistically significant. The Benjamini–Hochberg (BH) procedure is used for the false discovery rate (FDR) corrections (Jing et al. 2017). The important differentiating functional features are identified using the randomForest approach on imputed functional abundance profile data for the *Prakriti* and *Sasang* classes. The ranking of identified important functional features is performed on the basis of their importance in differentiating the classes. Only those important functional features identified by the randomForest classifier are used for the analysis which has a mean decrease in accuracy by a value of ≥ 5.

Criteria used to assess the similarity between constitutions

We have used multiple criteria to infer the commonly enriched functional features between the constitutionally similar classes of the *Prakriti* and *Sasang* systems, viz., *Vata–SE*, *Pitta–SY*, and *Kapha–TE*. These criteria include identification of (1) common enriched significantly differentiating functional features, (2) common important functional features identified by the randomForest, (3) common important functional features identified by the randomForest in one class and the most abundant functional features in the

other classes, and (4) the presence of previously identified functional signatures of *Prakriti* class (Mobeen et al. 2019) as among the most abundant functions in the *Sasang* classes (Fig. S2). In addition, we have also examined the functional features which are present in the highest abundance in the *Sasang* and *Prakriti* classes.

Prediction of organism-level microbiome traits

Apart from the predictive functional profiles, an organism based microbial phenotype is predicted which may shed light on various features of microbes including aerobic status, gram-stain type, pathogenic status, etc. The organism-level functional traits of the human gut microbiome are calculated using the BugBase web server by providing OTU abundance table and associated metadata with default parameters (Ward et al. 2017). The BugBase method performs a genomic content calculation using the predicted OTUs. The analysis is used to calculate the relative contribution of organism-level functional traits viz., aerobic, anaerobic, facultative anaerobic, gram-negative, gram-positive, potential pathogens, mobile genetic elements, biofilm-formation, and stress-tolerance across the *Prakriti* and *Sasang* classes. Besides, we have also calculated the relative contribution of taxa in the functional traits by employing various databases viz., IMG4 (Markowitz et al. 2011), KEGG (Kanehisa et al. 2013), and The Pathosystems Resource Integration Center (PATRIC) (Wattam et al. 2013). The statistical significance of the organism level functional traits was carried out by Mann–Whitney–Wilcoxon tests and Kruskal–Wallis test for the pair-wise-group and multi-group comparisons, respectively.

Results and discussion

OTU identification in the human gut microbiomes of the *Prakriti* and *Sasang* systems

The current study presents a comparative human gut microbiome analysis using the 16S rRNA genes of samples classified as per the *Prakriti* and *Sasang* constitutional systems of traditional medicine. The taxonomic profiling and imputed functional analysis of the human gut microbiome across the *Prakriti* classes have been carried out previously by our group (Chauhan et al. 2018; Mobeen et al. 2019). The taxonomic analysis of the human gut microbiome across the *Sasang* classes has been carried out previously by Kim et al. (2013). The current study explores the similarity between the *Prakriti* classes and the correspondingly similar *Sasang* classes in terms of taxonomy, imputed functional profiles, and organism-level functional traits of the gut microbiome.

To perform a comparative analysis between the *Prakriti* and *Sasang* systems, we applied the same metagenomic analysis pipeline to the two datasets. The pre-processing of the 16S rRNA gene sequences from the *Prakriti* ($n = 113$) and *Sasang* ($n = 40$) datasets resulted in 33,16,209 and 1,12,371 high quality reads in the two datasets, respectively. The average number of high-quality reads per sample was found to be ~ 22,409 (Table S1). A total of 34,28,554 reads were taxonomically annotated during the taxonomic analysis of the combined datasets (*Prakriti* and *Sasang*). The minimum and maximum number of OTUs predicted in the *Sasang* dataset was 249 and 910, respectively, whereas, it was 117 and 1545, respectively, in the *Prakriti* dataset.

Taxonomic composition analysis of the Indian (*Prakriti*) and Korean (*Sasang*) samples

The taxonomic abundance of the two dominating phyla of human gut microbiome viz., Firmicutes and Bacteroidetes were found to be different across the *Prakriti* (Indian) and *Sasang* (Korean) samples (Fig. S3). The Korean gut microbiome was found to harbour a high abundance of the phyla Firmicutes, Actinobacteria, and Tenericutes, whereas the phyla Bacteroidetes was predominantly present in the Indian gut microbiome. In addition, the taxa Firmicutes, Actinobacteria, and Tenericutes were significantly abundant in the Korean samples, whereas Bacteroidetes was abundantly present in the Indian samples (Fig. S3). Previous studies have also revealed a higher abundance of the phylum Firmicutes in the healthy Korean gut microbiome (Nam et al.2011). Further, the presence of a high abundance of the phylum Actinobacteria in the Korean gut microbiome may be attributed to the dietary intakes of these people (Jang et al. 2017). Similarly, a high abundance of the phylum Bacteroidetes was found to be present in the western Indian gut microbiome (Tandon et al. 2018).

The Indian gut microbiome revealed a comparatively lower alpha diversity than Korea, which also corroborates with earlier studies (Fig. S4) (Mobeen et al. 2018). The beta diversity analysis of the Indian and Korean gut microbiomes revealed a clear separation of the samples of the two populations using the unweighted taxa (Fig. S5). The variation within the population was found to be lower in the Korean samples than the Indian samples, however, that between the populations (Indian and Korean) was found to be higher (Fig. S6). However, the beta diversity analysis of the *Prakriti* and *Sasang* samples independently reveals a lack of distinct clusters specific to the *Vata*, *Pitta*, and *Kapha* and *TE*, *SE*, and *SY* classes. It is well known that the constitution based stratification of healthy individuals in many TMS are subjective and there is an utmost need for the development of objective classification methods based on the modern scientific advancements.

