Fecal microbiome composition in healthy adults in Ghana

Prince Kofi Parbie, Taketoshi Mizutani, Aya Ishizaka, Ai Kawana-Tachikawa, Lucky Ronald Runtuwene, Sayuri Seki, Christopher Zaab-Yen Abana, Dennis Kushitor, Evelyn Yayra Bonney, Sampson Badu Ofori, Satoshi Uematsu, Seiya Imoto, Yasumasa Kimura, Hiroshi Kiyono, Koichi Ishikawa, William Kwabena Ampofo, and Tetsuro Matano

Received: June 12, 2020. Accepted: June 18, 2020. Published online: June 30, 2020. DOI: 10.7883/yoken.JJID.2020.469
Fecal microbiome composition in healthy adults in Ghana

Running Head: Fecal microbiome in Ghanaians

Prince Kofi Parbie\textsuperscript{1,2,3}, Taketoshi Mizutani\textsuperscript{4}, Aya Ishizaka\textsuperscript{4}, Ai Kawana-Tachikawa\textsuperscript{1,2,4}, Lucky Ronald Runtuwene\textsuperscript{2}, Sayuri Seki\textsuperscript{2}, Christopher Zaab-Yen Abana\textsuperscript{3}, Dennis Kushitor\textsuperscript{3}, Evelyn Yayra Bonney\textsuperscript{3}, Sampson Badu Ofori\textsuperscript{5}, Satoshi Uematsu\textsuperscript{4,6,7}, Seiya Imoto\textsuperscript{4,7}, Yasumasa Kimura\textsuperscript{4}, Hiroshi Kiyono\textsuperscript{4,8,9}, Koichi Ishikawa\textsuperscript{2}, William Kwabena Ampofo\textsuperscript{3}, and Tetsuro Matano\textsuperscript{1,2,4,}\textsuperscript{‡}

\textsuperscript{1}Joint Research Centre for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan

\textsuperscript{2}AIDS Research Centre, National Institute of Infectious Diseases, Tokyo, Japan

\textsuperscript{3}Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana

\textsuperscript{4}The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

\textsuperscript{5}Regional Hospital Koforidua, Ghana Health Service, Koforidua, Ghana

\textsuperscript{6}Department of Immunology and Genomics, Osaka City University Graduate School of Medicine, Osaka, Japan

\textsuperscript{7}Collaborative Research Institute for Innovative Microbiology, The University of Tokyo, Tokyo, Japan

\textsuperscript{8}Institute for Global Prominent Research, Graduate School of Medicine, Chiba University, Chiba, Japan

\textsuperscript{9}CU-UCSD Center for Mucosal Immunology, Allergy and Vaccines (cMAV), Department of Medicine, University of California San Diego, San Diego, CA, USA

\textsuperscript{‡}Corresponding author: Tetsuro Matano, AIDS Research Center, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan (Phone: +81-3-4582-2811; Email: tmatano@nih.go.jp)

\textbf{Keywords}: gut microbiome, diversity, West Africa, Ghana
水谷壮利 4, 石坂彩 1, 立川（川名）愛 1,2,4, 関紗由里 2,
植松智 4,6,7, 井元清哉 4,7, 木村恭将 4, 清野宏 4,8,9, 石川晃一 2, 俣野 哲朗 1,2,4‡

1熊本大学ヒトレトロウイルス学共同研究センター
2国立感染症研究所エイズ研究センター
4東京大学医科学研究所
6大阪市立大学大学院医学研究科ゲノム免疫学
7東京大学微生物科学イノベーション連携研究機構
8千葉大学大学院医学研究院グローバルプロミネント研究基幹

†責任著者連絡先
俣野哲朗
国立感染症研究所エイズ研究センター
〒162-8640 新宿区戸山1-23-1
Tel: 03-4582-2811; Fax: 03-5285-1165; Email: tmatano@nih.go.jp
Summary

