J Biosci (2019) 44:112
DOI: 10.1007/s12038-019-9939-6

Understanding the association between the human gut, oral and skin microbiome and the Ayurvedic concept of *prakriti*

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Published online 20 September 2019

Ayurveda is one of the ancient systems of medicine which is widely practised as a personalized scientific approach towards the general wellness. Ayurvedic *prakriti* is broadly defined as the phenotypes which are determined on the basis of physical, psychological and physiological traits irrespective of their social, ethnic, dietary and geographical stature. *Prakriti* is the constitution of a person, which comprises *vata, pitta,* and *kapha* and is a key determinant of how one individual is different from the other. Human microbiome is considered the 'latest discovered' human organ and microbiome research reiterates the fundamental principles of Ayurveda for creating a healthy gut environment by maintaining the individual-specific microbiome. Hence, it is important to understand the association of human microbiome with the Ayurvedic *prakriti* of an individual. Here, we provide a comprehensive analysis of human microbiome from the gut, oral and skin samples of healthy individuals (n=18) by 16S rRNA gene-based metagenomics using standard QIIME pipeline. In the three different *prakriti* samples differential abundance of *Bacteroides, Desulfovibrio, Parabacteroides, Slackia,* and *Succinivibrio* was observed in the gut microbiome. Analysis also revealed *prakriti*-specific presence of *Mogibacterium, Propionibacterium, Pyramidobacter, Rhodococcus* in the *kapha prakriti* individuals *Planomicrobium, Hyphomicrobium, Novosphingobium* in the *pitta prakriti* individuals and *Carnobacterium, Robiginitalea, Cetobacterium, Psychrobacter* in the *vata prakriti* individuals. Similarly, the oral and skin microbiome also revealed presence of *prakriti*-specific differential abundance of diverse bacterial genera. *Prakriti*-specific presence of bacterial taxa was recorded and only 42% microbiome in the oral samples and 52% microbiome in the skin samples were shared. Bacteria known for preventing gut inflammation by digesting the resistant starch were abundant in the *pitta prakriti* individuals, who are more prone to develop gut-inflammation-related disorders. In summary, human gut, oral and skin microbiome showed presence or high abundance of few bacterial taxa across three *prakriti* types, suggesting their specific physiological importance.

Keywords. Ayurvedic *prakriti*; human microbiome; 16S rRNA gene; next generation sequencing

1. Introduction

The human microbiota consists of the 10–100 trillion symbiotic microbial cells harboured by each person, which possess ~10 times more bacterial cells than the number of human cells (Ley *et al.* 2006). Microbiome represents over 100 times the amount of genomic content compared to human genome (Qin *et al.* 2010). Human microbiome is emerging as a key player in maintaining human health and well-being by performing various functions ranging from digestion, protection against pathogen colonization to host immunity and central nervous system regulation (Huttenhower *et al.* 2012). Effect of different confounding factors on the human microbiome is well studied: the association of specific diet and the microbiome is well known (Turnbaugh *et al*. 2009; Singh *et al* 2017).

Electronic supplementary material: The online version of this article (https://doi.org/10.1007/s12038-019-9939-6) contains supplementary material, which is available to authorized users.

Ayurveda is one of the ancient well-written medical sciences, widely practiced in India (Chopra *et al.* 2010). It has an individualized approach towards management of health, and prevention and curing of illness/disease (Chopra *et al.* 2010). *Prakriti* is one of the important concepts of Ayurveda that defines personalized approach in health and diseases (Chopra *et al.* 2010). It is the basic constitution of an individual which is decided at the time of conception and remains unchanged throughout the life. According to Ayurveda, *prakriti* are classified into seven types: *vata*, *pitta*, *kapha*, *vata-kapha*, *vata-pitta*, *kapha-pitta* and *sama prakriti* (all three, i.e., *vata-pitta-kapha*). These *prakriti* exhibit specific functions, mainly structure, behaviour, response to environmental stimuli, susceptibility to diseases, etc. *Prakriti* types explain the physiological variations (Rotti *et al.* 2014). An individual may have a dominance of one or more *doshas* (bio physiological forces). Balance of the *doshas* results in homeostasis and good health, while vitiation or depletion of *doshas* leads to the disease (Govindaraj *et al.* 2015). Over time, the natural balance of the *doshas* in an individual can be disturbed by a number of factors, such as ageing, improper diet, lifestyle, stress levels and environmental pollution (Lakhotia 2014). Human microbiome is also known to differ in response to above-mentioned factors (Conlon and Bird 2015). The clinical manifestation of disease and its severity is determined by origin and mechanism of perturbation of *doshas* (Prasher *et al.* 2016). A recent study in the Indian rural population has showed that, although a substantial portion of gut microbiome is shared across the population, different *prakriti* types illustrate enrichment of specific bacterial taxa (Chauhan *et al.* 2018). With this preliminary information we decided to explore the association among the *prakriti* of an individual with the gut, skin and oral microbiome. In the present study, we provide a comprehensive analysis of the human microbiome from the gut, oral and skin ecosystems from 18 healthy individuals. 16S rRNA gene amplicon sequencing based microbiome analysis was done to understand the association between human microbiome and Ayurvedic *prakriti*.