One of the most important aspects of gut microbiome analysis of individuals stratified based on the underlying constitution in various medicinal systems is to provide the microbial signatures, which can easily discriminate these constitution groups. Towards this, we have identified the set of microbial taxa, which can maximize the discrimination between any two given constitutions (Table S2). A higher discrimination can be attained based on the microbial taxa between the *Vata–Pitta* followed by the *Vata–Kapha* and *Pitta–Kapha* classes. Similarly, in the *Sasang* constitution systems, *SE–SY* classes can attain the highest discrimination followed by the *SE–TE* and *SY–TE* classes based on the microbial taxa. In addition, similar gut microbiome characteristics of the *Prakriti* and *Sasang* systems based on the discriminatory pattern of individuals is observed. For example, the highest discrimination is found between the *Vata* and *Pitta* classes of the *Prakriti* system and *SE* and *SY* classes of the *Sasang* system. Similarly, the lowest discrimination is found between the *Pitta* and *Kapha* classes of the *Prakriti* systems and the *SY* and *TE* classes of the *Sasang* systems. Further, the two constitutions of the *Sasang* system can be discriminated slightly better than the *Prakriti* systems based on the microbial taxa.

The enterotype analysis of the samples of the Indian ($n=113$) and Korean ($n=40$) human gut microbiomes revealed the presence of two enterotypes clusters (Figs. S7 and S8). The enterotype taxa enriched in cluster 1 and cluster 2 samples were belonging to the genera *Bacteroides* and *Prevotella*, respectively. The numbers of samples present in the enterotype cluster 1 and enterotype cluster 2 were 57 and 96, respectively (Fig. 1). A majority of the Korean samples (95%) were present in the enterotype cluster 1, whereas a majority of the Indian samples (83%) were a part of the enterotype cluster 2. Therefore, the *Sasang* samples were found to harbour the enterotype taxon *Bacteroides,* whereas *Prevotella* was found as the enterotype taxa in the *Prakriti* system (Fig. 2a, b).

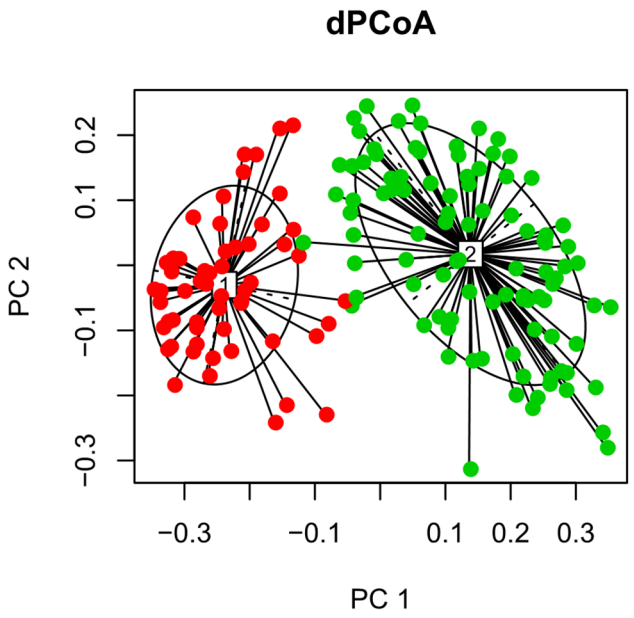

Fig. 1 The enterotypes identified in the Indian ($n=113$) and Korean ($n=40$) samples using the double principal coordinate analysis (dPCoA). *X* and *Y* axes show the PC1 and PC2 components, respectively. The number of *Prakriti* samples used in this analysis includes Vata (42 samples), Pitta (29 samples), and Kapha (42 samples) classes, whereas the *Sasang* samples include SE (13 samples), TE (14 samples), and SY (13 samples)

Organism-level functional analysis of healthy human gut microbiomes of the *Prakriti* and *Sasang* systems

We performed a comprehensive comparative organism-level functional analysis of the *Prakriti* and *Sasang* systems (Fig. 3). The *Sasang* samples exhibited a much larger number of facultative anaerobes as compared to the *Prakriti* samples ($p=3.77E^{-14}$) (Fig. 3). A majority of these microbes belonged to the phylum Tenericutes. In addition, the *Sasang* samples harboured a very large number of biofilm-forming microbes as compared to the *Prakriti* samples ($p=2.81E^{-12}$) which were mainly contributed by the phylum Actinobacteria. Another important difference was observed in the number of aerobic microbes, which were more abundant in the *Sasang* samples as compared to the *Prakriti* samples. These aerobic microbes were primarily attributed to the phylum Verrucomicrobia in the *Sasang* samples.

