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ORIGINAL ARTICLE

Basic Study

Gut microbiota profile in healthy Indonesians

Endang Sutriswati Rahayu, Tyas Utami, Mariyatun Mariyatun, Pratama Nur Hasan, Rafli Zulfa Kamil, Ryan Haryo Setyawan, Fathyah Hanum Pamungkaningtyas, Iskandar Azmy Harahap, Devin Varian Wiryohanjoyo, Putrika Citta Pramesi, Muhammad Nur Cahyanto, I Nengah Sujaya, Mohammad Juffrie

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Abstract

BACKGROUND

Recently, gut microbiota has been associated with various diseases other than intestinal disease. Thus, there has been rapid growth in the study of gut microbiota. Considering the numerous factors influencing gut microbiota such as age, diet, *etc*., area-based research is required. Indonesia has numerous different tribes and each of these tribes have different lifestyles. Hence, it is expected that each tribe has a specific gut microbiota. A deeper insight into the composition of gut microbiota can be used to determine the condition of gut microbiota in Indonesians and to consider which treatment may be suitable and effective to improve health status.

AIM

To investigate the gut microbiota of Indonesian subjects represented by Javanese and Balinese tribes by analyzing fecal samples.

METHODS

Fecal samples were collected from a total of 80 individuals with 20 in each of the young groups ranging from 25-45 years and the elderly group aged 70 years or more from two different regions, Yogyakarta and Bali. Fecal sample collection was performed at the end of the assessment period (day 14 ± 1 d) during which time the subjects were not allowed to consume probiotic or antibiotic products. The quantification of various *Clostridium* subgroups, *Lactobacillus* subgroups,

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Enterococcus, Streptococcus, Staphylococcus, Bacteroides fragilis group and *Prevotella, Bifidobacterium* and *Atopobium* cluster, *Enterobacteriaceae* and *Pseudomonas* was performed using the Yakult intestinal flora-scan (YIF-SCAN).

RESULTS

The bacterial population in younger subjects' feces was higher than that in the elderly population, with a total of approximately 10.0 – 10.6 $\log_{10}$ bacterial cells/g feces. The most abundant bacteria in all groups were *Clostridium*, followed by *Prevotella, Atopobium, Bifidobacterium* and *Bacteroides*. In the elderly, an increase in *Enterobacteriaceae*, Coliform and *Escherichia coli* was found. In terms of bacterial counts in Yogyakarta, total bacteria, *Clostridium coccoides* (*C. coccoides*) group, *Bifidobacterium*, *Prevotella*, *Lactobacillus plantarum* subgroup, and *Streptococcus* were significantly higher ($P < 0.05$) in younger than elderly subjects, while the *Lactobacillus gasseri* subgroup, *Lactobacillus casei* subgroup, and *Lactobacillus reuteri* subgroup counts were significantly lower ($P < 0.05$) in younger subjects. In Balinese subjects, total bacteria, *C. coccoides* group, *Clostridium leptum* subgroup, *Bacteroides fragilis* group, and *Prevotella* were significantly higher ($P < 0.05$) in younger compared to elderly individuals, while the *Lactobacillus ruminis* subgroup, and *Enterobacteriaceae* were significantly lower ($P < 0.05$) in younger subjects. The results also revealed that, besides the *C. coccoides* group and *Clostridium leptum* group being the most abundant gut microbiota in both Yogyakarta and Balinese people, the latter was indicated by a higher *Clostridium perfringens* count, which was almost 10 times that of Yogyakarta subjects. This may be a response to different lifestyles in the different tribes; however, this phenomenon requires further extensive study.

CONCLUSION

Bacterial populations were higher in younger than in elderly subjects. Most abundant bacterial groups were *Clostridium, Prevotella, Atopobium, Bifidobacterium*, and *Bacteroides*. The level of *Clostridium perfringens* in Yogyakarta subjects was lower than that in Balinese subjects.

Key words: Gut microbiota; Indonesian; Elderly; Young people; Enterotype

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Core tip: Research on gut microbiota has been growing rapidly due to their relationship with various diseases. Two factors influencing gut microbiota are age and location. Indonesia has numerous different tribes. Hence, it is expected that each tribe will have a specific gut microbiota. This research aimed to investigate the gut microbiota of Indonesians represented by Javanese and Balinese tribes. The results showed that bacterial populations were higher in younger than elderly subjects. The most common bacterial groups were *Clostridium, Prevotella, Atopobium, Bifidobacterium*, and *Bacteroides*. The level of *Clostridium perfringens* between the tribes was different, which might be associated with diet and lifestyle.

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INTRODUCTION

Indonesia has a population of 262 million people, with children (aged 0-14 years) comprising 25%, young-adults (aged 15-64 years) comprising 68%, and the elderly (aged 65 and over years) comprising 7% of the population, as suggested by Index Mundi (https://www.indexmundi.com/indonesia/age_structure. html). Moreover, Indonesia has hundreds of tribes each of which has its own lifestyle which has

resulted in an expected variety of gut microbiota. The development of gut microbiota composition starts when an infant is born; however, it changes markedly when an infant learns to eat, followed by a stable state starting from teenager and adult age and finally begins to undergo other changes in the elderly. In the child age group, fecal microbiota is not as complicated as that in advanced age. This was indicated by a decrease in *Bifidobacteria* species diversity and an increase in *Bacteroidetes* species diversity[1]. These microbial composition changes may affect gut microbiota, especially the individual's metabolic capacity, and have an important role in health. In addition, degenerative transformation effects on the physiology and function of the gut are possibly due to aging and associated with changes in colonic ecosystem composition and metabolic activities[2]. Previous studies have also found that *Bifidobacteria* is a protective intestinal microorganism, and decreases in the elderly group age. With regard to putatively detrimental microorganism populations, notably *Clostridia* and *Enterobacteriaceae*, these increase in the elderly age group[3].

Three principal human enterotypes were proposed based on a genus or group of specific bacteria[4]. These three enterotypes, which are *Bacteroides, Prevotella*, and *Ruminococcus*, were based on 39 individuals (22 European, 13 Japanese, 4 American) that were not nation or continent specific. The study showed that all three enterotypes were largely driven by a certain species composition, although most species are not obligated to provide a plethora of molecular functions. This indicates that functional analysis is needed to understand more about microbial composition. In addition[5], a total of 98 individuals were observed to detect how gut microbial composition is affected by diet, and supported the previously mentioned enterotypes[4]. However, the study showed that the dominant enterotypes, which are *Bacteroides* and *Prevotella*, better described gut composition. Both enterotypes have their own role on gut microbiota composition. The former enterotype was related to a diet containing high protein and animal fat, while the latter enterotype was associated with a high carbohydrate diet.