2. Materials and methods

2.1 *Approval of the study*

The study was approved by the ethics committees of National Centre for Cell Science (NCCS) and King Edward's Memorial Hospital Research Centre (KEMHRC). Written informed consent from the study participants or parents of the study participants was obtained. All methods and experiments were performed by following the approved guidelines.

2.2 *Sample collection site*

The Vadu HDSS (health and demographic surveillance system) study site lies within two administrative blocks (Shirur and Haveli), which is about 40 km from Pune city. *Prakriti* assessment and sample collection was done from 53 individuals belonging to the HDSS area.

2.3 *Assessment of Prakriti and participant recruitment*

Prakriti assessment of study participants were performed by Ayurveda physician who were trained and experienced in assessing the *prakriti* as per the traditional practice in Ayurveda. These Ayurveda physicians used few clinical parameters for assessing the *prakriti*, which primarily includes observation, palpation, percussion, auscultation and asking questions regarding appetite, likes–dislikes, exercise, mental strength and diseases. For this, a *prakriti* assessment questionnaire was prepared using Ayurveda *Samhita* references which describe detailed *lakshanas* (characteristics) of *prakriti* (Rotti *et al.* 2014).

2.4 *Sample collection*

A questionnaire was filled during sample collection, seeking answers to questions regarding use of antibiotics, general health status, and sanitary practices. Freshly voided, early morning faecal samples were collected in sterile containers, early morning oral washings before brushing or gargling was collected in the form of washings using freshly prepared sterile 1X PBS (pH 7.4) from each study participant in sterile container, and skin samples were collected from 11 different body sites including forearm volar, palm, umbilicals, popliteal fossa, forehead, retro-auricular crease, manubrium, armpit, antecubital fossa, back, and anterior nares belonging to three different regions, i.e. moist region, oily region and sebaceous region. All samples were stored at −80°C temperature until further processing.

2.5 *Sample processing and next generation sequencing*

DNA extraction from faecal (representative of gut), oral and skin samples was done using QIAamp stool DNA mini kit, QIAamp DNA mini kit and QIAamp blood and tissue DNA extraction kit, respectively (Qiagen, USA). DNA extraction was done according to manufacturer's instruction. 16S rRNA gene amplicon sequencing based microbiome analysis was done to understand the correlation of microbiome with the *prakriti* types. Sequencing of V3-V4 region of 16S rRNA gene (average read length = 450 BP) was done using illumina Miseq paired end (2 * 300) sequencing technology.

2.6 *Pre-processing of read and bioinformatics analysis*

Assembly of forward and reverse reads for each sample was carried out using FLASH (Fast Length Adjustment of SHort

reads) (Magoc and Salzberg 2011). Microbial diversity analysis was done using standard QIIME (v1.8.0) pipeline (Navas-Molina *et al*. 2013) on the high-quality sequences. Closed reference based OTU picking approach was used to cluster reads into Operational Taxonomic Units (OTUs) at 97% sequence similarity using UCLUST algorithm (Edgar 2010) and greengene database (13.8) and representative sequences (repset) from each OTU were selected for taxonomic assignment. For the beta diversity analysis R scripts and R packages such as Phyloseq (McMurdie and Holmes 2013), online tool Calypso (Zakrzewski *et al.* 2017) were used. Statistical analysis was also performed using STAMP (Parks *et al.* 2014) and graphpad Prism (*www.graphpad.com*). Additionally, gut microbiome data of western Indian population was also surveyed using similar analysis pipeline for the presence and abundance of specific bacterial taxa across different *prakriti* types (Chauhan *et al.* 2018).