Current studies have indicated association of gut microbiome composition with varieties of disorders including infectious diseases. The microbiome composition is different among races and countries, possibly resulting in diversified interaction between host immune and gut microbiome. Characterization of the baseline microbiota in healthy people is an essential step to understand this biological interaction in individual populations. However, data on gut/fecal microbiome has not been accumulated in West Africa. In the present study, we examined fecal microbiome composition in healthy adults in Ghana. The 16S rRNA gene libraries were prepared using bacteria fractions derived from 55 Ghanaian adults and subjected to next generation sequencing. Fecal microbiome of Ghanaian adults was dominated by *Firmicutes* (*Faecalibacterium, Subdoligranulum, and Ruminococcaceae UCG-014*), *Proteobacteria* (*Escherichia-Shigella and Klebsiella*), and *Bacteroidetes* (*Prevotella 9 and Bacteroides*), consistent with previous observations in African cohorts. Analysis found difference in composition and lower diversity of fecal microbiome in our cohort compared to non-African countries. This is the first study that describes substantial fecal microbiome data obtained by using high throughput metagenomic tools in Ghana. These data would be valuable as a basis for determination of the association between fecal microbiome and progression of varieties of diseases in West African populations.
Introduction

The gut microbiota, influencing host immune, metabolic, and nutritional functions, is known to be involved in human health conditions (1-3). Cumulative studies have indicated association of gut microbiome composition with varieties of disorders including infectious diseases (1-8). Characterization of the baseline healthy microbiome composition is essential for understanding of the biological interaction between host factors and microbiome in disease progression.

Gut microbiome compositions, which are influenced by factors including dietary habits linked with socio-cultural practices and geographic provenance, are different all over the world (9,10), and individual populations could show a wide variety of interaction between host factors and microbiome. For instance, association of dysbiosis in gut microbiome with disease progression in HIV-1 infected individuals has been indicated (6,7). In addition, influence of host genetics (HLA-B27 and HLA-DRB1) on gut microbial dysbiosis in ankylosing spondylitis and rheumatoid arthritis has been reported (11). It has been shown that higher colorectal cancer risk and mucosal proliferation rates in African Americans compared to native Africans are associated with dietary behavior (socioeconomic status) influencing gut microbial composition (12). Analysis of gut/fecal microbiome is thus important for our understanding of pathogenesis in varieties of diseases in individual regions.

Data on gut microbiome has been accumulated mainly in Europe and United States (13,14). However, the number of studies on gut microbiome in sub-Saharan African is limited and data on
gut/fecal microbiome has not been accumulated in West Africa. In the present study, we characterized fecal microbiome in healthy adults in Ghana, West Africa.

Materials and Methods

Study population
In a cross-sectional study, we enrolled 55 healthy Ghanaian adults above 18 years old from 6 communities (Akwadum, Jumapo, Koforidua, Oyoko, Suhum, and Tafo) in the Eastern Region of Ghana. Participants were recruited during a community health screening exercise. Participants who took antibiotics within 4 weeks prior to sample collection were not enrolled. This study was approved by the Institutional Review Board of Noguchi Memorial Institute for Medical Research (NMIMR) (approval number: 096/16-1; dated on May 3, 2017) and the Ethical Committee of National Institute of Infectious Diseases (NIID) (approval number: 685; dated on June 16, 2016). The written informed consent for sample collection and subsequent analysis was provided by all the participants.

Bacteria fraction preparation from fecal samples
Stool samples were collected from enrolled participants. All the samples were transported to NMIMR, processed within 24 hours of sample collection, and stored at -80°C until use. Bacterial pellets were prepared from frozen fecal samples as previously described (15) with minor modifications. Briefly, 1 g of stool was washed three times with 3 ml of SM-plus buffer (100 mM NaCl, 50 mM Tris-HCl [pH7.4], 8 mM MgSO4-7H2O, 5 mM CaCl2-2H2O, 0.01 % [w/v] Gelatin) and centrifuged at 6,000 x g for 5 min. Then, pellets were resuspended in 20 ml of SM-plus buffer
and filtered through a 100-μm cell strainer (Corning, Corning, NY). One ml out of the filtrated 20 ml of bacterial suspension was used for DNA extraction.