In the comparison of the *Vata*, *Pitta*, and *Kapha* classes of the *Prakriti* with the *SE*, *SY*, and *TE* classes of the *Sasang*, the most interesting results are observed for the biofilm-forming microbes and stress-tolerant microbes. The highest abundance of biofilm-forming microbes was present in the *TE* class (Mann–Whitney–Wilcoxon test $p=3.72E^{-6}$ for V, $p=2.32E^{-7}$ for P, $p=1.23E^{-7}$ for K, $p=5.77E^{-1}$for SE, and $p=2.59E^{-1}$ for SY) (Fig. 4a). Biofilm-formation helps the microbes to withstand and survive the operation of environmental stress. It is interesting to note that the relative abundance of stress-tolerant microbes is similar across the *Prakriti* classes (Fig. 4b). The lowest relative abundance of this functional trait was observed in the *SE* class with respect to the *Prakriti* classes ($p=0.03$ for V, $p=0.03$ for P, and $p=0.0456$ for K) and the other *Sasang* classes ($p=0.07$ for TE and $p=0.62$ for SY) (Fig. 4b). The *SE* class is known to be susceptible to upper respiratory infections (Chae et al. 2017). The recent progress in microbiome studies established a gut–lung axis for better health (McAleer and Kolls 2018). Thus, the presence of high potential pathogenic microbes in gut might distort the normal interaction between microbiome and lungs which in turn might lead to a higher susceptibility for upper respiratory diseases (Chae

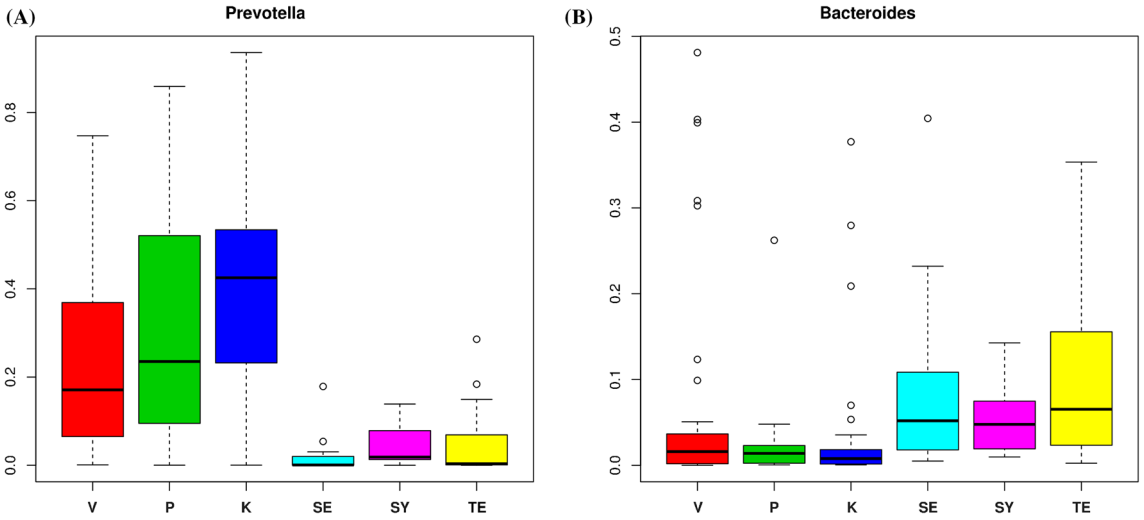

Fig. 2 The relative abundance of the enterotype taxa **a** *Prevotella* and **b** *Bacteroides* in the *Prakriti* (P, $n=113$) and *Sasang* (S, $n=40$) systems. *X*-axis shows the *Prakriti* and *Sasang* classes and *Y*-axis shows the relative abundance values. The number of *Prakriti* samples used in this analysis includes *Vata* (42 samples), *Pitta* (29 samples), and *Kapha* (42 samples) classes, whereas the *Sasang* samples include *SE* (13 samples), *TE* (14 samples), and *SY* (13 samples)

et al. 2017). The *TE* class exhibited a higher abundance of stress-tolerant microbes with respect to the *Prakriti* classes and the other *Sasang* classes (Fig. 4b). However, significant Mann–Whitney–Wilcoxon test *p* values could not be obtained either due to a very low abundance of this functional trait in the majority of the samples or the small number of the *Sasang* samples. The identification of the highest stress-tolerant microbes might provide an advantage to the *TE* class to withstand the stress. This is consistence with the fact, that the *TE* class is known to be lesser susceptible to environmental stress (Han et al. 2016). The identification of lowest stress-tolerance microbes in the *SE* class reveals contrasting features as compared to the *TE* class in context to the type-specific pathophysiological symptoms (TSPS). It has also been found that the *TE* class efficiently tolerates the psychological and oxidative stresses (Kim et al. 2015). These observations indicate that the *TE* class of the *Sasang* possesses a higher capability to tolerate environmental stress as compared to all other classes and the gut microbiota might contribute significantly towards this.

Further, we compared the abundance patterns of the functional traits between the *Prakriti* and *Sasang* classes based on three criteria viz., the highest, medium, and lowest abundance (Table 1). Our analysis revealed that the abundance patterns of aerobic microbes were similar in the *Kapha–TE*, *Vata–SE*, and *Pitta–SY* classes, though the observations are not statistically significant ($p=0.7$). Among the functional traits with statistically significant differences among the *Prakriti* and *Sasang* classes, *Vata–SY*, *Pitta–SE*, and *Kapha–TE* demonstrate the highest similarities in the abundance patterns. The similarity between the *Kapha–TE* classes corroborate with the overall constitution level similarity of these two functional classes, although the results should be considered with caution given the small number of samples being analysed for the *Sasang* dataset.