3. Results

Prakriti assessment of total 53 Individuals was done. Among these, 40 individuals having *pitta* pradhan *prakriti*, seven individuals has *vata* pradhan *prakriti* while only six study individuals has *kapha* pradhan *prakriti*. Randomly, six participants were selected from the *vata* and *pitta prakriti* to meet the number of participants in *kapha* pradhan *prakriti* for the microbiome analysis. Both male (n = 8) and female (n = 10) participants were included in the study (table 1).

3.1 *Association of human gut microbiome and prakriti type*

In total 28,31,418 good-quality sequences were obtained from the 35,49,865 raw sequences generated using 16S rRNA gene amplicon sequencing of the stool samples (n = 18). These sequences were clustered into 5,066 OTUs. Data normalization was done keeping 74,512 sequences per sample for the further microbiome analysis. Bacteria belonging to 21 different phyla were detected. Bacterial phyla Bacteroidetes (47%), Firmicutes (42%) and Proteobacteria (05%) were found to be highly dominant. Overall, higher abundance of bacterial genera *Prevotella* (43%), *Bacteroides* (14%) and *Dialister* (12%) in gut microbiome samples was observed. Preliminary investigations suggested high abundance of phyla Proteobacteria and Elusimicrobia in the *vata prakriti* samples, while Fusobacteria and Verrucomicrobia were highly abundant in the *pitta* and *kapha prakriti,* respectively (figure 1A). Presence of five statistically significant (ANOVA, $p \leq 0.05$) differentially abundant genera including *Bacteroides*, *Desulfovibrio, Parabacteroides, Slackia* and *Succinivibrio* (figure 1B) was observed across the three different *prakriti* types. *Bacteroides* and *Parabacteroides* were highly abundant in the *pitta prakriti* individuals while *Desulfovibrio, Slackia* and *Succinivibrio* were highly dominant in the *vata prakriti* individuals.

Analysis of gut microbiome data of earlier study by Chouhan *et al.* (2014) also showed the abundance of *Parabacteroides* and *Bacteroides* in the *pitta prakriti* individuals but those differences were not statistically significant (ANOVA, $p > 0.05$) (supplementary figure 1). Beta diversity analysis using bray-Curtis PCoA plot showed homogeneity in the microbiome composition of the *vata prakriti* samples by forming tight cluster separating the samples from *pitta* and *kapha prakriti* samples. *Pitta* and *kapha prakriti* samples were spread cross the plot (figure 1C). Shared and unique bacterial genera across the *prakriti* types revealed 53.6% sharing while 10%, 10.7% and 12.9% unique bacterial genera in the *vata*, *pitta* and *kapha prakriti* samples, respectively (figure 1D) (supplementary file 1). Genera *Enterobacter, Mogibacterium, Serratia, Pyramidobacter, Scardovia, Rhodococcus, Propionibacterium, Allobaculum, Methylobacterium, Eikenella, Zoogloea, Cronobacter* and *Dickeya* were only present in the *kapha prakriti* individuals. *Enterococcus, Lactococcus, Moryella, Pseudoramibacter, Cloacibacterium, Dermabacter, Flavisolibacter, Chlamydia, Planomicrobium, Trichococcus, Erysipelothrix, Hyphomicrobium, Novosphingobium, Acinetobacter, Anaeroplasma* and *Thermus* were only present in the *pitta prakriti* Individuals and genera *Anaerotruncus, Anaerofustis, Cetobacterium, Brachyspira, Robiginitalea, Alloiococcus, Carnobacterium, Sarcina, Pseudobutyrivibrio, Schwartzia, Gallicola, Desulfococcus, Psychrobacter and Meiothermus* were exclusively present in the *vata prakriti* individuals (supplementary file 1).

Table 1. Characteristics of the study participants

Sr. no	Participant ID	*Prakriti* type	Sex
1	D802	*Kapha*	Female
2	S102	*Kapha*	Female
3	S106	*Kapha*	Female
4	S107	*Kapha*	Male
5	S604	*Kapha*	Female
6	S618	*Kapha*	Male
7	D306	*Pitta*	Male
8	D308	*Pitta*	Female
9	D309	*Pitta*	Female
10	S104	*Pitta*	Female
11	S108	*Pitta*	Male
12	S607	*Pitta*	Female
13	D1001	*Vata*	Male
14	D101	*Vata*	Male
15	D106	*Vata*	Male
16	D302	*Vata*	Female
17	S105	*Vata*	Female
18	S601	*Vata*	Male

