HAYATI
Journal of Biosciences

Vol. 28 No. 4, October 2021 249-261
DOI:10.4308/hjb.28.4.249-261
ISSN: 1978-3019
EISSN: 2086-4094

Profiling Skin Microbiome in Healthy Young Adult Representing Javanese, Papuans, and Chinese Descent in Indonesia

Stella Vania, Amarila Malik*

Division of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, Universitas Indonesia, UI Depok Campus, Depok, Indonesia

ARTICLE INFO

Article history:
Received August 10, 2020
Received in revised form July 19, 2021
Accepted July 21, 2021

KEYWORDS:
Skin,
Microbiome,
Next Generation Sequencing,
Profiling,
Indonesia

ABSTRACT

Skin serves as the first physical barrier and biological barrier by the colonization of commensal bacteria to prevent pathogen invasion. It was known that the disruption on normal commensal microbiota composition or dysbiosis causes skin diseases, while the skin microbiota diversity itself is influenced by several factors, one of them is ethnicity. This study shows the influence of ethnicity factor in Papuans, Javanese, and Chinese descent young adults living in Jakarta on skin microbiome profiles. The microbiota genomic DNA are extracted from the face skin samples and sequenced with Next Generation Sequencing method to be further analyzed. The result shows that individuals with the same ethnic background share similar skin microbiome characteristics. The greatest skin microbiome alpha diversity is shown by the Papuans and the Chinese descent the smallest. Ethnicity factor that shows statistically significant differences in interindividual dissimilarities are independent of other intriguing factors such as age, geographical location, etc. Therefore the ethnic origin of individuals especially from three ethnics above is a factor to be considered in skin microbiome research and the skin microbiota composition can be used for potential future applications.

1. Introduction

As the largest and the outermost part of human body that directly exposed to the outer environment, skin serves as the first physical barrier and is colonised by skin commensal microbiota who serves as biological barrier to prevent pathogen invasion (Byrd *et al.* 2018). The balance interaction between microbiota either synergistically or competitively could prevent colonization of pathogenic bacteria. For example, *Staphylococcus aureus* is known as a pathogenic bacteria that is mostly identified as the cause of many skin infections such as impetigo, rosacea, etc. *Staphylococcus epidermidis*, who is known as part of skin commensal bacteria, produces serine protease glutamyl endopeptidase (GluSE) which degrades protein needed for biofilm making and epitel adhesion of *Staphylococcus aureus* (Iwase *et al.* 2010; Sugimoto *et al.* 2013). Another skin commensal bacteria such as *Staphylococcus lugdunensis* also produces antibiotic lugdunin which synergistically prevents colonization of *Staphylococcus aureus* (Zipperer *et al.* 2016).

* Corresponding Author
E-mail Address: amarila.malik@ui.ac.id

It was also known that skin diseases such as atopic dermatitis, psoriasis, rosacea, acne vulgaris, etc are often caused by dysbiosis or imbalance in normal skin microbiota composition rather than pathogens (Ong *et al.* 2002; Nomura *et al.* 2003; De Jongh *et al.* 2005; Gudjonsson *et al.* 2009; Grice and Segre 2011; Sanford and Gallo 2013; Gupta *et al.* 2017; Altonsy *et al.* 2020). Understanding the skin microbiome variation might explore the variation of susceptibility to certain pathologies or skin disorders (Chng *et al.* 2016; Gupta *et al.* 2017). Therefore profiling skin microbiota is interesting as it opens up a new skin therapeutic option in using probiotic and commensal skin microbiota to be developed as bacterial cocktail or postbiotic to maintain normal skin microbiota composition.

Microbiome composition on human body is affected by various factors such as age, gender, genetic, anatomical location, medication, lifestyle, and geographical location (Grice and Segre 2011; Oh *et al.* 2012; Mason *et al.* 2013; Perez *et al.* 2016; Gupta *et al.* 2017; Gaulke and Sharpton 2018). Studies show the ethnicity variations in human microbiome composition and diversity at gut, oral cavity, skin, and urogenital tract indicating that individuals with the same ethnic tend to have more

similarity in microbiome diversity rather than those with different ethnic (Gupta *et al.* 2017; Deschasaux *et al.* 2018). This study focused on the influence of ethnicity factor on skin microbiome composition in three ethnicities in Indonesia, which are Papuans, Javanese, and Chinese descent young adults. Determination of skin microbiota composition and diversity was conducted by employing Next Generation Sequencing (NGS) of gDNA obtained from face skin swab samples. The alpha and beta diversity were calculated quantitatively and qualitatively according to indices based on richness (observed OTUs) and divergence (phylogenetic diversity).

2. Materials and Methods

2.1. Participants

The participants consist of 3 men and 3 women (age 18–25, living in Jakarta, similar routine activities) from every ethnic (Papuans, Javanese, and Chinese descent) provided written informed consent. Data from previous study on "Simultaneous Profiling and Cultivation of the Skin Microbiome of Healthy Young Adult Skin for the Development of Therapeutic Agents" (Khayyira *et al.* 2020) was employed in this study as Javanese population sample. This study was approved by the Research Ethical Committee Faculty of Medicine University of Indonesia (0049/UN2.F1/ETIK/2019). All participants are free from dermatologic disease (i.e. atopic dermatitis and psoriasis) and not using antibiotics for at least 3 months before and during sampling period. The participants were asked not to wash their faces for a minimum of 5 hours prior to sampling and not to use skincare or cosmetic products on sampling day (Dekio *et al.* 2005; Jo *et al.* 2016).

One Javanese male and one Javanese female were later identified that they have Jambi descent from the father lines and Balinese descent lines from the mother lines consecutively. After sample normalization for Javanese, the number of subjects from the other ethnic groups (Papuans and Chinese descent) was also deducted to 4 subjects so the number of individuals from each population is equal.

2.2. Samples Collection and DNA Extraction

Skin microbiota samples were collected by using swabbing method on forehead and cheeks skin (21) of the participants making Z-stroke for at least 15 seconds (Leung *et al.* 2015). Swabbing was done with two sterile cotton swabs (each for different areas) which previously soaked with the recovery diluent (0.9% NaCl and 0.1% Tween-20 solution) (Ogai *et al.* 2018). The cotton swabs were cut aseptically, put into 1 ml of the recovery diluent, and homogenized by vortexing then stored in freezer -20°C. The sampling was carried out 4 times for each subject at different times within maximum 2 weeks.

The bacterial pellets from 4 samplings of each subject were collected into one microtube using centrifugation at 14,000 g for 2 minutes. The genomic DNA extraction from the bacterial pellet was carried out by Presto™ Mini gDNA Bacteria Kit (Geneaid, Taiwan) according to its protocol.