Imputed functional analysis of the human gut microbiomes of the *Prakriti* and *Sasang* systems

Taxonomic analysis revealed an overall difference in the Indian and Korean samples in terms of taxonomic abundance, diversity, and enriched enterotype taxa. However, it is well known that the functional redundancy is higher than the taxonomic redundancy in the human gut microbiome (Tian et al. 2017). Thus, the functional information might provide a better contribution to the identification of constitution-specific properties of the *Prakriti* and *Sasang* classes than the taxonomic information alone. Towards this, we have compared the Indian and Korean human gut microbiomes using imputed functional profiles to assess the similarities and differences between the *Prakriti* and *Sasang* systems.

The imputed functional alpha diversity analysis revealed the presence of a higher diversity in the *Prakriti* than the *Sasang* samples using the L2 and L3 levels of the KEGG pathways (Fig. S9a and S9b). The *Prakriti* associated higher functional diversity may be due to a unique Indian human gut microbiome, which also harbours additional novel genes and functions as revealed in a recent study (Dhakan et al. 2019). It is interesting to note that the taxonomic diversity of the Indian human gut microbiome is lesser than that of Korea. This implies that functional diversity may provide additional information about the phenotypic effect of human

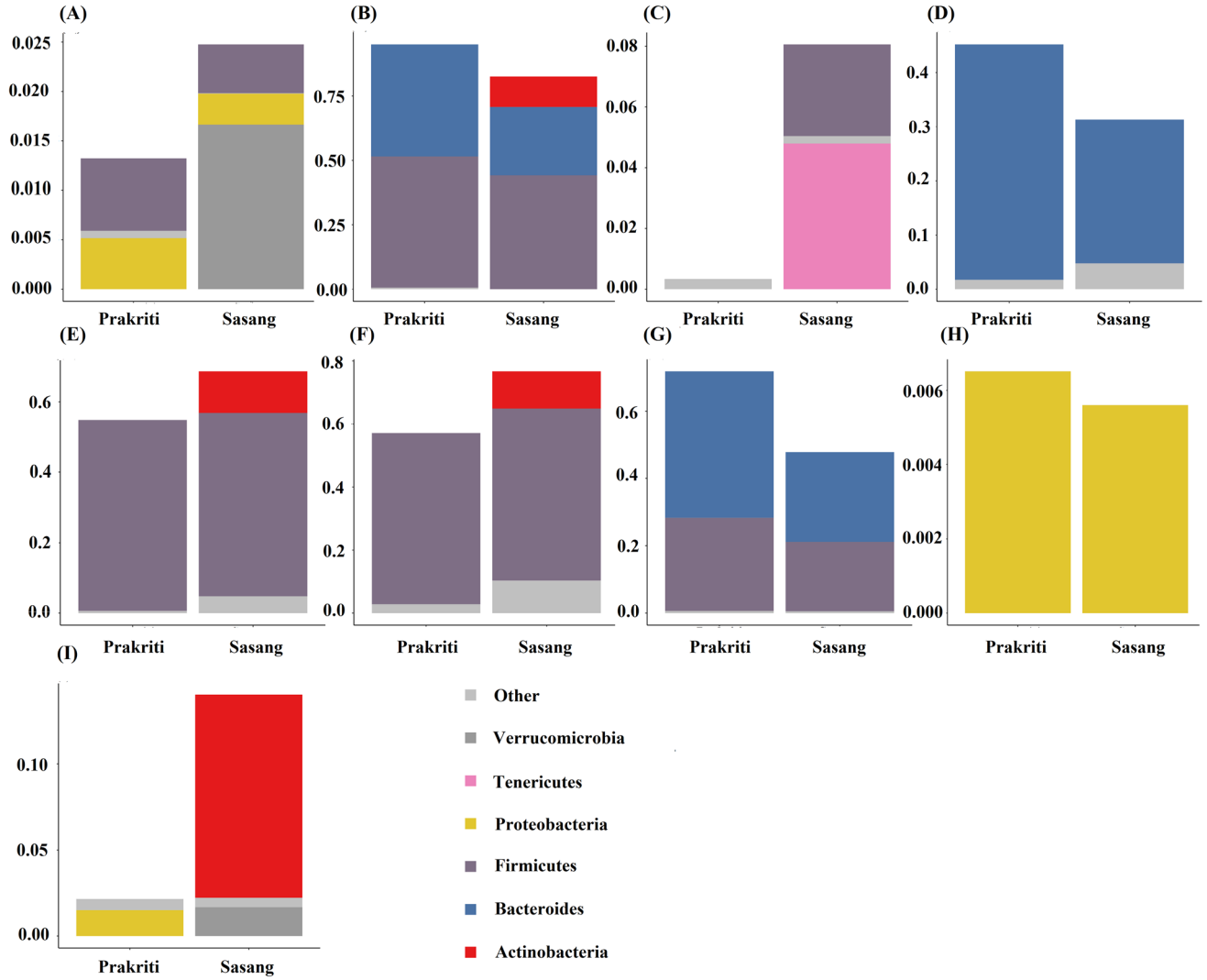

Fig. 3 The predicted organism-level functional traits viz., **a** aerobic, **b** anaerobic, **c** facultative anaerobic, **d** gram-negative, **e** gram-positive, **f** contains mobile elements, **g** potentially pathogenic, **h** stress tolerant, and **i** forms biofilms in the *Prakriti* and *Sasang* systems. *X*-axis shows the TMS and *Y*-axis shows the relative abundance of the functional traits. The number of *Prakriti* samples used in this analysis includes *Vata* (42 samples), *Pitta* (29 samples), and *Kapha* (42 samples) classes, whereas the *Sasang* samples include *SE* (13 samples), *TE* (14 samples), and *SY* (13 samples)