According to a study of 303 Asian children in which the subjects' gut microbiota community profiles were investigated, *Prevotella* (P-type) or *Bifidobacterium*/*Bacteroides* (BB-type) are able to encourage two classifications of enterotype-like clusters[6]. The P-type is mostly found in subjects from Indonesia and Thailand, while the BB-type is mainly found in Japan, Taiwan and China. Subjects with high P-type have diets rich in resistant starch. This explained the low bile acid biosynthesis and high carbohydrate digestion suggested by predictive metagenomics.

Previous results[6] revealed that gut microbiota in Indonesian schoolchildren was represented by two unique populations, which were quite distinct from those in other countries. Yogyakarta and Bali regions were selected to represent the Javanese tribe, the most abundant and widespread throughout the Indonesian archipelago and the Balinese tribe which is only concentrated in Bali Island. Both tribes have different lifestyles and diets as are strongly associated with different religions and beliefs. Based on these facts, Yogyakarta and Bali were selected as the sites for the current study as in the previous study[6]. Currently, various methods have been used to study gut microbiota populations. In this research, culture methods on various selective media reflecting living microorganisms are rarely used and replaced by culture independent methods, including quantitative real-time polymerase chain reaction (PCR). The objectives of the present study were to establish a baseline microbiota composition in both healthy young and elderly Indonesian subjects living in Yogyakarta and Bali. This baseline was then used to further study the correlation between gut microbiota and several diseases, such as obesity, type II diabetes, and liver diseases. Therefore, Yogyakarta and Bali were selected due to their different lifestyles and diet habits.

MATERIALS AND METHODS

Subject characteristics and ethic statement

The study subjects were selected from two different sites, Yogyakarta and Bali, Indonesia. Elderly subjects (aged 70 years and above) were recruited from Elderly Houses, while younger subjects (aged from 25-45 years) were volunteer university students and employees. All subjects signed an informed consent before the study. During pre-screening of the study subjects, they were instructed not to consume fermented milk products, probiotics, or prebiotics for the entire study (1-14 d). In addition, information on medical history, physical examination, demographic parameters and vital signs were obtained from each subject (Table 1). A pregnancy test was performed in women, where applicable. Ethical clearance was approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Universitas

Gadjah Mada-Dr. Sardjito General Hospital (Ref: KE/FK/988/EC/2016), on 2 September 2016.

Sample collection

During the assessment period (1-14 d), information on the intestinal microbiota and intestinal environment were collected. Before fecal collection, each subject was given a stool kit and the procedure was explained. On day 14 (± 1 d), the subjects were asked to defecate upon a trail paper (Eiken Chemical Co., Ltd) which was immediately transferred into a fecal tube containing RNAlater (Thermo Fischer Scientific). If the fecal samples were collected during the day, the subjects delivered the sample to the clinical center as soon as possible. However, if fecal samples were collected during the evening, the subjects stored the samples in an icebox containing ice bags and brought them to the clinical center the following morning. In the elderly group, the study team was involved in fecal sample collection and questionnaire completion.

Intestinal microbiota

The Yakult intestinal flora-scan (YIF-SCAN), an intestinal flora analysis system, was used to measure the intestinal microbiota. The basic principle of YIF-SCAN is the quantitative reverse transcription PCR method and it was conducted in Yakult Central Institute, Japan. The samples were brought to Japan by the investigating team from Indonesia. The gut microbiota composition was analyzed using a previously published method[7,16] as follows: Total Bacteria: *Clostridium coccoides* group, *Clostridium leptum* subgroup, *Bacteroides fragilis* group, *Bifidobacterium*, *Atopobium* cluster, *Prevotella*, *Clostridium perfringens*, *Clostridium difficile*, *Enterobacteriaceae*, *Lactobacillus* (6 subgroups and 3 species); *Lactobacillus casei* subgroup, *Lactobacillus gasseri* subgroup, *Lactobacillus plantarum* subgroup, *Lactobacillus reuteri* subgroup, *Lactobacillus ruminis* subgroup, *Lactobacillus sakei* subgroup, *Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus fructivorans*, *Enterococcus*, *Staphylococcus*, *Streptococcus* and *Pseudomonas*. The primer sets used for *C. difficile* and *Streptococcus* were previously described[8,9].

Culture method was also used to detect the composition of intestinal microbiotia in this study. By using selective media, this enabled bacterial growth and detection that may be missed. Therefore, the researchers used two methods and compared the results of total *Lactobacillus* to anticipate different results. Culture method was performed to detect the population of yeast and mold in general using the selective medium Malt Extract Agar (MEA), the population of *Lactobacillus plantarum* was calculated using *Lactobacillus plantarum* Selective Media (LPSM), while the population of *Escherichia coli*, and coliform non *E. coli* were calculated using Brilliance *E. coli*/Coliform Selective Agar from Oxoid.

Questionnaire

The subjects were given a questionnaire at the screening period (15-23 August 2016). The questionnaire was designed to obtain stool frequency (number of stools per day) and stool consistency. For stool consistency parameters, the Bristol Stool Form Scale[10] was used as the measurement scale.

Data analysis

Microbiota composition data were tabulated into a spreadsheet (Microsoft Excel, 2016) as $\log_{10}$ bacterial cells/g feces mean ± SD (detection rate %), and for demographic parameters as [median (min-max)], unless stated otherwise. All under limit detection data were excluded from statistical analysis. All statistical analyses were conducted using (SPSS/PC + 4.0, Chicago, IL, United States). A comparison of continuous variables was conducted with the Student's *t*-test for normally distributed data and the Mann Whitney test. Statistical analysis of the YIF-SCAN data was performed using the number of bacterial cells and the detection rate in the four groups (young subjects in Yogyakarta, elderly subjects in Yogyakarta, young subjects in Bali, elderly subjects in Bali), with the Mann-Whitney *U*-test and chi-square test, respectively. We used R software for the Mann-Whitney *U*-test and the chi-square test. The statistical methods and techniques mentioned were appropriate for the research.

RESULTS

Stool frequency and consistency

The data retrieved from the questionnaires regarding stool frequency and consistency are illustrated in Figures 1 and 2.