2.3. PCR Amplification and Purification

A region of the microbial 16S rRNA gene containing variable segments V3 and V4 were amplified using 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R primer (5'-GGACTACNNGGGTATCTAAT-3') (Takahashi *et al.* 2014). PCR was carried out using Phusion® High-Fidelity PCR Master Mix with HF buffer according to its protocol. The PCR product proceed to quality control by mixing with the same volume of 1X loading buffer containing SYBR green and operate electrophoresis on 2% agarose gel. The samples showing bright band on 400–450 bp passed and proceed to be purified using QIAquick® Gel Extraction Kit according to its protocol.

2.4. NGS Analysis

Library preparation was carried out using NEBNext® Ultra™ DNA Library Prep Kit for Illumina

according to its protocol and sample-specific barcode sequences were ligated to the PCR products. The libraries generated were quantified using Qubit and mixed at equal density ratios. Sequencing was carried out using Illumina NovaSeq 6000 sequencer with NovaSeq Control Software by Novogene Co, China, according to the manufacturer's protocol.

2.5. Data Analysis

The initial sequence reads were demultiplexed according to its barcode, then the primer and barcode from each reads were removed. Read 1 and read 2 from each sample were merged using FLASH (Magoč and Salzberg 2011) then quality filtering (Bokulich *et al.* 2013) was done with QIIME. Chimera sequences were removed (Haas *et al.* 2011) in accordance with GOLD database using the UCHIME algorithm (Edgar *et al.* 2011). Effective tags obtained were used for OTU clustering with closed reference types using QIIME based on SILVA SSU rRNA 138 database with 97% similarity (Caporaso *et al.* 2010).

The clustered sequences were assigned to its taxonomy annotation by the Classifier approach. The taxonomy classification results are visualized by barplot and circular representation with KRONA (Ondov *et al.* 2011). The phylogenetic tree is also made based on the clustered sequences using QIIME with Multiple Alignment using Fast Fourier Transform (MAFFT) algorithm, masking alignment, and FastTree algorithm.

Alpha and beta diversity analysis was calculated using QIIME, including observed OTUs, Shannon's index, and Faith's Phylogenetic Diversity for alpha diversity, and Bray-Curtis dissimilarity, Jaccard Index, Weighted and Unweighted Unifrac for beta diversity (Lozupone and Knight 2008). Samples were rarefied at 74,443 (minimum read number among samples). Significances of alpha diversity distribution between groups were calculated with Kruskal-Wallis. Significances of beta diversity were calculated based on PERMANOVA with pseudo-F test. Alpha rarefaction plot and visualization by principal coordinates analysis for beta diversity indices were carried out using QIIME. Linear discriminant analysis was calculated with LEfSE algorithm and plot in cladogram.

3. Results

Skin microbiota samples from each subject were coded according to their ethnicity (J–Javanese, P–Papuans, C–Chinese descent). After sequenced with next generation sequencing and analyzed with QIIME, total reads and OTUs from each sample are summarized in Figure 1. Taxonomic annotation at kingdom until genus level are visualized with column graph as presented in Figure 2.