gut microbiome than taxonomy alone. In addition, we also noted an overall higher functional alpha diversity in all the *Prakriti* classes than the *Sasang* classes. Among the *Prakriti* classes, the *Kapha* samples revealed a higher diversity than the other *Prakriti* classes. And, among the *Sasang* classes, the *TE* class revealed a higher diversity. The beta diversity analysis of imputed functions at the L2 level of the KEGG pathways revealed a distribution of the *Prakriti* samples along the PC1 axis while the *Sasang* samples were oriented along the PC2 axis (Fig. S10). The analysis also indicates a comparatively higher functional dissimilarity among the *Prakriti* than the *Sasang* samples. Besides, the constitution-specific class-wise clustering of samples was not evident from this analysis which might be due to a higher functional redundancy among the samples. The samples of the *Prakriti* classes were more scattered than the *Sasang* classes.

The common functional features between the *Prakriti* and *Sasang* classes were identified by four different strategies as described in the methods section. Using strategy 1, we identified four significant functional features across the *Prakriti* classes at the L2 level of the KEGG pathways, including "Signal Transduction" (*Vata*), "Lipid metabolism" (*Vata*), "Poorly characterized" (*Kapha*), and "Nucleotide metabolism" (*Kapha*) (Table 2). However, no significant functional feature was identified across the *Sasang* classes at this level. At level 3 of the KEGG pathways, only one significant functional feature was identified in the *SE* class of the *Sasang* system, viz., "Carbon fixation in a photosynthetic organism".

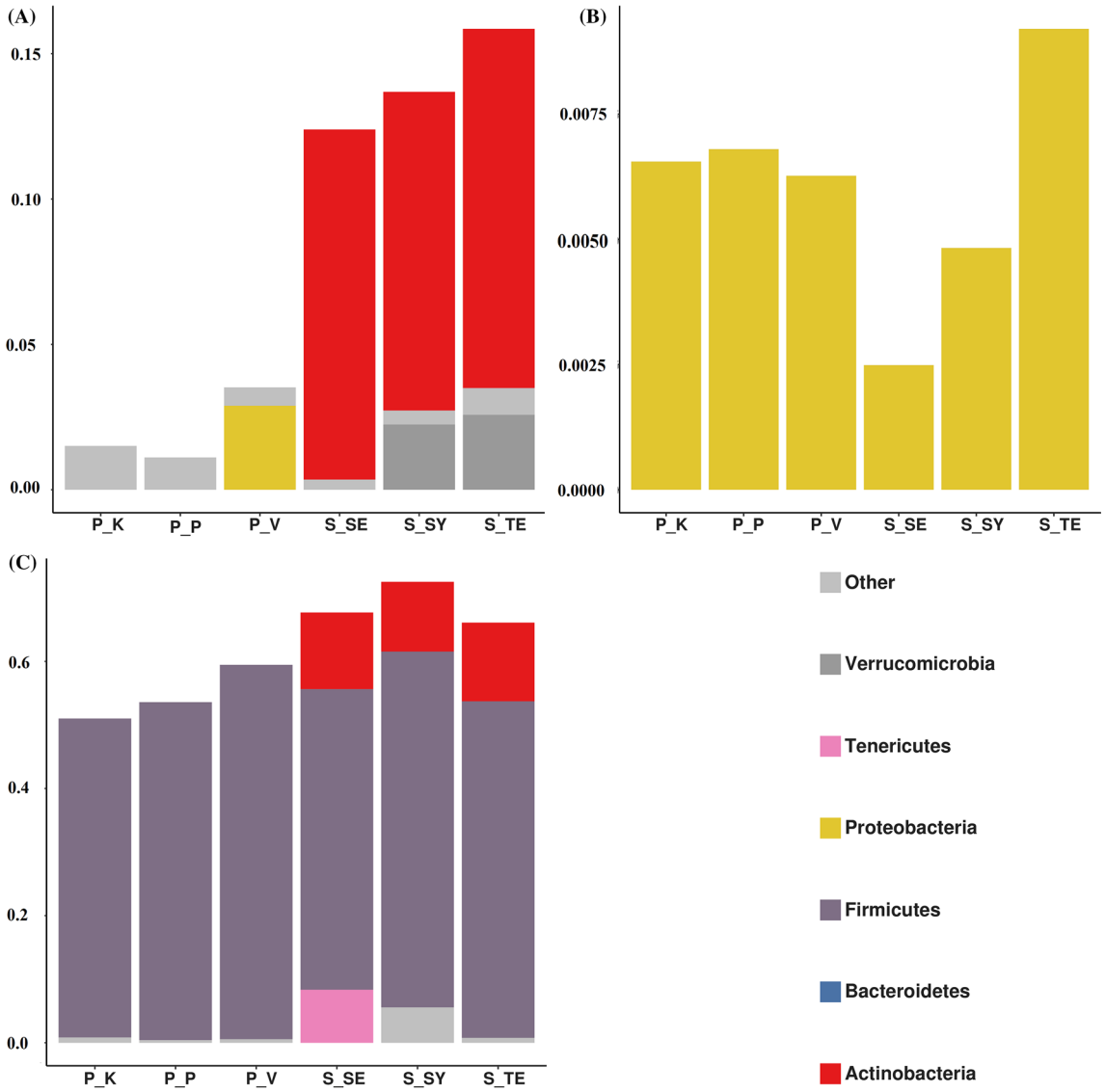

Fig. 4 Relative abundance of the **a** forms biofilm, **b** stress tolerant, and **c** gram-positive microbes in the *Prakriti* and *Sasang* classes. *X*-axis shows the *Prakriti* and *Sasang* classes and *Y*-axis shows the relative abundance. The number of *Prakriti* samples used in this analysis includes *Vata* (42 samples), *Pitta* (29 samples), and *Kapha* (42 samples) classes, whereas the *Sasang* samples include *SE* (13 samples), *TE* (14 samples), and *SY* (13 samples)

It is surprising to find the occurrence of this function in the gut microbiome. However, recent reports have suggested the presence of cynobacteria, which might be responsible for the occurrence of this function, in the human gut microbiome (Almeida et al. 2019).