**DNA extraction, amplification, and 16S rRNA gene sequencing**

DNA was extracted from fecal sample-derived bacteria fraction as previously described (16). The 16S rRNA gene libraries were prepared according to the 16S Metagenomic Sequencing Library Preparation guide (Illumina, San Diego, CA; Part # 15044223 Rev. B). Briefly, the hypervariable V3-V4 regions of the 16S rRNA gene were amplified by using specific primers, Forward (5’-ACACGACGCTCTTCCGATCTCCTACGGGNGGCWGCAG-3’) and Reverse (5’-GACGTGTGCTCTTCCGATCTGACTACHVGGGTATCTAATCC-3’), including Illumina adapter overhang nucleotide sequences (indicated by underlines) (17). Posteriorly, adaptor ligation for PCR amplicons were performed using NEB Next Multiplex Oligos for Illumina (Dual Index Primers Set 1) (NEB Japan, Tokyo, Japan). Sequencing was performed on the Illumina MiSeq (Illumina, USA) using MiSeq Reagent Kit v3 (600-cycle) with a 20% PhiX (Illumina) spike-in at NMIMR.

**Sequence analyses**

Sequences were quality filtered, denoised and analyzed with the Quantitative Insights Into Microbial Ecology 2 (QIIME 2™ version 2019.4) (18). Briefly, paired-end reads were denoised into amplicon sequence variants with DADA2 (19). Taxonomy was assigned to the resulting amplicon sequence variants (ASVs) against the SILVA database (release 132) (20), trimmed to the V3-V4 region of the 16S rRNA gene, using Naive Bayesian classifier (21).
Statistical analyses

GraphPad Prism version 7.04 and R were used for statistical analyses. Comparison was performed using Wilcoxon rank-sum test or Kruskal Wallis test with Benjamini, Krieger and Yekutieli FDR correction. *p* values less than 0.05 were considered significant.

Results

Analysis of fecal microbiome in healthy adults in Ghana

A total of 55 participants of Ghanaian adults were enrolled from the Eastern Region of Ghana in the present study. The median age of participants was 45 years old (IQR [interquartile range], 33-51) and 42 (76%) were females. Stool samples were collected from these participants. The 16S rRNA gene libraries were prepared using bacteria fractions derived from fecal samples and subjected to next generation sequencing.

Analysis revealed fecal microbiome compositions in the healthy Ghanaian adults (Fig. 1). Mean relative abundance showed that *Firmicutes* is dominant at the phylum level (more than half) while *Ruminococcaceae* is dominant at the family level (more than one-third) (Table 1). Top seven abundant genera were *Faecalibacterium* (20%), *Subdoligranulum* (11%), *Escherichia-Shigella* (7%), *Prevotella 9* (4%), *Ruminococcaceae UCG-014* (3%), *Bacteroides* (3%), and *Klebsiella* (3%) (Table 1 and Fig. 2). *Faecalibacterium*, *Subdoligranulum*, and *Ruminococcaceae UCG-014* are belonging to the family of *Ruminococcaceae*, the phylum of *Firmicutes*. *Escherichia-Shigella* and *Klebsiella* are belonging to the family of *Enterobacteriaceae*, the phylum of *Proteobacteria*. *Prevotella 9* and *Bacteroides* are belonging to the phylum of *Bacteroidetes*. Analysis of Shannon's
index (22) showed no significant difference in alpha diversity of fecal microbiome between females and males, indicating no clear impact of difference in gender on fecal microbiome diversity (Fig. 3).

Comparison of data on fecal microbiome composition in our cohort with those in non-African countries

To compare data on fecal microbiome in our Ghana cohort with those in non-African countries, we used data on fecal microbiome in US and Papua New Guinea (PNG), available from MG-RAST, metagenomics analysis server (https://www.mg-rast.org/) (accession number: mgp10381) (23).

Comparison of top 20 abundant genera of fecal microbiome revealed large difference in fecal microbiome composition among Ghanaian, Papua New Guineans, and US people (Table 1 and Fig. 2). Faecalibacterium, Subdoligranulum, Agathobacter, [Eubacterium] coprostanoligenes group, Bifidobacterium, Streptococcus, and Collinsella were included in top 20 in all the three Ghana, PNG, and US cohorts. In particular, Faecalibacterium and Subdoligranulum were relatively abundant in these three cohorts. However, Ruminococcaceae UCG-014 (belonging to the family of Ruminococcaceae) and Escherichia-Shigella and Klebsiella (belonging to the family of Enterobacteriaceae) were included in top 7 abundant genera in our Ghana cohort, but these were not in top 20 in PNG or US. Conversely, Lachnospiraceae unclassified, Catenibacterium, Blautia, Dorea, Romboutsia, Erysipelotrichaceae UCG-003, and [Eubacterium] hallii group, were included in top 20 in both PNG and US, but these were not in top 20 in our Ghana cohort.
Regarding the remaining genera included in top 7 in our Ghana cohort, *Prevotella* was in top 20 in PNG but not in US, whereas *Bacteroides* was in top 20 in US but not in PNG.