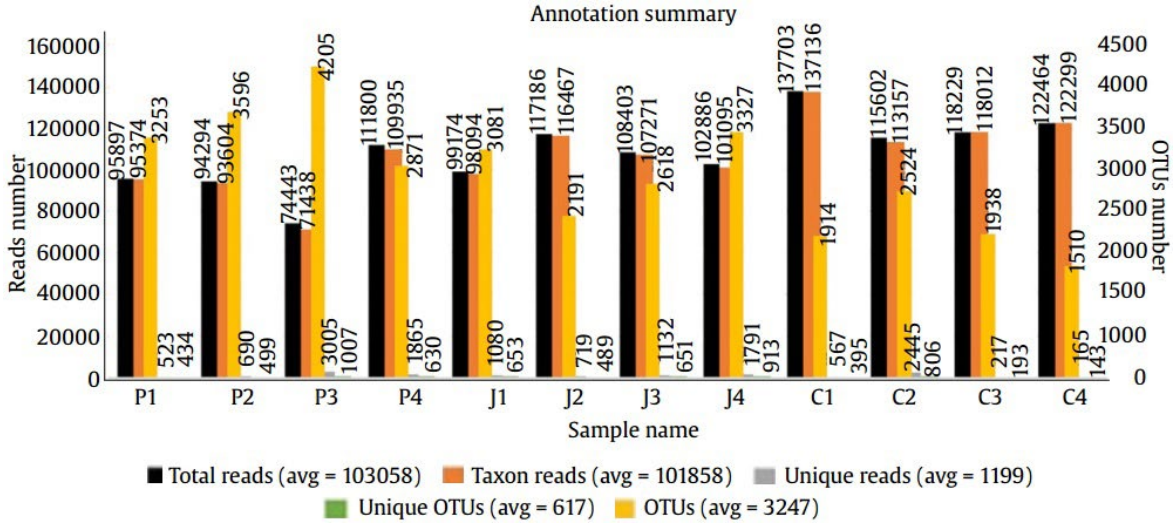

Figure 1. Annotation summary

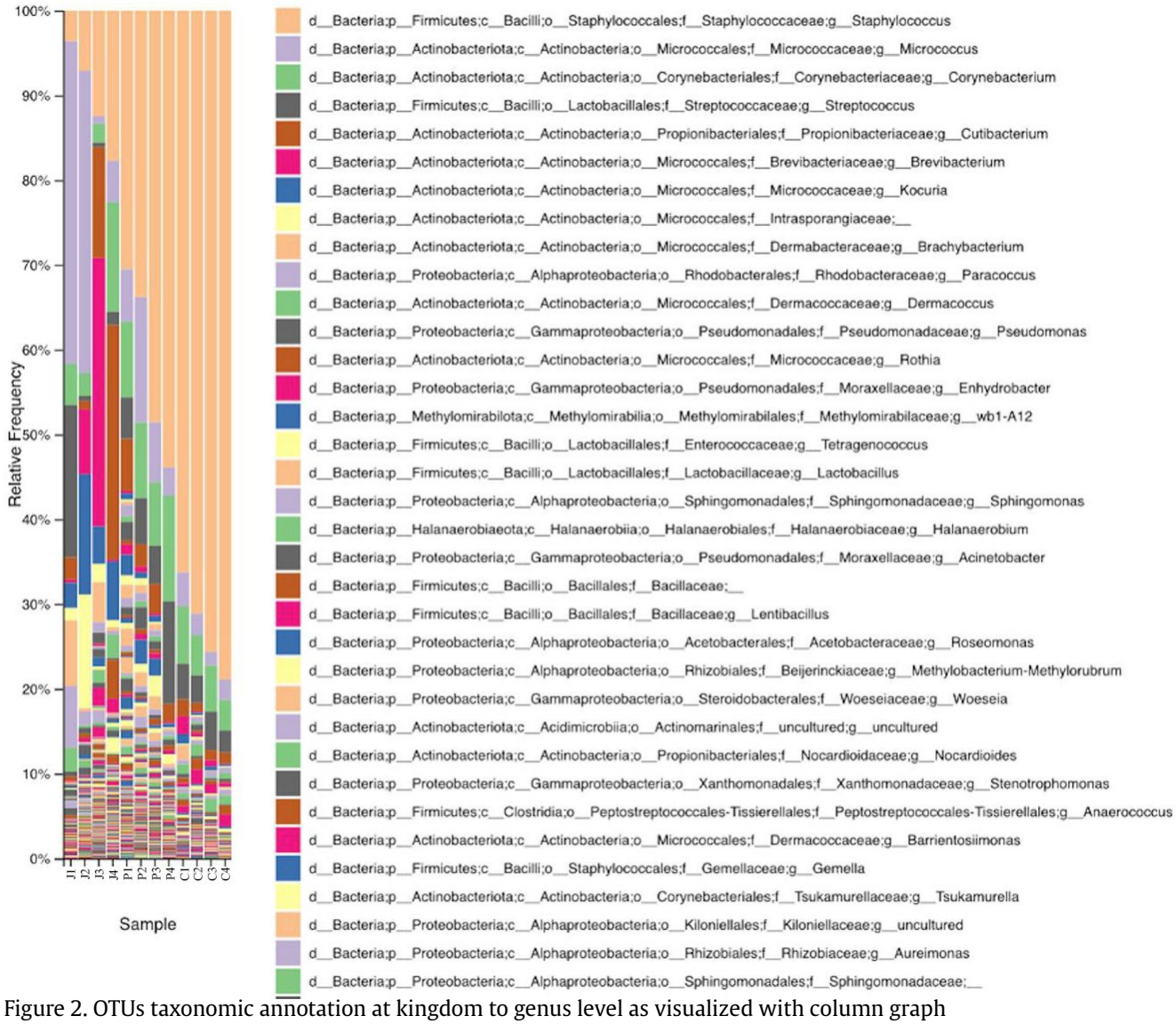

Figure 2. OTUs taxonomic annotation at kingdom to genus level as visualized with column graph

Taxonomic annotation for 50 highest abundance OTUs in every ethnic group are summarised in Table 1. Microbiota composition in every ethnic group is also visualized in circular representation by Krona in genus level as it has high confidence for its annotation, since not every OTU can produce high identity percentage in species level (Figure 3).

Alpha diversity based on observed OTUs, Shannon index, and Faith's Phylogenetic Diversity are visualized by boxplot in Figure 4a. Alpha rarefaction curves based on observed OTUs and Shannon index with minimum sampling depth can be seen in Figure 4b. Beta diversity analysis based on Bray Curtis dissimilarity, Jaccard index, weighted Unifrac and unweighted Unifrac are visualized with PCoA method resulted in graph plotting shown in Figure 5.

Table 1. 50 Highest OTU taxonomic annotation in every ethnic group

Papuans			
Staphylococcus epidermidis	*Lactobacillus rennini*	*Woeseia*	*Janibacter*
Staphylococcus capitis	*Pseudomonas stutzeri*	*Staphylococcus haemolyticus*	*Sphingomonas molluscorum*
Streptococcus gallolyticus	*Staphylococcus pettenkoferi*	*Corynebacterium singulare*	*Staphylococcus aureus*
Staphylococcus	*Tetragenococcus halophilus*	*Kocuria palustris*	*Corynebacterium*
Micrococcus	*Corynebacterium jeikeium*	*Methylobacterium-Methylorubrum*	*minutissimum*
Corynebacterium tuberculostearicum	*Streptococcus*	*Roseomonas mucosa*	*Corynebacterium ureicelerivorans*
Cutibacterium acnes	*Moraxella osloensis*	*Staphylococcus caprae*	*Corynebacterium imitans*
Staphylococcus cohnii	*Brachybacterium paraconglomeratum*	*Aureimonas altamirensis*	*Rothia amarae*
			Brevibacterium pityocampae
Methylomirabilaceae	*Dermacoccus nishinomiyaensis*	*Clostridium tarantellae*	*Sphingomonas*
Corynebacterium	*Staphylococcus saprophyticus*	*Virgibacillus*	
Javanese			
Micrococcus	*Streptococcus gallolyticus*	*Barrientosiimonas humi*	*Micrococcus luteus*
Brevibacterium casei	*Staphylococcus capitis*	*Corynebacterium*	*Cutibacterium granulosum*
Cutibacterium acnes	*Staphylococcus*	*Micrococcus terreus*	*Staphylococcus warneri*
Janibacter	*Staphylococcus caprae*	*Staphylococcus aureus*	*Staphylococcus epidermidis*
Kocuria rhizophila	*Streptococcus*	*Roseomonas mucosa*	*Nocardioides rotundus*
Brachybacterium paraconglomeratum	*Dermacoccus*	*Dermacoccus nishinomiyaensis*	*Pseudomonas stutzeri*
Corynebacterium tuberculostearicum	*Staphylococcus haemolyticus*	*Rothia mucilaginosa*	*Gordonia*
Staphylococcus saprophyticus	*Strenotrophomonas maltophilia*	*Micrococcus yunnanensis*	*Knoellia*
Kocuria palustris	*Corynebacterium suicordis*	*Tsukamurella*	*Truepera*
Paracoccus	*Moraxella osloensis*	*Propionibacterium* sp.	
Chinese			
Staphylococcus epidermidis	*Halanerobium prevalens*	*Methylobacterium-Methylorubrum*	*Sphingomonas molluscorum*
Staphylococcus capitis	*Staphylococcus caprae*	*Kocuria palustris*	*Brevibacterium*
Staphylococcus cohnii	*Virgibacillus*	*Brevibacterium pityocampae*	*Corynebacterium singulare*
Staphylococcus	*Moraxella osloensis*	*Staphylococcus saprophyticus*	*Sphingomonas*
Streptococcus gallolyticus	*Dermacoccus nishonomiyaensis*	*Pseudomonas stutzeri*	*Lentibacillus juripiscarius*
Corynebacterium tuberculostearicum	*Brachybacterium paraconglomeratum*	*Halanaerobium fermentans*	*Paracoccus*
Micrococcus	*Staphylococcus haemolyticus*	*Corynebacterium jeikeium*	*Tetragenococcus halophilus*
Staphylococcus pettenkoferi	*Streptococcus*	*Staphylococcus warneri*	*Staphylococcus cohnii*
Cutibacterium acnes	*Corynebacterium*	*Roseomonas mucosa*	*Streptococcus salivarius*
Lentibacillus sp.	*Staphylococcus aureus*		