3.2 *Association of human oral microbiome and prakriti type*

Genera level microbiome analysis in the oral samples revealed abundance of *Neisseria* (22%), *Streptococcus*

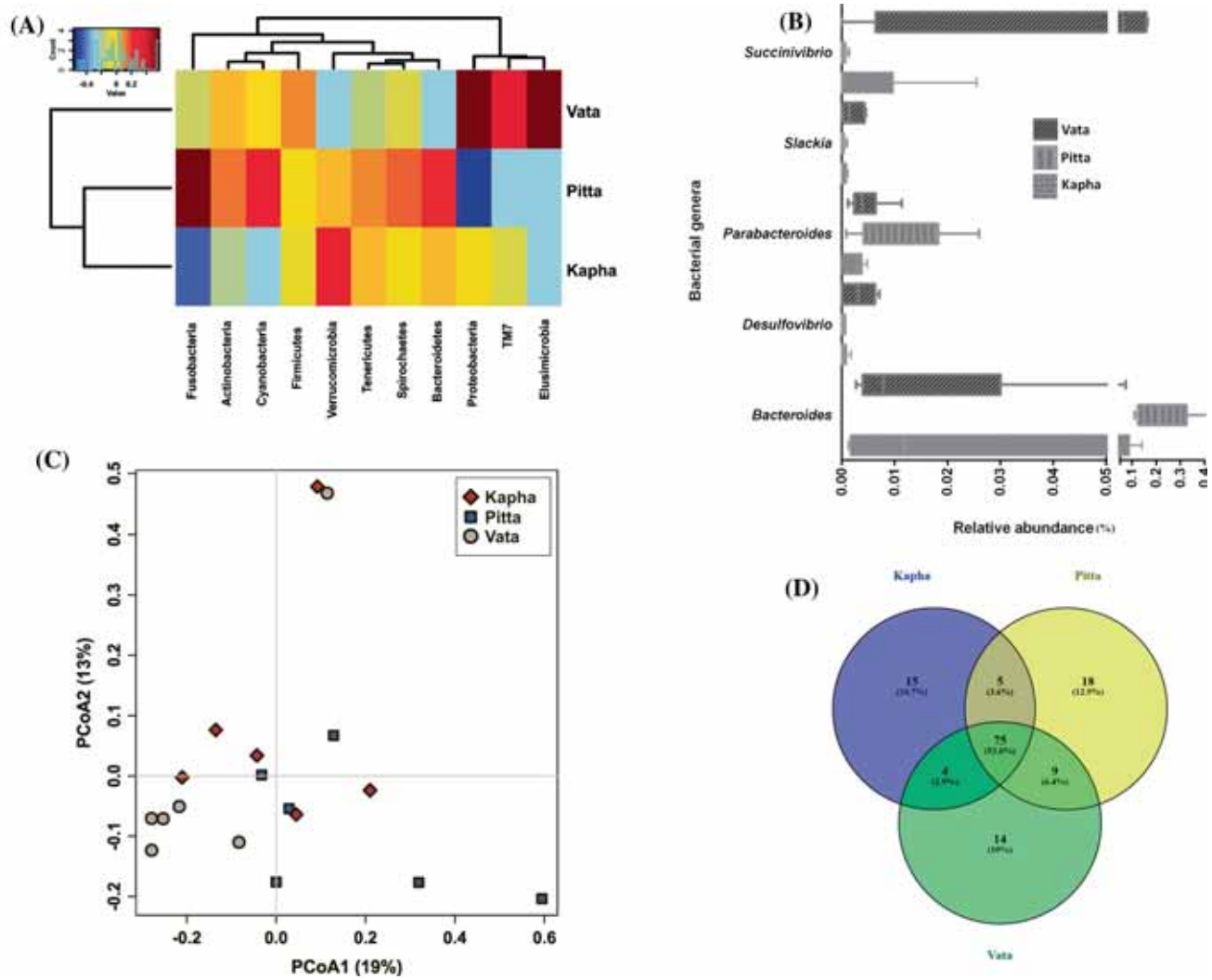

Figure 1. (**A**) Phylum-level distribution of bacterial populations in the *vata*, *pitta* and *kapha prakriti* types. (**B**) Differentially abundant bacterial genera in human gut microbiome across the *vata*, *pitta* and *kapha prakriti* individuals. (**C**) Multivariate analysis of beta-diversity: two-dimensional scatter plots of bacterial community (PCoA of 16S rDNA data) composition across the three different sample groups. Blue, red and grey colour represents samples from *vata*, *pitta* and *kapha prakriti* individuals, respectively. (**D**) Venn diagram of shared and *prakriti*-specific unique bacterial genera in the human gut microbiome. The numbers represent the *prakriti*-specific genera and total no of shared genera across all the study groups.