We observed eleven significant functional features across the *Prakriti* classes at the L3 level of the KEGG pathways using strategy 1 (Table 2). Polycyclic aromatic hydrocarbon (PAH) is an organic pollutant conferring toxic and hazardous effects leading to an adverse effect on human health (Ghosal et al. 2016). The enrichment of PAH-degradation pathway in the *Pitta* class highlights a xenobiotic metabolism capacity, which is already known to be high in this class. Peptidoglycan biosynthesis, which is found to be a functional feature of the *Kapha* class, has been shown to be enriched in gut microbiome samples of cervical cancer (Kwon et al. 2019) and symptomatic atherosclerosis (Karlsson et al. 2012). The *Kapha* class is known to be susceptible to soft tissue cancer (Mobeen et al. 2019) and atherosclerosis (Prasher et al. 2008). Thus, the presence of a high abundance of peptidoglycan biosynthesis function in the *Kapha* class may contribute to the predisposition of the individuals to these diseases. Common significant functional features between the *Prakriti* and *Sasang* classes were not identified at any level of the

Table 1 Abundance patterns of the organism-level functional traits across the *Prakriti* and *Sasang* classes

Functional trait	*Vata*	*Pitta*	*Kapha*	*SE*	*SY*	*TE*	Kruskal–Wallis test
Aerobic	L	M	H	L	M	H	0.713865
Anaerobic	L	H	M	M	L	H	4.94E−07
Facultative anaerobic	L	H	M	L	M	H	1.13E−11
Gram-negative	L	M	H	M	L	H	0.028126
Gram-positive	H	M	L	M	H	L	0.028126
Biofilm	H	L	M	L	M	H	2.74E−10
Mobile genetic elements	H	M	L	M	H	L	0.000156
Stress-tolerance	L	H	M	L	M	H	0.140444
Potential pathogens	L	M	H	H	L	M	9.36E−09

The number of *Prakriti* samples used in this analysis includes *Vata* (42 samples), *Pitta* (29 samples), and *Kapha* (42 samples) classes, whereas the *Sasang* samples include *SE* (13 samples), *TE* (14 samples), and *SY* (13 samples) classes. (Functional traits with $p < 0.05$ is considered as statistically significant)

H highest, *M* medium, *L* lowest

Table 2 List of significantly enriched functional features present in the *Prakriti* and *Sasang* classes

Significant functional features (strategy 1)						
KEGG pathway level	*Vata*	*Pitta*	*Kapha*	*SE*	*SY*	*TE*
L2	Signal transduction		Poorly characterized			
	Lipid metabolism		Nucleotide Metabolism			
L3	Protein export	Polycyclic aromatic hydrocarbon degradation	Purine metabolism	Carbon fixation in photosynthetic organism		
	Valine leucine and isoleucine biosynthesis	DNA repair and recombination proteins	Chromosome			
	Histidine metabolism	Pyrimidine metabolism	Protein folding and associated processing			
	Two component System		Peptidoglycan biosynthesis			

The number of *Prakriti* samples used in this analysis includes *Vata* (42 samples), *Pitta* (29 samples), and *Kapha* (42 samples) classes, whereas the *Sasang* samples include *SE* (13 samples), *TE* (14 samples), and *SY* (13 samples)

KEGG pathways using strategy 1 which might be due to the stringent criteria of statistical significance.

It is already known from previous reports that the *Prakriti* classes exhibit similar constitutions with respect to the *Sasang* classes. For example, the *Vata*, *Pitta*, and *Kapha* classes of the *Prakriti* are similar in constitution to the *SE*, *SY*, and *TE* classes of the *Sasang* system, respectively. Thus, we deduced the conserved functional features between the constitutionally similar *Prakriti* and *Sasang* classes, viz., *Vata–SE*, *Pitta–SY*, and *Kapha–TE* using the afore-mentioned strategies viz., 2, 3, and 4 (Table 3). In addition, we extracted the functional features which were either highly abundant in both, the *Prakriti* class and their correspondingly similar *Sasang* class or were low abundant in the corresponding classes of both the constitution types (Tables S3 and S4). This information may provide additional insights into the similarity between the *Prakriti* and *Sasang* classes in terms of the common functional features despite various factors influencing human gut microbiome. Since the number of samples used for *Sasang* TMS are very small, these results may be interpreted with caution. The text given below describes the common functional features and their roles in constitution-specific properties in the similar *Prakriti* and *Sasang* classes.