Gut microbiota composition of healthy Indonesians

Table 1 General characteristics of the subjects (*n* = 80)

	n (%)
Gender	
Male	30 (37.50)
Female	50 (62.50)
Ethnicity	
Javanese	34 (42.50)
Balinese	40 (50.00)
Chinese	3 (3.75)
Ambonese	1 (1.25)
Bugis	1 (1.25)
Melayu	1 (1.25)
Weight (kg) (mean ± SD)	55.65 (14.35)
Height (cm) (mean ± SD)	156.07 (10.54)
Body mass index (kg/m^2) (mean ± SD)	22.57 (4.54)

Before conducting gut microbiota composition analysis, subject characteristics consisting of gender, ethnicity, weight, height and body mass index (BMI) were obtained and are described in Table 1. Microbiota profiles (based on the YIF-SCAN) in young and elderly subjects living in Yogyakarta and Bali, Indonesia are presented in Table 2 and Figure 3. Populations of bacteria in the feces of young subjects were higher than in the elderly group. The total amount was approximately $\log_{10}$ 10.0-10.6 bacterial cells/g feces, and the most abundant bacteria in all groups were *Clostridium*, followed by *Prevotella, Atopobium, Bifidobacterium*, and *Bacteroides*. These bacteria are obligate anaerobic bacteria. Interestingly, even though *Prevotella* showed the second highest population following *Clostridium*, its prevalence was not 100%. Furthermore, its prevalence in the elderly group from Yogyakarta was only 75%.

Among the analyzed *Clostridia*, the most dominant was the *Clostridium coccoides* group, followed by *Clostridium leptum* subgroup and *Clostridium perfringens*, whereas the pathogenic *Clostridium difficile* was not found in any group. *Enterobacteriaceae* (phylum *Proteobacteria*), which is a facultative anaerobe had a population of $\log_{10}$ 7.3 ± 0.8 to 8.4 ± 0.8 bacterial cells/g feces, less than the other obligate anaerobic phylum.

In terms of the bacterial counts in Yogyakarta subjects, total bacteria, *Clostridium coccoides* group, *Bifidobacterium, Prevotella, Lactobacillus plantarum* subgroup, and *Streptococcus* were significantly higher ($P < 0.05$) in younger than in elderly subjects, while the *Lactobacillus gasseri* subgroup, *Lactobacillus casei* subgroup, and *Lactobacillus reuteri* subgroup counts were significantly lower ($P < 0.05$) in younger subjects. On the other hand, in Balinese subjects, the younger groups had significantly higher ($P < 0.05$) total bacteria count, *Prevotella* subgroup, *Clostridium leptum* subgroup, *Bacteroides fragilis* group and *Clostridium coccoides* group. The counts for *Lactobacillus ruminis* subgroup and *Enterobacteriaceae* were significantly lower ($P < 0.05$) in younger subjects.

A comparison within young subjects showed that the *Bifidobacterium* count in Yogyakarta was significantly higher ($P < 0.05$) than that in Bali, while the *Clostridium perfringens* count was significantly lower ($P < 0.05$) in Yogyakarta. On the other hand, a comparison of elderly subjects from both sites showed significantly low ($P < 0.05$) counts for *Clostridium perfringens, Lactobacillus plantarum* subgroup and *Enterococcus* in Yogyakarta, while the counts for *Lactobacillus casei* subgroup and *Lactobacillus fermentum* were significantly higher ($P < 0.05$) in Yogyakarta. Counts for *Lactobacillus plantarum* subgroup were significantly higher ($P < 0.05$) in young subjects, while the counts for *Lactobacillus reuteri* subgroup and *Enterobacteriaceae* were significantly lower ($P < 0.05$) in young subjects.

In a comparison of young and elderly, younger subjects tend to have higher concentrations of microbiota based on the YIF-SCAN, compared to the elderly as shown in Table 3 and Figure 3, and a significant difference between younger and elderly subjects was found. Younger subjects had significantly higher ($P < 0.05$) total bacteria, *Clostridium coccoides* and *Bacteroides fragilis* groups, and *Clostridium leptum, Bifidobacterium, Prevotella*, and *Lactobacillus plantarum* subgroups, while *Lactobacillus reuteri* subgroup and *Enterobacteriaceae* were significantly lower ($P < 0.05$) in younger subjects. No significant difference was found in the distribution of total *Lactobacillus*. Additionally, *Atopobium* cluster showed a constant number.

In terms of the bacterial counts in Yogyakarta subjects, total bacteria, *Clostridium*

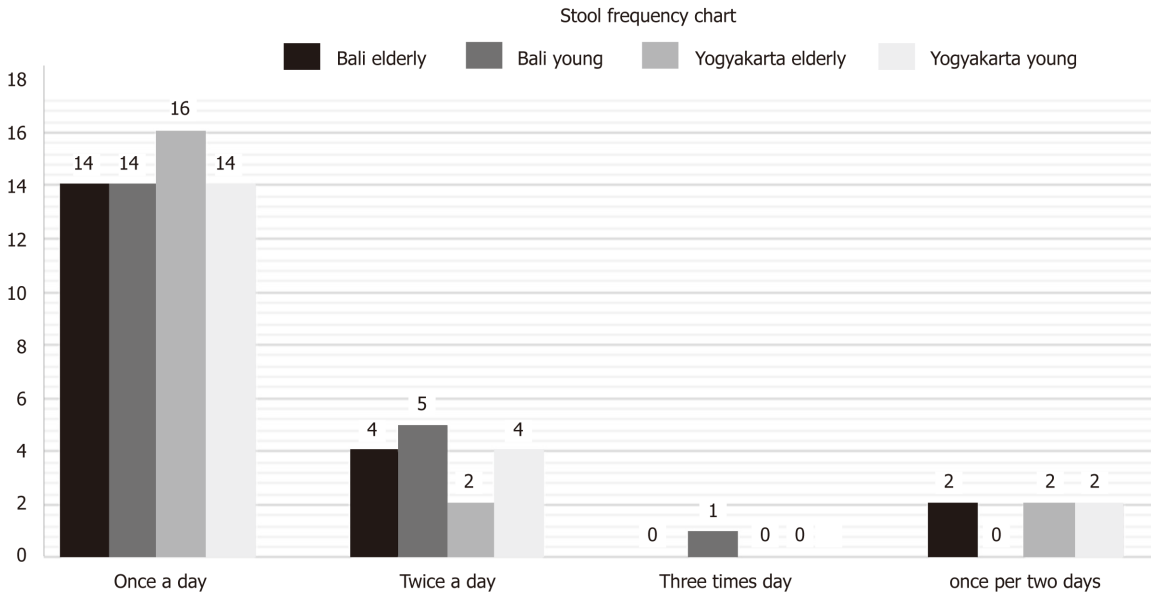

Figure 1 Data on stool frequency in young and elderly subjects from Bali and Yogyakarta.