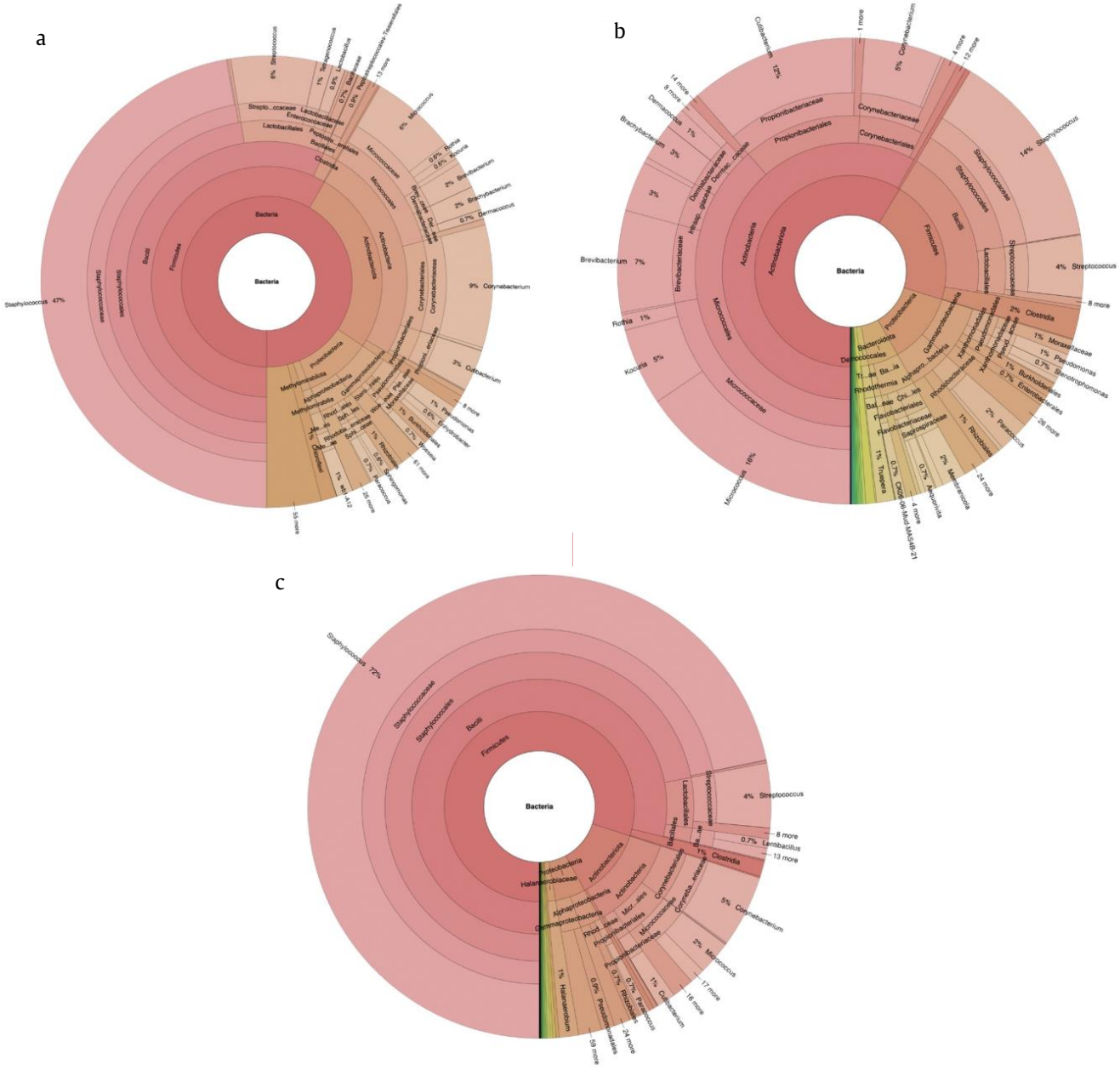

Figure 3. Microbiota composition of skin microbiome of three ethnic groups visualized in circular representation by Krona at genus level; (a) Papuans, (b) Javanese, and (c) Chinese descent