(15%), *Prevotella* (13%), *Haemophilus* (10%) and *Porphyromonas* (09%) across the samples. ANOVA analysis showed three statistically significant (ANOVA, $p < 0.05$) differently abundant genera based on the *prakriti* type. Wherein, genus *Leptotrichia* showed higher abundance in *kapha prakriti* samples, while *Gemella* and *Enhydrobacter* were dominant in *pitta prakriti* and *Campylobacter* and *Bifidobacterium* were dominant in *vata prakriti* samples (figure 2A). These three genera in addition to *Salenomonas* have showed differential abundance in the 2 group comparisons across the three *prakriti* types (figure 2B). The shared and unique bacterial genera analysis showed that 64 bacterial genera were common to all the three *prakriti* types and 21 genera were unique to *kapha*, 30 were unique to *pitta* and six genera, i.e. *Geobacillus, Lachnospira, Caulobacter, Hyphomicrobium, Mesorhizobium* and *Stenotrophomonas* were unique to *vata prakriti* samples (figure 2C) (supplementary file 2).

3.3 *Association of human skin microbiome and prakriti type*

Microbiome analysis of the skin samples revealed the differences in the mean relative abundance of bacterial genera *Corynebacterium*, *Streptococcus, Staphylococcus, Peptoniphilus, Alloiococcus, Anaerococcus, Porphyromonas, Paracoccus, Novosphingobium* and *Neisseria* in *vata, pitta* and *kapha prakriti* samples (figure 3A). Analysis of differentially abundant bacterial genera revealed presence of five

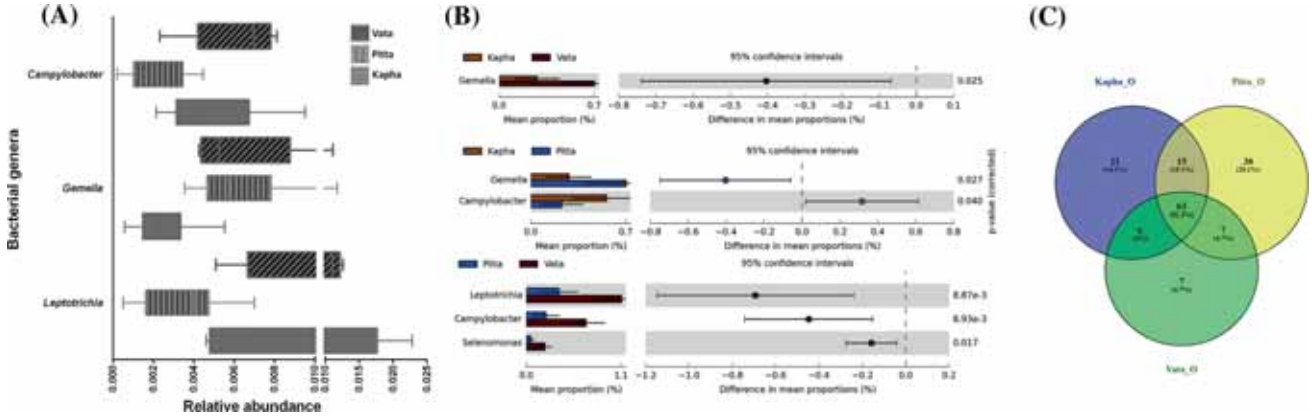

Figure 2. (**A**) Differentially abundant bacterial genera in human oral microbiome across the *vata*, *pitta* and *kapha prakriti* individuals. (**B**) Differentially abundant bacterial genera in human oral microbiome across the *vata*, *pitta* and *kapha prakriti* individuals in the 2 group comparisons using Welch t test analysis. (**C**) Venn diagram of shared and *prakriti*-specific bacterial genera in the human oral microbiome. The numbers represent the *prakriti*-specific genera and total no of shared genera across all the study groups.

bacterial genera (ANOVA, P<0.05). These genera include *Ruminococcus, Lysobacter, Mesorhizobium, Brevundimonas* and *Salegentibacter*, and all showed higher abundance in the skin microbiome of the *pitta prakriti* samples. Total 250 bacterial genera in the skin microbiome were shared in the three *prakriti* types, while 25, 72 and 22 genera were unique to the *pitta, kapha* and *vata prakriti,* respectively (figure 3B) (supplementary file 3). Beta dispersion analysis showed that *pitta prakriti* samples having less inter-sample variation, while *kapha prakriti* samples showed high inter-sample variation in the skin microbiome (figure 3C).