coccoides group, *Bifidobacterium, Prevotella, Lactobacillus plantarum* subgroup, and *Streptococcus* were significantly higher ($P < 0.05$) in young subjects compared to the elderly, while the counts for *Lactobacillus gasseri*, *Lactobacillus casei*, and *Lactobacillus reuteri* subgroups were significantly lower ($P < 0.05$) in young subjects. Bacterial counts in Balinese younger subjects were significantly higher ($P < 0.05$) including total bacteria, *Clostridium coccoides*, *Clostridium leptum*, *Bacteroides fragilis*, and *Prevotella* compared to elderly subjects, while the counts for *Lactobacillus ruminis* subgroup and *Enterobacteriaceae* were significantly lower ($P < 0.05$) in younger subjects.

Differences between Prevotella, Bifidobacterium, and Bacteroides and Enterobacteriaceae

Populations of *Prevotella*, *Bifidobacterium* and *Bacteroides fragilis* group in young and elderly subjects in Yogyakarta and Bali are shown in Figure 4. From the figure, it can be seen that *Prevotella* was the most abundant bacteria in each group, and *Prevotella* enterotype was found in each group. It should also be noted that the populations of *Prevotella*, *Bifidobacterium* and *Bacteroides fragilis* group were fewer in elderly subjects. The bacteria which provide health benefits, namely *Bifidobacterium* were not widespread in elderly subjects. On the other hand, *Enterobacteriaceae* which has a disadvantageous effect on health was more common in the elderly.

The Lactobacillus group and others

The *Lactobacillus* subgroups which were dominant and had high prevalence (> 85%) were *Lactobacillus plantarum, Lactobacillus gasseri*, and *Lactobacillus reuteri*, while *Lactobacillus ruminis* although it showed a high population (6.4±1.7 – 7.6±0.9 $\log_{10}$ bacterial cells/g), its prevalence in Balinese subjects was only 70%-80% and as few as 35%-45% in Yogyakarta subjects. With regard to *Lactobacillus casei* subgroup, the number and prevalence were low.

From Table 2 and Table 3, it can be seen that in the elderly, there was an increase in *Enterobacteriaceae* (qPCR) together with *Coliform* and *E. coli*. Similar results were found for yeasts and molds, notably molds. Initially, molds were not found in younger subjects but were later found in the elderly. This may indicate that the elderly is more prone to mold infection. Also, it should be noted that *Coliform* and *E. coli*, which belong to *Enterobacteriaceae* were analyzed using culture methods, had a lower count in younger subjects compared to the elderly, and this was in accordance with the YIF-SCAN results. Using the culture method, the amount of yeast in feces was observed to be 4.17 $\log_{10}$ bacterial cells/g (younger subjects) and 4.28 $\log_{10}$ bacterial cells/g (elderly subjects), respectively, with a prevalence of 53% and 43%, respectively. Mold was not found in the feces of younger subjects, although a prevalence of 8% was found in the elderly with the highest number accounting for 3.68 $\log_{10}$ mold cells/g.

A comparison of *Lactobacillus* analysis between qPCR and culture method was conducted and the results are presented in Table 4. It was shown that no significant difference between the results of the two methods was detected.

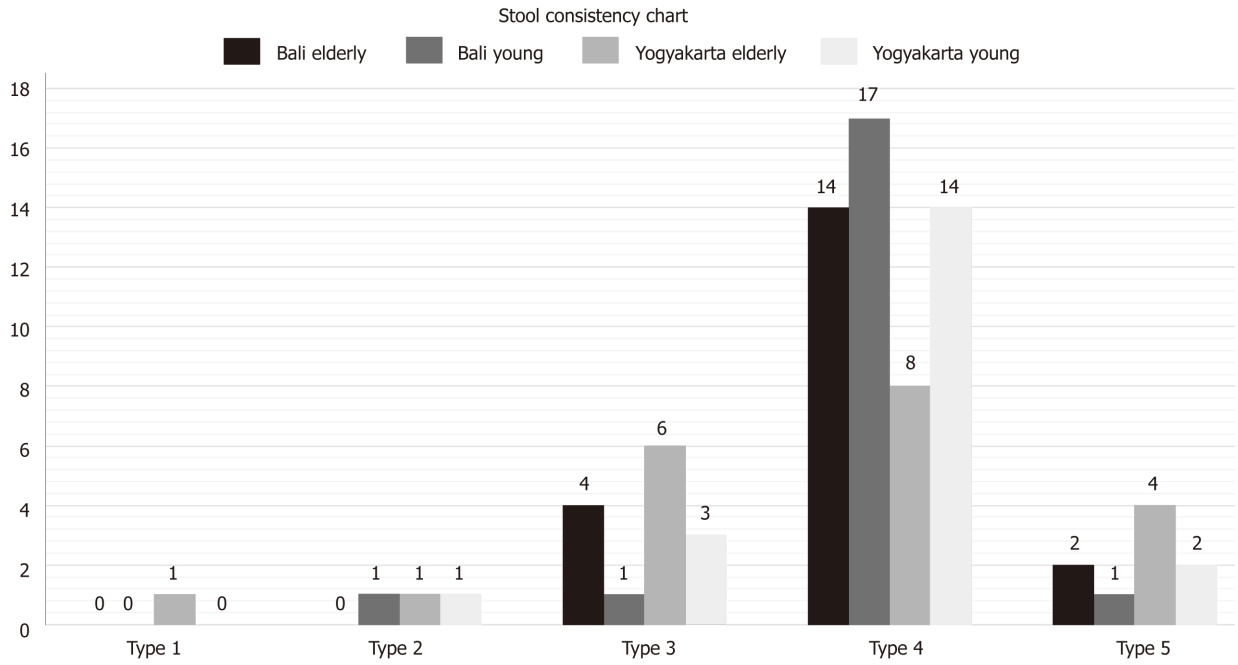

Figure 2 Data on stool consistency in young and elderly subjects from Bali and Yogyakarta.