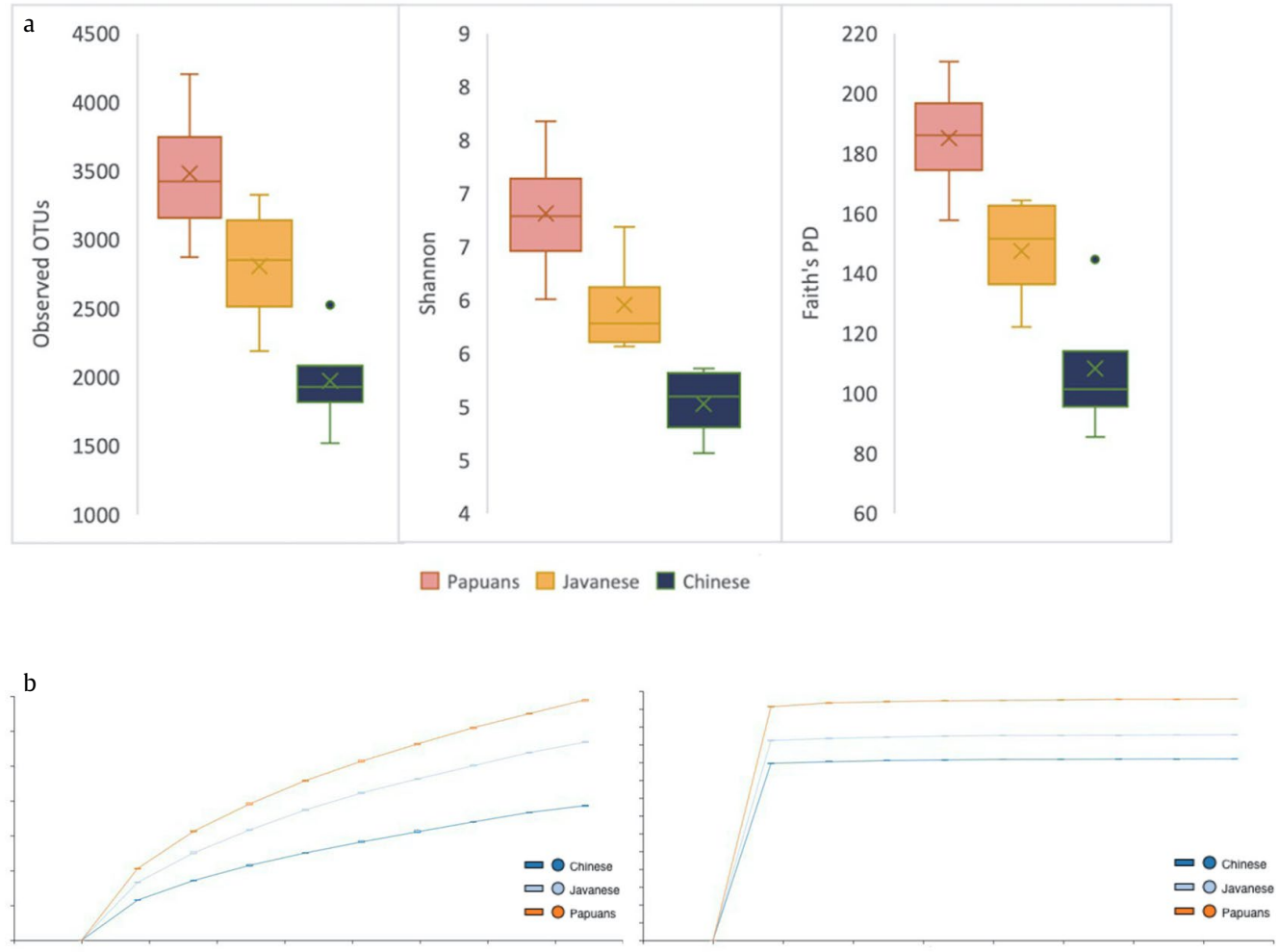

Figure 4. Microbiota composition of skin microbiome of three ethnic groups analyzed by (a) Alpha diversity based on observed OTUs, Shannon index, and Faith's Phylogenetic Diversity, from left to right, respectively, and visualized by boxplot; and by (b) Alpha rarefaction curves based on observed OTUs (left) and Shannon index (right)

Linear discriminant analysis to shows distinctive factors between ethnic groups is done with Linear Discriminant Analysis Effect Size (LEfSE) algorithm. The analysis result is visualized in cladogram shown in Figure 6.

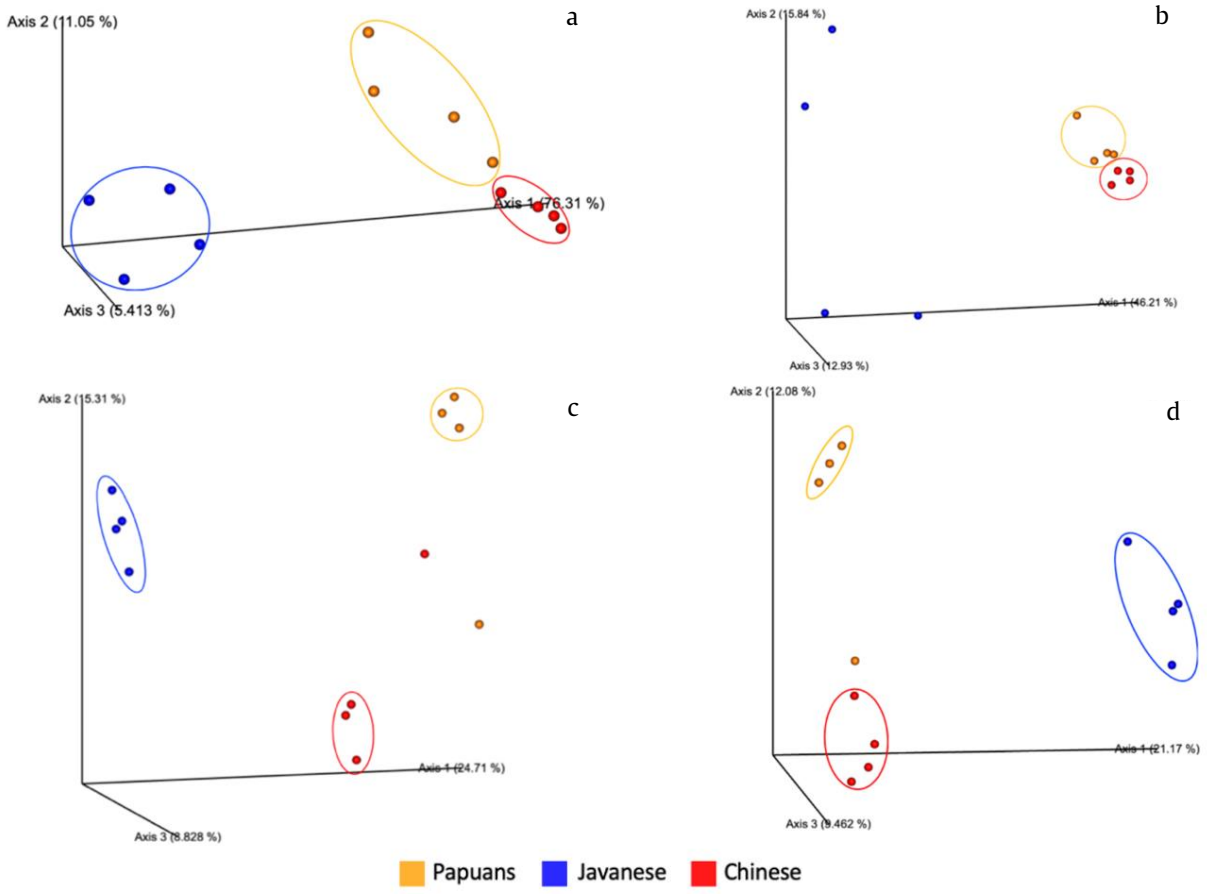

Figure 5. PCoA on beta diversity based on (a) weighted Unifrac, (b) Bray Curtis dissimilarity, (c) unweighted Unifrac, (d) Jaccard index