4. Discussion

Ayurveda is one of the oldest health sciences of the world. It is based on the concepts of *tridosha* and *prakriti* as the central philosophies. The primary aim of Ayurveda is maintenance of health and improvement of disorders in diseased people. Modernized practices derived from Ayurveda traditions are on similar lines with modern clinical practices (Sen and Chakraborty 2017). The present study is the first report explaining the detailed correlation of predominant Ayurvedic *prakrities* (*vata*, *pitta* and *kapha*) with the multiple human microbiomes (gut, oral and skin) of the individuals for unravelling the microbiome and *prakriti* associations. Ayurveda describes three fundamental entities that govern our inner and outer environments, viz. movement, transformation and structure, and are known in Sanskrit as *vata*, *pitta* and *kapha,* respectively (Pal 1991). These primary forces are responsible for the characteristics of our mind and body. And each of us has a unique proportion of these three forces that shapes our nature.

Here, analysis of human gut microbiome revealed five differentially abundant genera across the three *prakriti* types. Wherein, *Bacteroides* and *Parabacteroides* were found to be dominant in the *pitta prakriti* individuals (figure 1B). Earlier studies on the human gut microbiome reported that *Bacteroides* is one of the most abundant anaerobic organisms in the human gut (Wexler 2007). Members of genus *Parabacteroides* were found more in the *pitta prakriti* individuals. Gut microbiome studies exhibited that members of genus *Parabacteroides* play a key role in preventing gut inflammation by digesting the resistant starch (Hu *et al.* 2016), and are low abundant or absent in the IBD and ulcerative colitis patient (Noor *et al.* 2010). Ayurveda literature suggests that *pitta prakriti* individuals are more prone to develop gut inflammation related disorders like gastric ulcers (Dey and Pahwa 2014). Together, the altered human gut microbiome structure and Ayurveda literature substantiates high abundance of gut inflammation preventing organisms (*Parabacteroides*) in the *pitta prakriti* individuals. Microbiome data analysis of the study by Chauhan *et al.*, exploring gut microbiome and *prakriti* association, showed high abundance of *Parabacteroides* (statistically non-significant, ANOVA, $p > 0.05$) in the *pitta prakriti* individuals (supplementary figure 1) based on 4,000 reads per samples (Data normalization at 4000 reads/samples) using 29 samples each for *vata, pitta* and *kapha* (Chauhan *et al.* 2018). While, here in the present study, statistically significant differences were observed. We analysed less number of samples at high sequence depth ($\sim$ 74,000 sequences per sample), while in the earlier study, Chauhan *et al.* have analysed relatively more samples at lower sequence depth ($\sim$ 4,000 per sample). For this reason, future studies with additional samples at higher sequence depth are needed for precise understanding of microbiome and *prakriti* association. It is known that enteric nervous system is responsible for digestion and interaction with gut microbiome for modulation and activity of immune functions (Sharon *et al.* 2016). The *vata* brain-type exhibits a high range of digestive power, leading to an irregular appetite, bowel movements and frequent gas (Travis and Wallace 2015), and bacterial genus *Desulfovibrio* known for their ability for the production of H_2S (Motamedi and Karsten 1998) and methane were specifically