DISCUSSION

Several factors are reported to have important roles that affect the normal gut microbiota[3,11]. These factors are: delivery type (vaginal or cesarean)[1]; infancy (breast milk or formula feeds) and adult (vegetable based or meat based) diet[2]; and antibiotics or antibiotic-like molecules derived from gut environment intake[3]. Of these, diet is the main factor affecting the change in gut microbiota following aging.

Changes in gut microbiota start when babies are weaned and consume solid foods, and these changes become stable in teenage and adult years[12-14]. Changes occur again in the elderly. Intestinal movements are weaker with age, extending transit time and changes in nutrient turn-over dynamics. The elderly possibly have reduced dentition and chewing strength affecting their diet that causes low support of microbial growth. Therefore, when compared, the elderly group was different from the younger group as the elderly have different physical aspects, such as reduced bowel function. This is in accordance with the results of the present study in subjects from Yogyakarta and Bali. The results showed that differences in gut microbiota were only seen between age groups, as some bacteria such as *Bifidobacteria, Prevotella* and *Lactobacillus plantarum* were reduced, while *Enterobacteriaceae* and *Lactobacillus reuteri* increased in elderly subjects. Both locations did not show a difference in gut microbiota composition, as rice is consumed as a staple carbohydrate source in both locations. Even though there was a slight difference in the main protein source, this did not result in a significant difference in the gut microbiota.

This difference in gut microbiota concentration may have been influenced by the difference in eating habits between younger and elderly subjects[15]. In terms of eating habit, most elderly subjects eat regularly (3 times a day) compared to younger subjects. The difference in daily eating frequency is significant. Younger people tend to consume probiotic and fruit or vegetable juices more than the elderly. However, milk, vegetables and fruits are more frequently consumed among younger subjects compared to the elderly. This might explain why there was a higher concentration of *Bacteroides* in the gut microbiota of younger subjects[15]. Our study had a limitation in study population heterogeneity; therefore, our subjects may not be representative of the Indonesian population. However, the subjects included in this study may provide a background description of the gut microbiota profile in young compared to elderly Indonesians.

Based on 16S rRNA gene sequencing, *Firmicutes* is the most abundant bacteria living in the human gastrointestinal tract, followed by *Bacteroidetes, Actinobacteria* and *Proteobacteria*[16-19]. The results of the present study showed that the gut microbiota is dominated by *Firmicutes, Bacteroidetes*, and *Actinobacteria* (which are obligate anaerobic bacteria) and *Proteobacteria* (which is a facultative anaerobic bacterium). This was in line with the results of previous research. Based on the bacterial composition, it

Table 2 Microbiota profile comparison (based on Yakult intestinal flora-scan)

No	Type of Bacteria	Log10 bacterial cells/g feces mean ± SD (detection rate %)			
		Yogyakarta		Bali	
		Young	Elderly	Young	Elderly
Phylum Firmicutes					
1	*Clostridium coccoides* group	9.9 ± 0.5 (100)	9.3 ± 1.2[a] (100)	9.9 ± 0.5 (100)	9.4 ± 0.5[c] (100)
2	*Clostridium leptum* subgroup	9.7 ± 0.4 (100)	9.3 ± 1.1 (100)	9.6 ± 0.5 (100)	9.4 ± 0.6[c] (100)
3	*Clostridium perfringens*	5.6 ± 0.9 (100)	5.0 ± 1.1 (85)	6.7 ± 1.2[e] (100)	6.9 ± 1.4[f] (95)
4	*Clostridium difficile*	- (0)	- (0)	- (0)	- (0)
5	Total *Lactobacillus*	6.7 ± 1.1 (95)	7.2 ± 1.2 (95)	6.8 ± 1.1 (100)	7.1 ± 1.2 (100)
6	*Lactobacillus plantarum* subgroup	5.0 ± 1.0 (100)	4.1 ± 0.8[a] (85)	5.1 ± 1.1 (100)	4.8 ± 0.8[f] (100)
7	*Lactobacillus gasseri* subgroup	5.4 ± 1.1 (100)	6.4 ± 1.6[a] (90)	5.5 ± 1.2 (100)	5.6 ± 1.7 (90)
8	*Lactobacillus reuteri* subgroup	4.6 ± 0.8 (95)	5.3 ± 1.4[a] (90)	4.5 ± 0.9 (90)	4.9 ± 1.3 (100)
9	*Lactobacillus ruminis* subgroup	6.8 ± 1.6 (45)	7.6 ± 0.9 (35)	6.4 ± 1.7 (80[e])	7.4 ± 1.3[c] (70[f])
10	*Lactobacillus casei* subgroup	3.7 ± 0.6 (40)	4.9 ± 1.0[a] (25)	3.8 ± 0.8 (40)	3.6 ± 000.8[f] (35)
11	*Lactobacillus sakei* subgroup	3.6 ± 0.5 (35)	0[b]	3.1 ± 0.6 (55)	3.0 ± 0.6 (30[f])
12	*Lactobacillus fermentum*	5.3 ± 0.8 (75)	5.9 ± 1.1 (80)	5.3 ± 0.8 (55)	5.1 ± 0.8[f] (60)
13	*Lactobacillus brevis*	3.9 ± 0.8 (50)	4.0 ± 0.9 (15[a])	4.7 ± 0.9 (35)	3.7 ± 0.8 (40)
14	*Lactobacillus fructivorans*	- (0)	- (0)	- (0)	- (0)
15	*Enterococcus*	7.4 ± 0.7 (90)	7.1 ± 1.0 (90)	7.4 ± 0.9 (95)	7.9 ± 1.1[f] (95)
16	*Streptococcus*	8.4 ± 0.5 (95)	7.5 ± 1.6[a] (75)	8.0 ± 0.7 (95)	8.2 ± 0.7 (45[d])
17	*Staphylococcus*	5.3 ± 0.5 (100)	5.1 ± 1.1 (90)	5.0 ± 0.6 (100)	5.2 ± 1.0 (100)
Phylum Bacteroidetes					
18	*B. fragilis* group	8.9 ± 0.7 (100)	8.3 ± 1.1 (100)	9.3 ± 0.6 (100)	7.9 ± 1.1[c] (100)
19	*Prevotella*	10.0 ± 1.2 (85)	9.1 ± 1.6[a] (75)	9.4 ± 1.9 (80)	9.0 ± 1.1[c] (85)
Phylum Actinobacteria					
20	*Atopobium* cluster	9.1 ± 0.6 (100)	8.9 ± 0.7 (100)	8.9 ± 0.7 (100)	9.0 ± 0.5 (100)
21	*Bifidobacterium*	9.4 ± 0.6 (100)	8.4 ± 1.3[a] (100)	8.6 ± 0.9[f] (100)	8.4 ± 1.2 (100)
Phylum Proteobacteria					
22	*Enterobacteriaceae*	7.3 ± 0.8 (100)	7.8 ± 1.6 (100)	7.6 ± 0.9 (100)	8.4 ± 0.8[c] (100)
23	*Pseudomonas*	4.1 ± 0.6 (25)	4.7 ± 1.0 (30)	3.1 ± 0.0 (10)	4.2 ± 0.8 (55[d])
24	Total Bacteria	10.6 ± 0.4 (100)	10.0 ± 1.0[a](100)	10.5 ± 0.5 (100)	10.1 ± 0.5[c] (100)