Vata- and *SE*-associated common functional features

As evident from Table 3, the common enriched functional pathways in the *Vata* and *SE* classes are "Carbohydrate metabolism", "Chloroalkane and chloroalkene degradation", "Phenylpropanoid biosynthesis", "Valine leucine and isoleucine biosynthesis", "Bacterial chemotaxis" and "Amino acid metabolism", and "Amino sugar and nucleotide

Table 3 List of common enriched functional features present in the *Prakriti* classes and their corresponding similar *Sasang* classes

Important functions in both classes (*Prakriti* and *Sasang*) (strategy 2)			
	Vata–SE	*Pitta–SY*	*Kapha–TE*
L3	Amino sugar and nucleotide sugar metabolism		
Important functions in one class and most abundant in other (*Prakriti* and *Sasang*) (strategy 3)			
	Vata–SE	*Pitta–SY*	*Kapha–SE*
L2	Amino acid metabolism		Enzyme families
	Carbohydrate metabolism		Signalling molecule and interaction
L3	Amino sugar and nucleotide metabolism	Polycyclic aromatic hydrocarbon degradation	Inorganic ion transport and metabolism
	Carbohydrate metabolism		Novobiocin synthesis
	Chloroalkane and chloroalkene degradation		
	Phenylpropanoid biosynthesis		
	Valine leucine and isoleucine biosynthesis		
Enrichment of the earlier identified female *Prakriti* class-specific functional signatures in *Prakriti* and *Sasang* classes (strategy 4)			
	Vata–SE	*Pitta–SY*	*Kapha–TE*
L2			Glycan biosynthesis and metabolism
			Infectious disease
			Metabolism of other amino acids
			Metabolism of terpenoids and polyketides
			Signalling molecules and ineractions
			Enzyme families
	Vata–SE	*Pitta–SY*	*Kapha–TE*
L3	Bacterial chemotaxis		Drug metabolism other enzymes
	Carbohydrate metabolism		Glutathione metabolism
			Glycine serine and threonine metabolism
			Lipopolysaccharide biosynthesis
			Peptidases
			Pores ion channels
			Protein digestion and absorption
			Ubiquinone and other terpenoid quinine biosynthesis

The number of *Prakriti* samples used in this analysis includes *Vata* (42 samples), *Pitta* (29 samples), and *Kapha* (42 samples) classes, whereas the *Sasang* samples include *SE* (13 samples), *TE* (14 samples), and *SY* (13 samples)

metabolism". A majority of short-chain fatty acids contributing to energy production are mainly produced through a fermentation process in the small intestine by the action of microbes (Mancabelli et al. 2017). The production of energy mainly from carbohydrates and a higher utilization of energy in the physiological processes might limit the accumulation of body fat. This might play an important role in the physiological characteristic of the *Vata* and *SE* classes of the *Prakriti* and *Sasang* systems, respectively, which are known to harbour lean phenotype as compared to the other constitution classes. Besides, amino acid metabolism was also found to be high in these classes. This also corroborates well with the finding of the Phenylpropanoid biosynthesis in this class which is known to be formed from protein fermentation in the human gut microbiome (Russell et al. 2013). This might also contribute to meeting with the higher energy demands of the *Vata* and *SE* individuals. The prevalence of Sarcopenia disease is found to be the highest in the *SE* class and a low availability of dietary amino acids is considered as one of the potential factors for its pathogenesis (Lee et al. 2015). In Ayurveda, Sarcopenia, a musculoskeletal type of disease, is known to be associated with the *Vata* class (Patwardhan et al. 2015). A potential role of the human gut microbiome in the Sarcopenia disease is recently hypothesized (Ticinesi et al. 2019). The individuals of the *SE* class have been found to consume less food as compared to those of the other classes of the *Sasang* system (Duc Pham et al. 2012). The *Vata* individuals have also been found to exhibit irregular appetite than the other *Prakriti* classes (Travis and Wallace 2015). Thus, the high energy requirements of the

SE individuals may be compensated by the high metabolism of carbohydrates by the gut microbes.

Further, we also observed that some commonly enriched functional features of *Kapha*, including "Protein folding and associated processing", "Enzyme families", and "Signalling molecules and interactions" were present in lowest abundance in the *Vata* and *SE* classes (Tables 3, S3). An opposite trend identified in the enriched functional features corroborates with the presence of a general opposite trend in the constitution-specific properties in the *Vata* and *Kapha* of the *Prakriti* constitution and *SE* and *TE* of the *Sasang* constitution. Further, our organism-level functional analysis revealed the lowest abundance of aerobic ($p = 0.2816$) and facultative anaerobic ($p = 1.33E^{-4}$) microbes in the *Vata* and SE classes of *Prakriti* and *Sasang*, respectively.

Pitta- and *SY*-associated common functional features

The "Polycyclic aromatic hydrocarbon degradation" was found to be commonly enriched functional feature present in the *Pitta* and *SY* classes of the *Prakriti* and *Sasang* constitutions, respectively (Table 3). PAHs are known to be toxic substances for human health and their metabolism via the action of gut microbes is well known (Van de Wiele et al. 2004). The enrichment of this pathway in the *Pitta* and *SY* classes highlights the microbial role in the metabolism of highly toxic substances leading to the diminished effect of these toxins on overall human health. A recent study revealed the enrichment of plasma metabolic markers associated with xenobiotic metabolism in the *Pitta* class (Shirolkar et al. 2018). A quick metabolism of toxic substances is a well-known phenotype associated with the *Pitta* class (Prasher et al. 2008).