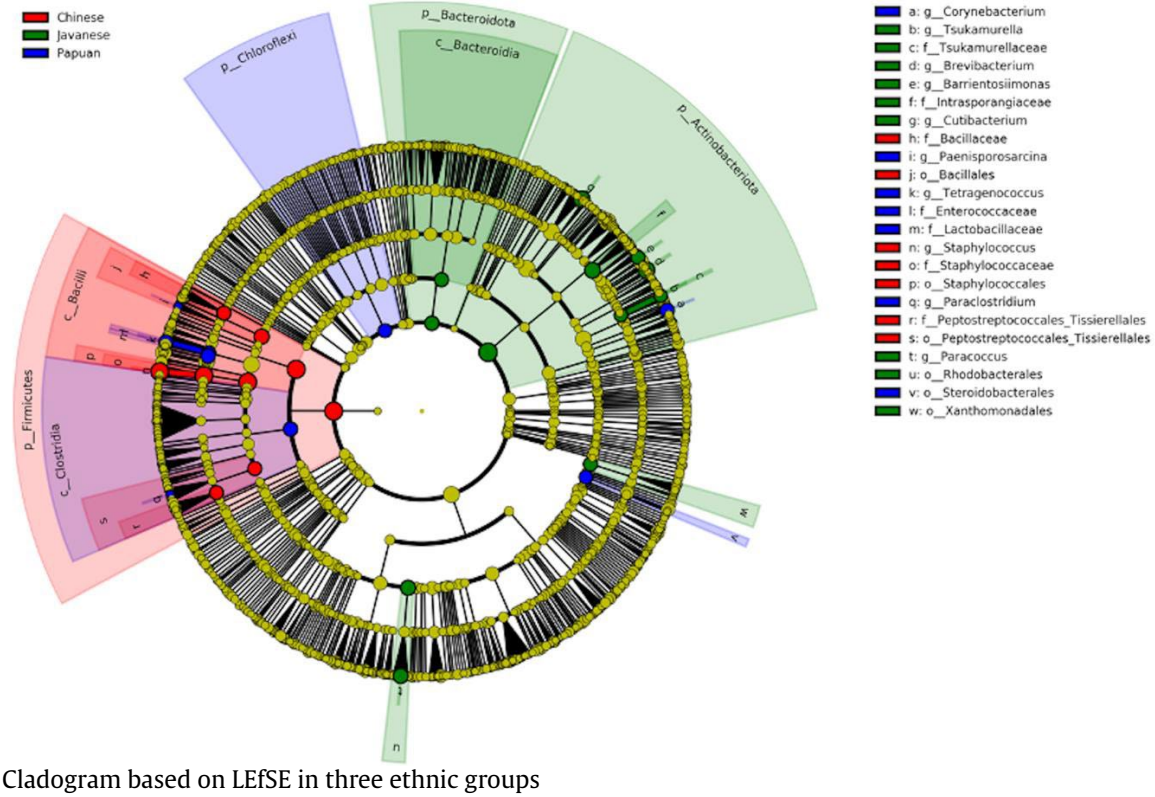

Figure 6. Cladogram based on LEfSE in three ethnic groups

4. Discussion

In order to minimize confounding factors, inclusion and exclusion criteria apply for participants. All participants live in shared geography, Jakarta, and are college students in the same university so they have similar routine activity in which the extreme one might affect the skin microbiome profile. All participants are young adults with age within 21–22 years old so the skin microbiome is expected to have stabilized. As it is difference with gastrointestinal microbiota which stabilize since 3 years old (Yatsunenko *et al.* 2012), the skin microbiome diversity is restructured during puberty when the increasing hormone levels stimulates sebaceous glands to produce more sebum so it is expected to have more lipophilic microorganisms such as Propionibacterium and Corynebacterium during post-puberty (Oh *et al.* 2012). Subjects with healthy skin and doesn't have any skin diseases are chosen so the normal skin microbiota composition is not affected as studies show that diseases such as atopic dermatitis, psoriasis, rosacea, acne, etc are often caused by disruption on normal skin microbiota (Ong *et al.* 2002; Nomura *et al.* 2003; De Jongh *et al.* 2005; Gudjonsson *et al.* 2009).

To investigate the relation between host and microbiota in stabilized physiological conditions, the face area is chosen as the place of sampling which includes forehead and cheeks. Other than the main purpose to develop skin microbial therapeutic which mainly focuses on face skin, the site is considered to maintain a tight host–parasite relationship, through enriched nutrient supplementation and by the antibiotic peptides of sebaceous secretions (Dekio *et al.* 2005). The site is also not covered by clothes and not likely to contact with anything compared to other body parts such as palm. The site is also exposed to the outer environment so the individuals' forehead are expected to experience the same environmental condition. During sample collection periods, subjects are not allowed to wash their faces with water or soap for at least 5 hours before the sample is taken to increase the gaining of normal skin microbiota. Nevertheless, all subjects are needed to take shower and wash their faces in the morning to prevent microbiota transfer from pillow used.

Ethnicity significantly contributed to the interindividual dissimilarities in all indices based on both OTUs and phylogenetic tree ($p<0.005$; Table 2). Beta diversity was calculated quantitatively with Bray Curtis dissimilarity and Weighted Unifrac, and qualitatively with Jaccard index and Unweighted Unifrac. The grouping and clear separation were observed in visualization by Principal Coordinate Analysis (PCoA) based on Weighted Unifrac. It shows that individuals with same ethnic group tend to share similar skin microbiota composition quantitatively. On PCoA based on Bray Curtis dissimilarity, individuals in Javanese groups do not show a great grouping, this might explain that the abundance in Javanese groups are concentrated in skin microbiota which are closely related in phylogenetic tree. As additional data, clear separation based on ethnicity is also shown qualitatively, which also shows grouping based on ethnicity. Although one or two subjects are separated from its group, it might show that those individuals have unique OTUs but the abundance is not significant, therefore it does not show in PCoA based on quantitative indices.

Alpha diversity between ethnic groups is analyzed with observed OTUs, Shannon index, and Faith's Phylogenetic Diversity. The observed OTUs index analyzed qualitatively the richness of every individual based on the number of OTUs, while Shanonn index also counts the evenness of the OTUs abundances. Faith's Phylogenetic Diversity calculated qualitatively the richness based on its phylogenetic tree. Similar to beta diversity, skin microbiota alpha diversity differed between ethnic groups and is statistically significant with p-value from all indices <0.005 (Table 3). The greatest alpha diversity is shown in Papuans and the smallest in Chinese descent. The Papuans show high richness in observed OTUs and Faith's PD, followed by the Javanese and Chinese descent. Unevenness shows the most by the Chinese descent group with the lowest Shannon index, as seen on the taxonomical circular representation, the 72% composition of the Chinese group consists of Staphylococcus.

According to linear discriminant analysis, it shows that Chinese descent group has distinctive features from Javanese and Papuans in phylum Firmicutes class Bacilli order Bacilli family Staphylococcaceae genus Staphylococcus and order Bacillales family Bacillaceae; also order and family Peptostreptococcales tissierellales. While Papuans have distinctive features with phylum Chloroflexi; class Clostridia genus Paraclostridium; family Lactobacillaceae; family Enterococcaceae genus Tetragenococcus; genus Paaenisporosarcina; genus Corynebacterium; and order Steroidobacterales. Distinctive features on Javanese are phylum Bacteroidota class Bacteroidia; phylum Actinobacteriota genus Cutibacterium; family Intrasporangiaceae genus Barrientosiimonas, genus Brevibacterium, and family Tsukamurellaceae genus Tsukamurella; order Xanthomonadales; and order Rhodobacterale genus Paracoccus.

Although those three ethnicities showed distinctive features on microbiota's composition as discussed above, it is revealed that they do share similarities as much as 791 OTUs as analyzed and visualized in the Venn diagram in Figure 7. This population can be assumed as basic skin microbiome features of commensal microbiotas.