[a]$P < 0.05$ Yogyakarta Young *vs* Yogyakarta Elderly.
[b]$P < 0.01$ Yogyakarta Young *vs* Yogyakarta Elderly.
[c]$P < 0.05$ Bali Young *vs* Bali Elderly.
[d]$P < 0.01$ Bali Young *vs* Bali Elderly.
[e]$P < 0.05$ Yogyakarta Young *vs* Bali Young.
[f]$P < 0.05$ Yogyakarta Elderly *vs* Bali Elderly.
Mann-Whitney *U*-test for bacterial cells/g feces, chi-square test for detection rate.

should be noted that each group had a *Prevotella* enterotype. From the recent gut microbiota profiling of children in Yogyakarta and Bali, it was stated that *Prevotella* was the most dominant bacteria[6]. In addition, Papua New Guineans, which is the neighboring country to Indonesia, showed a gut composition dominated by *Prevotella* over *Bacteroides*[20]. Therefore, it can be concluded that Indonesians, from children to the elderly had a *Prevotella* enterotype.

Bifidobacterium-Enterobacteriaceae. The low abundance of *Proteobacteria* in younger subjects was due to the strict anaerobic environment of the colon. The facultative species may represent 0.1% in this strict anaerobic environment[21]. Interestingly, during aging, the population of *Enterobacteriaceae* from phyla *Proteobacteria* increased, followed by a reduction in protective bacteria *Bifidobacterium*. This also agrees with the results of prior research, notably by[22] where even though there was a difference in population between *Bacteroides, Prevotella* and *Bifidobacterium* in each country and age group, in common was that higher proportions of *Enterobacteriaceae* were found in all elderly volunteers independent of the location.

Probiotic supplementation contributes to gut microbiota composition alterations from adults to the elderly[13]. As humans age, factors related to their diet are highly

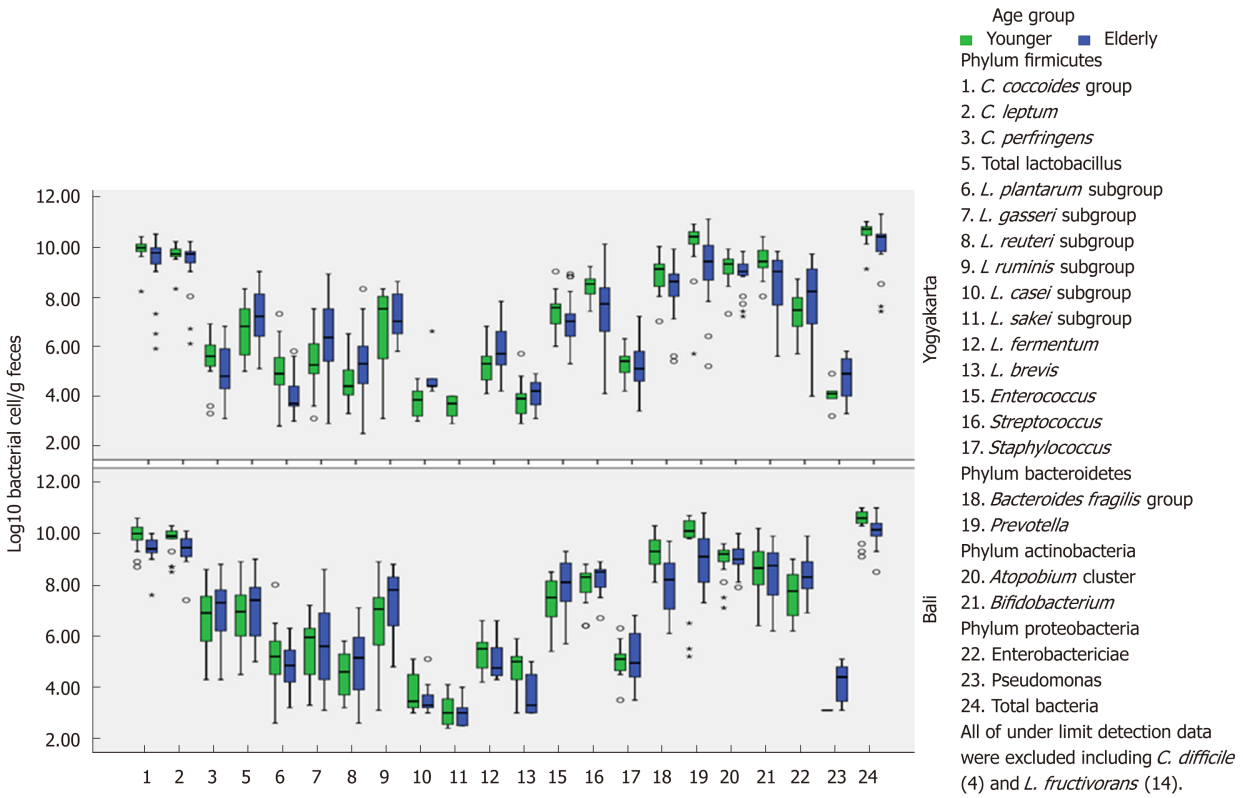

Figure 3 Comparison of gut microbiota composition in young and elderly subjects from Bali and Yogyakarta.