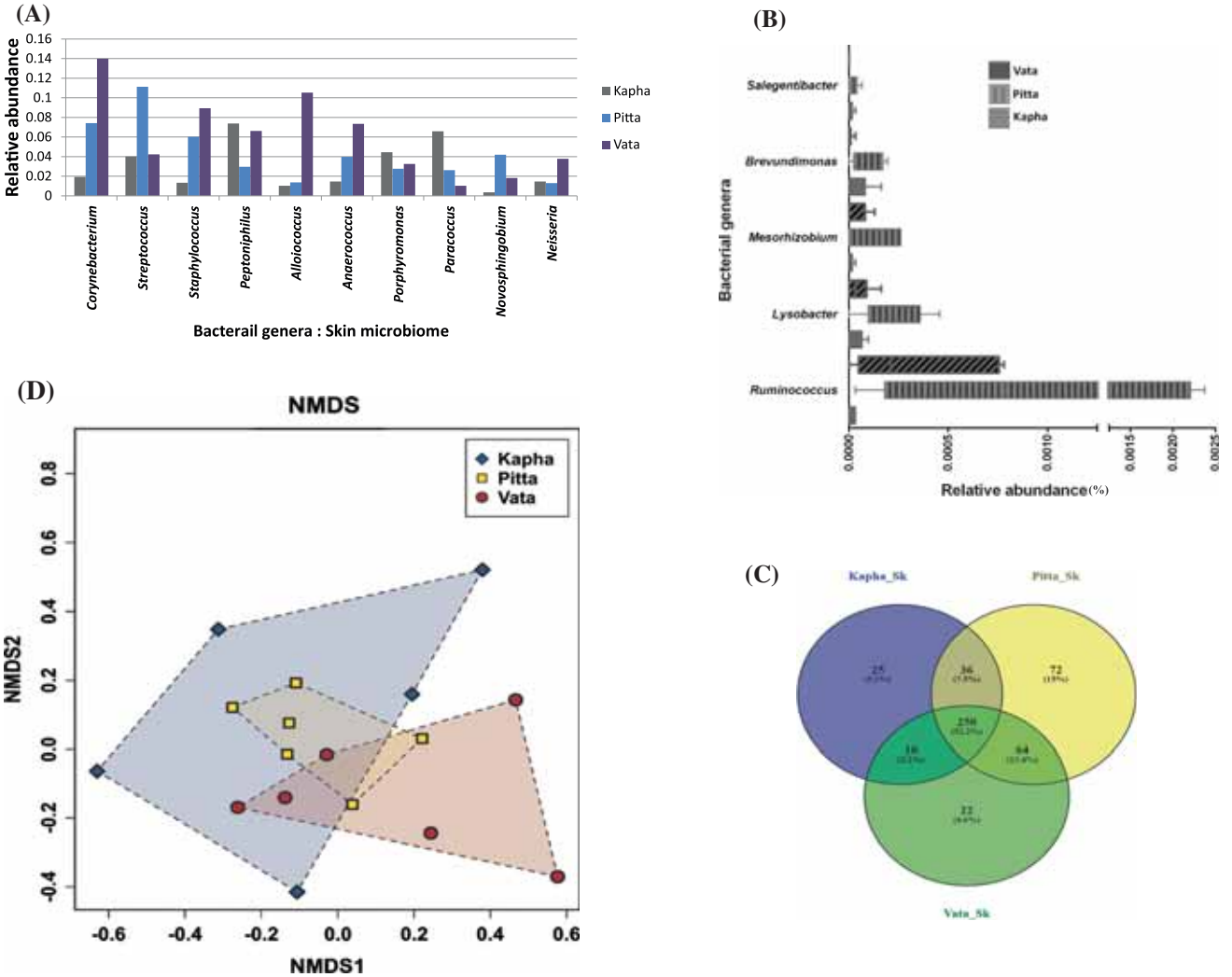

Figure 3. (**A**) Difference in the mean relative abundance in the bacterial genera in human skin microbiome across *the vata*, *pitta* and *kapha prakriti* individuals. (**B**) Differentially abundant bacterial genera in human skin microbiome across the *vata*, *pitta* and *kapha prakriti* individuals. (**C**) Venn diagram of shared and *prakriti*-specific bacterial genera in the human skin microbiome. The numbers represent the *prakriti*-specific genera and total no of shared genera across all the study groups. (**D**) Multivariate analysis of beta-diversity: two-dimensional scatter plots of bacterial community (NMDS of 16S rDNA data) composition across the three different sample groups. Blue, red and yellow colour represents samples from *vata*, *pitta* and *kapha prakriti* individuals respectively.