Table 2. Beta diversity

		J1	J2	J3	J4	P1	P2	P3	P4	C1	C2	C3	C4
C4	w	0.736	0.659	0.739	0.649	0.281	0.426	0.461	0.229	0.059	0.073	0.133	0.000
	u	0.746	0.691	0.713	0.734	0.673	0.690	0.697	0.653	0.552	0.633	0.528	0.000
C3	w	0.641	0.551	0.644	0.540	0.168	0.308	0.347	0.118	0.120	0.105	0.000	
	u	0.727	0.668	0.686	0.703	0.646	0.659	0.677	0.644	0.563	0.624	0.000	
C2	w	0.698	0.616	0.698	0.607	0.227	0.371	0.406	0.185	0.069	0.000		
	u	0.748	0.719	0.724	0.740	0.613	0.608	0.622	0.643	0.642	0.000		
C1	w	0.725	0.637	0.727	0.627	0.257	0.399	0.434	0.206	0.000			
	u	0.722	0.682	0.692	0.707	0.657	0.674	0.671	0.653	0.000			
P4	w	0.643	0.520	0.644	0.516	0.148	0.273	0.314	0.000				
	u	0.749	0.723	0.725	0.736	0.634	0.638	0.656	0.000				
P3	w	0.522	0.463	0.553	0.435	0.211	0.145	0.000					
	u	0.745	0.743	0.731	0.736	0.545	0.530	0.000					
P2	w	0.500	0.396	0.495	0.410	0.165	0.000						
	u	0.743	0.724	0.727	0.728	0.451	0.000						
P1	w	0.585	0.497	0.592	0.489	0.000							
	w	0.747	0.731	0.729	0.732	0.000							
J4	u	0.290	0.288	0.313	0.000								
	w	0.621	0.564	0.566	0.000								
J3	u	0.285	0.237	0.000									
	w	0.602	0.559	0.000									
J2	u	0.330	0.000										
	w	0.622	0.000										
J1	u	0.000											
	w	0.000											

w = weighted unifrac (pseudo-F 19.6837, p-value 0.001)
u = unweighted unifrac (pseudo-F 2.6903, p-value 0.001)

	J1	J2	J3	J4	P1	P2	P3	P4	C1	C2	C3	C4
C4	0.887	0.893	0.883	0.828	0.398	0.527	0.547	0.478	0.323	0.143	0.178	0.000
C3	0.851	0.825	0.845	0.777	0.317	0.441	0.470	0.407	0.319	0.183	0.000	
C2	0.884	0.875	0.873	0.828	0.367	0.496	0.518	0.449	0.348	0.000		
C1	0.872	0.869	0.883	0.812	0.427	0.539	0.558	0.528	0.000			
P4	0.874	0.823	0.863	0.778	0.369	0.397	0.419	0.000				
P3	0.842	0.801	0.815	0.719	0.302	0.278	0.000					
P2	0.841	0.716	0.757	0.747	0.250	0.000						
P1	0.835	0.791	0.800	0.740	0.000							
J4	0.722	0.802	0.742	0.000								
J3	0.709	0.571	0.000									
J2	0.794	0.000										
J1	0.000											

Bray curtis dissimilarity: pseudo-F 5.2374, p-value 0.001

	J1	J2	J3	J4	P1	P2	P3	P4	C1	C2	C3	C4
C4	0.878	0.848	0.865	0.860	0.751	0.767	0.791	0.732	0.699	0.726	0.665	1.000
C3	0.855	0.817	0.837	0.824	0.739	0.732	0.771	0.720	0.700	0.719	1.000	
C2	0.882	0.859	0.868	0.867	0.753	0.754	0.775	0.753	0.747	1.000		
C1	0.875	0.847	0.861	0.855	0.761	0.768	0.795	0.753	1.000			
P4	0.881	0.858	0.864	0.856	0.743	0.740	0.767	1.000				
P3	0.881	0.874	0.872	0.868	0.684	0.675	1.000					
P2	0.873	0.857	0.863	0.851	0.612	1.000						
P1	0.881	0.863	0.868	0.861	1.000							
J4	0.797	0.750	0.761	1.000								
J3	0.784	0.754	1.000									
J2	0.804	1.000										
J1	1.000											

Jaccard index: pseudo-F 2.12787, p-value 0.001

Table 3. Alpha diversity

	Observed OTUs	Shannon index	Faith's phylogenetic diversity
Papuans			
P1	3253	6.60975397782824	179.868950203
P2	4596	6.96167684274831	192.118216562
P3	4205	7.67533232153036	210.511236287
P4	2871	6.00593074734042	157.734765021
Javanese			
J1	3081	5.93165180683389	103.960978909
J2	2191	5.56528090154216	144.523357872
J3	2618	5.62161934593371	98.857889868
J4	3327	6.68709506405382	85.364012729
Chinese			
C1	1914	4.88865414525585	103.960978909
C2	2524	5.29934363362385	144.523357872
C3	1938	5.35651812561711	98.857889868
C4	1510	4.56047117421394	85.364012729

Kruskal-wallis (all groups)

	Observed OTUs	Shannon index	Faith's phylogenetic diversity
H	7.65384615384615	8.76923076923077	7.53846153846154
p-value	0.021776517	0.012467682	0.023069802

Kruskal-wallis (pairwise)

Group 1	Group 2	Observed OTUs			Shannon			Shannon		
		H	p-value	q-value	H	p-value	q-value	H	p-value	q-value
Chinese	Javanese	4.08333	0.0433081428107921	0.06496221421618815	5.33333	0.020921335337794052	0.031382003006691084	3.000000	0.0832645166635504	0.0832645166635504
	Papuans	5.33333	0.020921335337794052	0.06276400601338217	5.33333	0.020921335337794052	0.031382003006691084	5.333333	0.0209213353377940	0.0627640060133821
Javanese	Papuans	2.08333	0.14891467317876178	0.14891467317876178	3.00000	0.08326451666355042	0.08326451666355042	3.000000	0.0832645166635504	0.0832645166635504

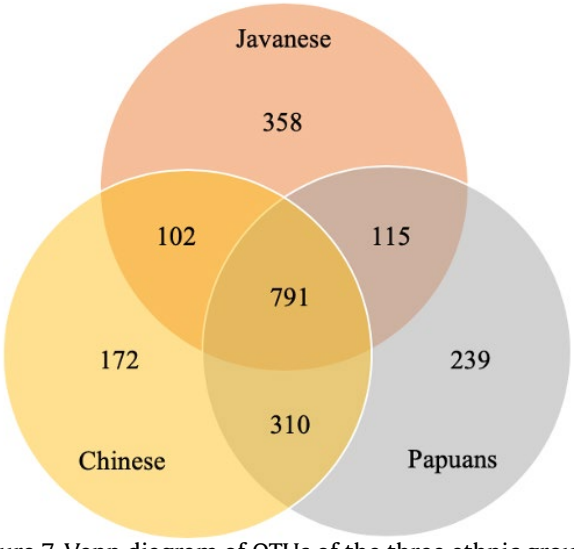

Figure 7. Venn diagram of OTUs of the three ethnic groups

Conflicts of Interest

The authors declare that there is no conflict of interest in this research.