linked with changes in the gut microbiome. However, a connection between these factors has not been proven. An increase in medication intake is also prevalent in the elderly and can support reduced gut microbiota composition. On the other hand, the use of anti-diabetic drugs has been shown to have a positive impact on gut microbiota, which was shown by increasing populations of beneficial microbiota and their metabolites[23,24]. Several studies have reported that the gut microbiota has an important impact not only in colorectal cancer development, but also in the treatment of colorectal cancer. It was reported that the efficacy of anticancer agents is regulated by gut microbiota and improves the killing effect of 5-fluorouracil, an anticancer drug[25]. Nevertheless, chemotherapy treatments can disrupt the equilibrium of gut microbiota and cause damage to gut microbiota[26,27]. In specific diseases such as heartburn and other symptoms caused by gastroesophageal reflux disease and functional dyspepsia, proton pump inhibitors (PPIs) are used worldwide. However, it was shown that long-term use of PPIs lowered *Faecalibacteria* and increased *Streptococci* thought to be associated with enteric infection[28]. Alterations in gut microbiota following the use of pharmaceutical agents, which is associated with disease, possibly recovers after the administration of pre-and probiotics. The manipulation of gut microbiota to improve health status using foods composed of prebiotic oligosaccharide components is being investigated. A previous study found that ingestion of fermented milk containing probiotics was able to enhance stool quality and defecation frequency[29].

Commensal *Clostridia*-known to be the microbial with the highest population found in the gut, was represented by *Clostridium cocoides* and *Clostridium leptum* subgroup in the present study (Table 2). The number of *Clostridia* decreased in elderly subjects in both locations. The Gram-positive bacteria, *Clostridia*, start to colonize in the first month in breastfed infants, and populate a certain area of the intestinal mucosa within intestinal cells[30]. Therefore, *Clostridia* can play an important role in affecting the physiologic, metabolic and immune processes. On the other hand, *Bifidobacterium* is believed to be one of the first microbes to colonize the human gastrointestinal tract and provide beneficial health effects as they have the ability to produce butyric acid[31,32]. The *Atopobium* cluster belongs to phylum *Actinobacteria* alongside *Bifidobacterium*. The population of this bacterium in adults was the same as that of *Bifidobacterium*, approximately 10^9, and unlike *Bifidobacterium* which decreased in the elderly, *Atopobium* counts were constant[16].

Lactobacillus plantarum was found to have the highest prevalence among the higher-

Table 3 Microbiota profile (culture method) in young and elderly subjects

Microbiota (Log_{10} CFU/g feces)	mean ± SD (detection rate %)		*P* value
	Younger (*n* = 40)	Elderly (*n* = 40)	
Coliform	6.80 ± 0.83 (100)	7.20 ± 0.73 (100)	0.028
Escherichia coli	6.87 ± 0.74 (100)	7.29 ± 0.78 (100)	0.016
Yeast	4.17 ± 0.51 (53)	4.28 ± 0.50 (43)	0.411
Mold	0.0 ± 0.0 (0)	3.68 ± 0.27 (8)	-
Lactobacillus plantarum	4.16 ± 0.85 (8)	4.16 ± 0.03 (5)	1.000
Total LAB	7.03 ± 0.83 (100)	7.67 ± 1.23 (100)	0.008

Comparison of continuous variable was carried out using the Independent *T* test for normally distributed data and the Mann Whitney test for non-normally distributed data.

level species of *Lactobacillus* within the gut. This bacterium was related to high BMI and blood glucose. However, other groups and species of *Lactobacillus* have been found at various glucose levels in adults and the elderly[2]. Additionally, *Lactobacillus plantarum* is abundant in fermented Indonesian traditional food[33], and has been found to have probiotic potential[34,35].

A culture method was used in this research to determine some microorganisms. However, another culture-independent method based on cloned 16S rRNA sequence was introduced[21]. Moreover, with profiling culture enriched molecules, most human gut microbiota, especially bacteria, are able to be cultured[36]. In addition, using selective media will enable bacterial growth and detection that may be missed. The culture method which relied on various specific media was compared with molecular-based analysis in this research and a significant difference was not found between the two methods. With the culture method, elderly subjects tended to have higher concentrations of *Coliform*, *E. coli* and total lactic acid bacteria. Thus, our findings were not similar to those in previous studies in an Asian population[37,38]. However, these data were obtained from an East Asian population (Japanese) and the study was conducted in the early 1970s. Our study population, both young and elderly subjects showed a different pattern where these bacteria were found in relatively higher concentrations (Table 3). The different distribution of gut microbiota in our study showed that the gut microbiota may change over time, and the gut microbiota of Indonesians was unique.

Probiotics and prebiotics have the potential to maintain gut microbiota balance. Analysis of the gut microbiota was carried out to observe its potential to modulate and improve health, particularly in the elderly when protective bacteria (*Bifidobacteria)* declined and potential pathogenic *Enterobacteriaceae* increased. Numerous conditions could trigger the shift in elderly gut microbiota, such as weakened chewing ability, a decline in intestinal physiological condition to digest, lowered appetite, and limited ability to prepare food. Consumption of probiotics and prebiotics could be used as a way to retain the balance of microbiota in the elderly, in order to maintain health.

Table 4 Comparison between qPCR and culture method

Lactobacillus **(Log10 CFU/g feces)**	**mean ± SD (detection rate %)**			
	Younger (*n* = 40)		**Elderly (*n* = 40)**	
	Bali	Yogyakarta	Bali	Yogyakarta
Culture method	7.04 ± 0.87 (100)	7.01 ± 0.81 (100)	8.17 ± 0.99[b] (100)	7.17 ± 1.33[a] (100)
Yakult intestinal flora-scan	6.7 ± 1.1 (95)	7.2 ± 1.2 (95)	6.8 ± 1.1 (100)	7.1 ± 1.2 (100)

[a]$P < 0.05$ Yogyakarta Elderly *vs* Bali Elderly;
[b]$P < 0.01$ Bali Young *vs* Bali Elderly.