Analysis of Shannon’s index indicated significantly lower alpha diversity of fecal microbiome in our Ghana cohort compared to the PNG and US cohorts, although no significant difference was observed between PNG and US (Fig. 4).

**Discussion**

The gut microbiota is known to be influenced by factors such as dietary behavior linked with socio-cultural practices (9,10,12,24). It is imperative to contextually describe the microbiota in relation to disease considering such factors. It is thus important to obtain data on microbiome in individual populations. This study presents data on fecal microbiome composition in healthy Ghanaian adults.

Generally, it has been indicated that gut microbiome of sub-Saharan populations is dominated by genera belonging to the phyla *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* (9,10,24). In particular, dominance of *Prevotella* has been indicated in African cohorts in contrast to non-African populations (10,24-27). High fiber-carbohydrates diet also found in our population could be attributable to this observation as previously reported (10,24,26). Consistent with these previous observations in African cohorts, fecal microbiome of Ghanaian adults was dominated by *Firmicutes* (*Faecalibacterium*, *Subdoligranulum*, and *Ruminococcaceae UCG-014*); *Proteobacteria* (*Escherichia-Shigella* and *Klebsiella*) and *Bacteroidetes* (*Prevotella* 9 and *Bacteroides*). Our observation that *Escherichia-Shigella* and *Klebsiella* included in top 7 in our
cohort were not detected in top 20 in PNG or US should be stressed. The fecal microbial signature of our cohort suggests the pattern of dietary habit in transition from rural to industrialized area, which has been suggested in a previous report (28). This is consistent with the socioeconomic characteristic of our cohort consisting of peri-urban communities.

Comparison between our Ghana cohorts and US and PNG cohorts revealed that fecal microbiome composition in Ghana is largely different from that in US and PNG, non-African countries. Remarkably, fecal microbiome of Ghanaians showed significantly lower alpha diversity compared to US and PNG. These data would be important as a basis for determination of the association between fecal microbiome and progression of varieties of diseases in West African populations.

In summary, this is the first study that describes the fecal microbiome in Ghanaian adults using high throughput metagenomic tools. Our results provide valuable data in West Africa, where data on enteric microbiome has not yet been systematically accumulated, and thus contribute to understanding of the interaction between the host and enteric microbiota in a population specific manner.
Competing interests

The authors have no conflict of interest to declare.

Acknowledgements

We thank staff of NMIMR-Virology Department, RHK, and NIID-ARC for their administrative and technical support. We are sincerely grateful to all the individuals who consented to participate in this study.

This study was supported by Japan Agency for Medical Research & Development (AMED) (grant number: JP18fk0410003, JP20fk0410011, JP20fk0108125, JP20fk0108139, and JP20jk0210002), AMED-JICA (the Science and Technology Research Partnership for Sustainable Development [SATREPS]; JP19jm0110012), and the Ministry of Education, Culture, Sports, Science, and Technology in Japan (18H02666). The Kumamoto University International Scholarship Program generously provided travel support during this study.
References

1. Shreiner AB, Kao JY, Young VB. The gut microbiome in health and in disease. Curr Opin Gastroenterol. 2015;31:69–75.

2. Thaiss CA, Zmora N, Levy M, et al. The microbiome and innate immunity. Nature. 2016;535:65-74.

3. Valdes AM, Walter J, Segal E, et al. Role of the gut microbiota in nutrition and health. BMJ. 2018;361:36–44.

4. Vijay-Kumar M, Aitken JD, Carvalho FA, et al. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. Science. 2010;328:228–31.

5. Honda K, Littman DR. The microbiome in infectious disease and inflammation. Annu Rev Immunol. 2012;30:759–95.

6. Vujkovic-Cvijin I, Dunham RM, Iwai S, et al. Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. Sci Transl Med. 2013;5:193ra91.