recorded in high abundance in the *vata prakriti* individuals, explaining the association of gut microbiome and Ayurvedic *prakriti*. Analysis suggested that overall most of the microbiome members are common across the samples of different *prakriti* and difference exists in the presence and abundance of key bacterial genera as largely the samples of the three *prakriti* types were dispersed across the PCoA plot and only 32% variation was explained (figure 1C). Bacteria interact exclusively within and between the species while they are responding to external stimuli. Human physiological responses changes with the *prakriti* type and it is the reason for *prakriti*-specific presence of few bacteria in the oral, gut and skin microbiome. However, studying one bacterium at a time or known consortium of bacteria and host physiological responses will help in understanding their precise associations. In addition to the gut microbiome, oral and skin microbiomes of these individuals were also studied. Oral microbiome analysis revealed the abundance of genera *Neisseria, Streptococcus, Prevotella, Haemophilus* and *Porphyromonas.* These are the most abundant organism's plays most important role in the formation of the healthy oral microbiome (Chen and Jiang 2014). Larger proportion the oral microbiome (63%) is shared across the three *prakriti* types, whereas *pitta prakriti* individuals showed most number of the unique bacterial genera (30 genera) and high inter-individual variation in the microbiome of *pitta prakriti* individuals is one possible explanation.

Analysis based on the abundance of the organisms revealed three differentially abundant genera (figure 1A). Among the three, *Leptotrichia* were found dominant in the

kapha prakriti individuals. These organisms are common inhabitant of the oral cavity. These organisms are opportunistic pathogens and ferment carbohydrates and produce lactic acid which may eventually be involved with tooth decay (Emenike and Olsen 2017). Genera *Gemella* and *Campylobacter* were also relatively high in the *pitta prakriti* individuals. *Gemella* were also the opportunistic pathogens (Jayananda *et al.* 2017). *Pitta prakriti* individuals are more prone to the disorders related to gums and teeth; here the abundance of opportunistic pathogens represents the putative *prakriti*-specific characteristics (Pravin *et al.* 2015) and the human oral cavity is a reservoir for the different *Campylobacter* species.

Complexion of the skin changes with the *prakriti* (Umarkar *et al.* 2013). *Vata prakriti* individuals show lustreless skin having hairs, skin and nails rough in texture and develop cracks due to dryness (Umarkar *et al.* 2013). Similarly, *pitta prakriti* person has fair body colour; they have a tendency for wrinkles and the hairs to turn gray at an early age, while *kapha prakriti* individuals have oily skin. Human skin microbiome showed the association with the characteristics of the skin (Prasuna and Srinivasulu 2013). Skin microbiome analysis of the abundant genera showed the differences in their relative abundance across the three *prakriti* type. Among the 479 bacterial genera, 5 genera showed statistically significant differential abundance in the skin microbiome. All these 5 genera have high abundance in the *pitta prakriti* samples (figure 3B). '*Pittam sasneha tikshnoshnam laghu visram, saram dravam*'(Ashtanga Hrdayam: Sutrasthana I:11) explains the main characteristics of the *pitta prakriti,* i.e. *pitta* is oily, sharp, hot, light, fleshy-smelling, spreading, and liquid. Here, the oily quality allows skin softness, the liquid quality exhibits excess sweating and fleshy-smelling indicates strong body odor. Bacteria including *Lysobacter, Rhizobium, Ruminococcus* were relatively high in the skin microbiome of *pitta prakriti* individuals (figure 3B). The bacteria like *Lysobacter* and *Rhizobium* (Yan *et al.* 2016) detected in agricultural soils and *Ruminococcus* is associated with the domestic animals like cattle (Guo *et al.* 2010). The present study population is rural agricultural population and the dominant bacteria present in their environment get adhered to the skin due to oily skin of the *pitta prakriti* individuals. However, samples from only three *pradhan* (dominant) *prakriti* types were included in the present study. Thus, microbiome analysis of study participants from all the seven *prakriti* types with additional sample size will be helpful for the precise understanding of these associations. In summary, human gut, oral and skin microbiome showed *prakriti*-specific presence and relative abundance (high or low abundance) of signature bacterial taxa. Microbiome structure of healthy individuals belonging to three different *prakriti* is explored so the findings of the study will further help in understanding and treating the specific diseases in future using Ayurveda treatment regimes by keeping microbiome knowledge as a base.

Acknowledgements

This work was supported by funding from the Department of Biotechnology, India, through a project entitled 'PUNE MICROBIOME STUDY – Molecular analysis of human microbiome' (DBT Grant Number: BT/PR3461/BRB/10/968/2011). The authors would like to acknowledge the Director, KEMHRC, Pune, and Director, NCCS, Pune, for their support. The authors would like to thank Mr. Shreyas Kumbhare for his suggestions in data analysis. DSC would like to thank the KEMHRC field staff for assisting in sample collection and the study participants.

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