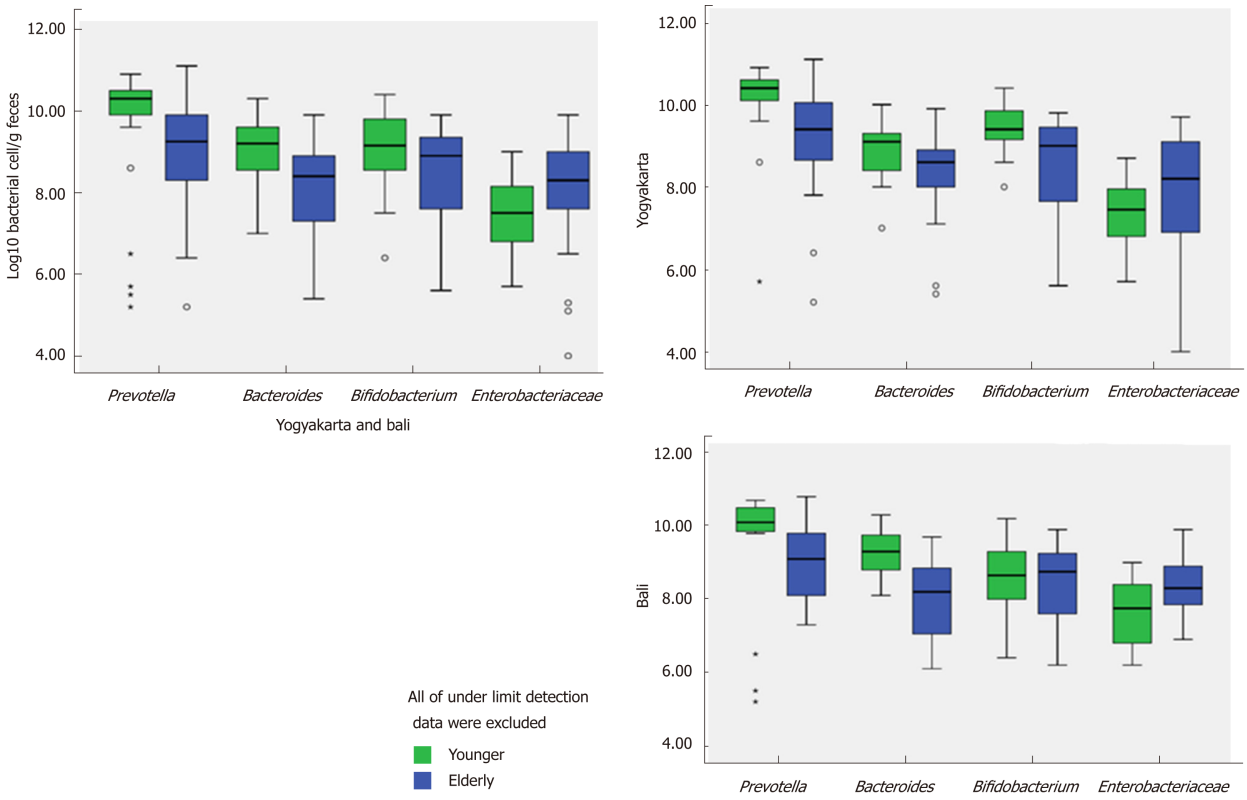

Figure 4 Populations of *Prevotella*, *Bifidobacterium*, *Bacteroides* and *Enterobacteriaceae* in young and elderly subjects from Yogyakarta and Bali.

ARTICLE HIGHLIGHTS

Research background

Studies on gut microbiota are growing rapidly, as the gut microbiota has been associated with various diseases. There are numerous factors influencing gut microbiota composition; hence, further research is needed. Indonesia has a high diversity of tribes and lifestyles. Therefore, it is expected that each tribe has its specific gut microbiota. Thus, a deeper insight into the composition of gut microbiota can be further used to determine the condition of gut microbiota in Indonesians and assess which treatment may be suitable and effective to improve health status.

Research motivation

This research aimed to investigate gut microbiota composition in two different groups in relation to age and tribe. The results obtained may be further used to determine the condition of gut microbiota in Indonesians and to assess which treatment may be suitable and effective to improve health status.

Research objectives

The objective of this study was to compare gut microbiota composition in Indonesian subjects represented by two different age groups (elderly and young) from the Javanese and Balinese tribes, by the analysis of fecal samples. The results obtained may be further used to determine the condition of gut microbiota in Indonesians and to assess which treatment may be suitable

and effective to improve health status.

Research methods

Fecal samples were collected from a total of 80 subjects with 20 in each young group ranging from 25-45 years and elderly group ranging from 70 years or more from two different regions, Yogyakarta and Bali. The collection of fecal samples was performed at the end of the assessment period (day 14 ± 1 d) and during this period, the subjects were not allowed to consume probiotic and antibiotic products. The quantification of various *Clostridium* subgroups, *Lactobacillus* subgroups, *Enterococcus, Streptococcus, Staphylococcus*; *Bacteroides fragilis* group and *Prevotella*, *Bifidobacterium* and *Atopobium* cluster; *Enterobacteriaceae* and *Pseudomonas* was performed using the Yakult intestinal flora-scan (YIF-SCAN).

Research results

Bacteria populations in younger subjects' feces were higher than in elderly subjects. The most abundant bacteria in both age groups were *Clostridium*, followed by *Prevotella, Atopobium, Bifidobacterium* and *Bacteroides*. In the elderly, an increase in *Enterobacteriaceae, Coliform* and *E. coli* was found. In terms of bacterial counts in Yogyakarta, total bacteria, *Clostridium coccoides* group, *Bifidobacterium*, *Prevotella*, *Lactobacillus plantarum* subgroup, and *Streptococcus* were significantly higher ($P < 0.05$) in younger than in elderly subjects, while the counts for *Lactobacillus gasseri* subgroup, *Lactobacillus casei* subgroup, and *Lactobacillus reuteri* subgroup were significantly lower ($P < 0.05$) in the younger group. In Balinese subjects, total bacteria, *Clostridium coccoides* group, *Clostridium leptum* subgroup, *Bacteroides fragilis* group, and *Prevotella* were significantly higher ($P < 0.05$) in younger compared to elderly subjects, while the counts for *Lactobacillus ruminis* subgroup and *Enterobacteriaceae* were significantly lower ($P < 0.05$) in younger subjects. The results also revealed that the *Clostridium perfringens* count in Balinese subjects was 10 times that in Yogyakarta subjects. This may have been a response to the lifestyles of the different tribes although this phenomenon requires further extensive study.

Research conclusions

Bacterial populations were higher in younger than in elderly subjects. The most dominant bacterial groups in these subjects were *Clostridium* followed by *Prevotella, Atopobium, Bifidobacterium, and Bacteroides*. The gut microbiota of the two tribes in this study were similar, but it was observed that *Clostridium perfringens* in Yogyakarta subjects was almost ten times lower than that in Balinese due to lifestyle and diet. However, the effect of lifestyle and diet on gut microbiota composition requires further investigation.

Research perspectives

These results proved that gut microbiota composition within different age groups and tribes is different. Different tribes may have different lifestyles and diets; therefore, future studies should investigate how different diets contribute to gut microbiota composition.

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