Kapha- and *TE*-associated common functional features

The common enriched functional features present in the *Kapha* and *TE* classes are given in Table 3. Gut microbiome analysis of obese and normal individuals revealed an over-representation of the functional category "Glycan biosynthesis and metabolism" (Hou et al. 2017). The enrichment of this functional category in the *Kapha* and *TE* classes might explain their higher predisposition to the onset of obesity. Further, a lower abundance of glycine is observed in obese people, thus a higher production of glycine may provide additional beneficial effects to prevent obesity (Hou et al. 2017). Towards this, we have observed "Glycine serine and threonine metabolism" as a common functional feature in the *Kapha* and *TE* classes which may contribute to reducing the risk of obesity in these classes. It is noteworthy here that the individuals included in this study are all healthy. Thus, they may carry functions predisposing them to obesity but also harbour other important traits that help in maintaining their overall health.

Our analysis revealed an enrichment of the functional category "Metabolism of the other amino acids" and the subcategory "Glutathione metabolism" in *Kapha* and *TE* classes. Glutathione is known to be an important antioxidant and plays an important role in the maintenance of human health (Wilson and Nicholson 2017). Further, we also identified the enrichment of the functional feature "Drug metabolism and other enzymes" in the *Kapha* and *TE* classes. The metabolism of drug compounds has been shown to be carried out by gut microbes in the presence of glutathione, indicating an important role of glutathione in drug metabolism. It is known from previous reports that glycine is required for glutathione synthesis (Alves et al. 2019). Towards this, we have observed "Glycine serine and threonine metabolism" as the common functional feature in the *Kapha* and *TE* classes. In addition, we observed "Novobiocin synthesis" as another common functional feature between the *Kapha* and *TE* classes which may have primarily contributed to a high abundance of the functional category "Drug metabolism and other enzymes". Earlier reports have suggested a higher drug metabolism enzyme activity in the TE class (Lee et al. 2007). Novobiocin has been known to be effective against gram-positive pathogens (Liang et al. 2018). The contribution of this abundant pathway may also be corroborated by the less abundance of gram-positive microbes in the *Kapha* and *TE* classes (Fig. 4c).

The enrichment of the functional feature "Ubiquinone and other terpenoid-quinone biosynthesis" has been observed in the gut microbiome of colorectal cancer patients (Dai et al. 2018). The *Kapha* class is known to be highly predisposed to the onset of soft tissue cancer (Mobeen et al. 2019). However, the incidence of cancer in the *TE* class of *Sasang* has been found to be less than the other classes (Lee et al. 2013). Comprehensive research is needed to explain the existing differences between the *Kapha* and *TE* with respect to the incidence of soft tissue cancer. Further, our organism-level functional traits analysis revealed the highest abundance of gram-negative microbes and the lowest abundance of gram-positive microbes in the *Kapha* and *TE* classes of the *Prakriti* and *Sasang*, respectively. In addition, the lowest abundance of mobile genetic elements carrying microbes is found in the *Kapha* and *TE* classes of *Prakriti* and *Sasang*, respectively.

Our analysis revealed the presence of a higher number of common functional categories in the *Kapha* and *TE* categories followed by the *Vata* and *SE* and *Pitta* and *SY*. This trend is obvious as the *Vata* and *Kapha* reveal striking differences between them than with the *Pitta* in the *Prakriti* classes and the same is true for the *SE* and *TE* than *SY* in the *Sasang* classes. The presence of common functional

categories between the *Prakriti* classes and their correspondingly similar *Sasang* classes revealed the important contribution of the human gut microbiome in the phenotypes of *Prakriti* and *Sasang* classes, despite the effect of different factors, including geography, genetics, diet, and environment, etc. This study provides a baseline for the evaluation of the gut microbiome-based similarity between the *Prakriti* and *Sasang* classes. Given a very few studies exploring the human gut microbiomes of individuals classified by the Traditional Medicinal Systems, the availability of datasets is very limited which poses a limitation of our study. A comprehensive analysis is required to firmly establish the conclusions of this study by including more number of samples belonging to different age-groups and gender in each category, increasing the sequencing-depth, and by employing more sophisticated statistical analysis techniques.

Conclusions

This is the first attempt to explore the human gut microbiome level (taxa and imputed-functions) similarities between the constitutionally similar classes of the *Prakriti* and *Sasang* systems. The taxonomic level differences between the *Prakriti* and *Sasang* systems are evident from the enrichment of different phyla and enterotype taxa. These differences are well anticipated due to the other factors, including geography, diet, environment, genetics, etc. operating on the human gut microbiome. One of the interesting exploratory outcomes of this study is the finding of a lesser impact of the constitution on the *Sasang* than *Prakriti* system. It is also interesting to note that the gut adaptive functions, including stress-tolerance, biofilm-formation, and presence of facultative anaerobic microbes, etc., play important roles in the pathophysiological conditions of the constitution in both the *Prakriti* and *Sasang* systems. In addition, the *TE* class is found to be the most different than all the other *Sasang* and *Prakriti* classes with respect to the gut adaptive functions. We also conclude that some functional level similarity in gut microbiota is observed between the constitutionally similar *Prakriti* and *Sasang* classes. Particularly, the *Kapha* class is found to be highly similar to the *TE* class with respect to the gut microbiome functional features contributing to their constitution-specific properties and disease predisposition patterns. The present study provides useful information about the relatedness of the *Prakriti* and *Sasang* classification systems in terms of the gut-associated taxa and functions and can be utilized for the translation of the knowledge between the *Prakriti* and *Sasang* systems for the diagnosis of health and disease and the prescription of appropriate treatment regime.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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