Acknowledgements

This study was funded and supported by Universitas Indonesia research grant PUTI 3 2020 (NKB-1807/UN2.RST/HKP.05.00/2020), and partially by PMDSU-DIKTI Research Grant (NKB-427/UN2.RST/HKP.05.00/2020 with addendum NKB-3026/UN2.RST/HKP.05.00/2020) to A.M.

References

Altonsy MO *et al.* 2020. Corynebacterium tuberculostearicum, a human skin colonizer, induces the canonical nuclear factor-_B inflammatory signaling pathway in human skin cells. *Immunity, Inflammation and Disease* 8:62-79. DOI:10.1002/iid3.284

Bokulich NA *et al.* 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nature Methods* 10:57-59. DOI10.1038/nmeth.2276

Byrd AL *et al.* 2018. The human skin microbiome. *Nature Reviews Microbiology* 16:143-155. DOI:10.1038/nrmicro.2017.157

Caporaso JG *et al.* 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7:335-336. DOI:10.1038/nmeth.f.303

Chng KR *et al.* 2016. Whole metagenome profiling reveals skin microbiome-dependent susceptibility to atopic dermatitis flare. *Nature Microbiology* 1:16135. DOI:10.1038/nmicrobiol.2016.106

De Jongh G *et al.* 2005. High expression levels of keratinocyte antimicrobial proteins in psoriasis compared with atopic dermatitis. *Journal of Investigative Dermatology* 125:1163–1173. DOI:10.1111/j.0022-202X.2005.23935.x

Dekio I *et al.* 2005. Detection of potentially novel bacterial components of the human skin microbiota using culture-independent molecular profiling. *Journal of Medical Microbiology* 54:1231–1238. DOI:10.1099/jmm.0.46075-0

Deschasaux M *et al.* 2018. Depicting the composition of gut microbiota in a population with varied ethnic origins but shared geography. *Nature Medicine* 24:1526–1531. DOI:10.1038/s41591-018-0160-1

Edgar RC *et al.* 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27:2194–2200. DOI:10.1093/bioinformatics/btr381

Gaulke CA, Sharpton TJ. 2018. The influence of ethnicity and geography on human gut microbiome composition. *Nature Medicine* 24:1495-1496. DOI:10.1038/s41591-018-0210-8

Grice EA, Segre JA. 2011. The skin microbiome. *Nature Reviews Microbiology* 9:244–253. DOI:10.1038/nrmicro2537

Gudjonsson JE *et al.* 2009. Global gene expression analysis reveals evidence for decreased lipid biosynthesis and increased innate immunity in uninvolved psoriatic skin. *Journal of Investigative Dermatology* 129:2795–2804. DOI:10.1038/jid.2009.173

Gupta VK *et al.* 2017. Geography, ethnicity or subsistence-specific variations in human microbiome composition and diversity. *Frontiers in Microbiology* 8:1162. DOI:10.3389/fmicb.2017.01162

Haas BJ *et al.* 2011. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Research* 21:494– 504. DOI:10.1101/gr.112730.110

Iwase T *et al.* 2010. *Staphylococcus epidermidis* Esp inhibits *Staphylococcus aureus* biofilm formation and nasal colonization. *Nature* 465:346–349. DOI:10.1038/nature09074

Jo JH *et al.* 2016. Research techniques made simple: bacterial 16S ribosomal RNA gene sequencing in cutaneous research. *Journal of Investigative Dermatology* 136:23–27. DOI:10.1016/j.jid.2016.01.005

Khayyira AS *et al.* 2020. Simultaneous profiling and cultivation of the skin microbiome of healthy young adult skin for the development of therapeutic agents. *Heliyon* 6:e03700. DOI:10.1016/j.heliyon.2020.e03700

Leung MHY *et al.* 2015. Insights into the pan-microbiome: skin microbial communities of Chinese individuals differ from other racial groups. *Scientific Reports* 5:1–14. DOI:10.1038/srep11845

Lozupone CA, Knight R. 2008. Species divergence and the measurement of microbial diversity. *FEMS Microbiology Reviews* 32:557–578. DOI:10.1111/j.1574-6976.2008.00111.x

Magoč T, Salzberg SL. 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 27:2957–2963. DOI:10.1093/bioinformatics/btr507

Mason MR *et al.* 2013. Deep sequencing identifies ethnicity-specific bacterial signatures in the oral microbiome. *PLoS ONE* 8:1–7. DOI:10.1371/journal.pone.0077287

Nomura I *et al.* 2003. Distinct patterns of gene expression in the skin lesions of atopic dermatitis and psoriasis: a gene microarray analysis. *Journal of Allergy and Clinical Immunology* 112:1195–1202. DOI:10.1016/j.jaci.2003.08.049

Ogai K *et al.* 2018. A comparison of techniques for collecting skin microbiome samples: swabbing versus tape-stripping. *Frontiers in Microbiology* 9:2362. DOI:10.3389/fmicb.2018.02362

Oh J *et al.* 2012. Shifts in human skin and nares microbiota of healthy children and adults. *Genome Medicine* 4:77. DOI:10.1186/gm378

Ondov BD *et al.* 2011. Interactive metagenomic visualization in a web browser. *BMC Bioinformatics* 12:385. DOI:10.1186/1471-2105-12-385

Ong PY *et al.* 2002. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *New England Journal of Medicine* 347:1151–1160. DOI:10.1056/NEJMoa021481

Perez Perez GI *et al.* 2016. body site is a more determinant factor than human population diversity in the healthy skin microbiome. *PLoS ONE* 11:e0151990. DOI:10.1371/journal. pone.0151990

Sanford JA, Gallo RL. 2013. Functions of the skin microbiota in health and disease. *Seminars in Immunology* 25:370-377. DOI:10.1016/j.smim.2013.09.005

Sugimoto S *et al.* 2013. *Staphylococcus epidermidis* Esp degrades specific proteins associatedwith *staphylococcus aureus* biofilm formation and host-pathogen interaction. *Journal of Bacteriology* 195:1645-1655. DOI:10.1128/JB.01672-12

Takahashi S *et al.* 2014. Development of a prokaryotic universal primer for simultaneous analysis of bacteria and archaea using next-generation sequencing. *PLoS ONE* 9: e105592. DOI:10.1371/journal.pone.0105592

Yatsunenko T *et al.* 2012. Human gut microbiome viewed across age and geography. *Nature* 486:222–227. DOI:10.1038/nature11053

Zipperer A *et al.* 2016. Human commensals producing a novel antibiotic impair pathogen colonization. *Nature* 535:511–516. DOI:10.1038/nature18634