7. Nwosu FC, Avershina E, Wilson R, et al. Gut microbiota in HIV infection: implication for disease progression and management. Gastroenterol Res Pract. 2014;2014:803185.

8. West CE, Renz H, Jenmalm MC, et al. The gut microbiota and inflammatory noncommunicable diseases: Associations and potentials for gut microbiota therapies. J Allergy Clin Immunol. 2015;135:3–13.

9. Senghor B, Sokhna C, Ruimy R, et al. Gut microbiota diversity according to dietary habits and geographical provenance. Hum Microbiome J. 2018;7–8:1–9.

10. Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. Nature. 2012;486:222–7.

11. Gill T, Asquith M, Brooks SR, et al. Effects of HLA–B27 on gut microbiota in experimental
spondyloarthritis implicate an ecological model of dysbiosis. Arthritis Rheumatol. 2018;70:555-65.

12. O'Keefe SJ, Chung D, Mahmoud N, et al. Why do African Americans get more colon cancer than Native Africans?. J Nutr. 2007;137:175S-82S.

13. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010;464:59-65.

14. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature. 2012;486:207-214.

15. Morita H, Kuwahara T, Ohshima K, et al. An improved DNA isolation method for metagenomic analysis of the microbial flora of the human intestine. Microbes Environ. 2007;22:214-22.

16. Kim SW, Suda W, Kim S, et al. Robustness of Gut Microbiota of Healthy Adults in Response to Probiotic Intervention Revealed by High-Throughput Pyrosequencing. DNA Res. 2013;20:241-53.

17. Klindworth A, Popp L, Pruesse E, et al. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 2013;41:e1.

18. Bolyen E, Rideout JR, Dillon MR, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37:852-7.

19. Callahan BJ, McMurdie PJ, Rosen MJ, et al. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13:581-3.

20. Quast C, Pruesse E, Yilmaz P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2012;41:D590-6.
21. Fabian P, Varoquaux G, Gramfort A, et al. Scikit-learn: Machine learning in Python. J Mach Learn Res. 2011;12:2825-30.

22. Shannon CE. A mathematical theory of communication. Bell System Technical Journal. 1948;27:379-423.

23. Martinez I, Stegen JC, Maldonado-Gomez MX, et al. The gut microbiota of rural Papua New Guineans: composition, diversity patterns, and ecological processes. Cell Rep, 2015;11:527–38.

24. Hansen ME, Rubel MA, Bailey AG, et al. Population structure of human gut bacteria in a diverse cohort from rural Tanzania and Botswana. Genome Biol. 2019;20:16.

25. Monaco CL, Gootenberg DB, Zhao G, et al. Altered virome and bacterial microbiome in human immunodeficiency virus-associated acquired immunodeficiency syndrome. Cell Host Microbe. 2016;19:311–22.

26. Morton ER, Lynch J, Froment A, et al. Variation in rural African gut microbiota is strongly correlated with colonization by Entamoeba and subsistence. PLoS Genet. 2015;11:e1005658.

27. Nowak RG, Bentzen SM, Ravel J, et al. Rectal microbiota among HIV-uninfected, untreated HIV, and treated HIV-infected in Nigeria. AIDS. 2017;31:857–62.

28. Schnorr SL, Candela M, Rampelli S, et al. Gut microbiome of the Hadza hunter-gatherers. Nat Commun. 2014;5:3654.
**Figure Legends**

**Figure 1. Taxa bar plots showing top 10 abundant genera in fecal microbiome in the whole participants.**

Individual bars represent frequencies of the genera in fecal microbiome of individual healthy Ghanaians (n = 55).

**Figure 2. Abundant genera in fecal microbiome of Ghanaians, Papua New Guineans, and US people.**

(A) Top 20 abundant genera found in fecal microbiome of cohorts in Ghana, PNG, and US. The top 7 abundant genera in our Ghana cohort are shown by bold. Genera included in top 20 abundant genera in all the three cohorts are shown by blue. Genera included in top 20 only in Ghana but not in PNG or US are shown by red. Genera included in top 20 in both PNG and US but not in Ghana are shown by pink. Genera included in top 20 in Ghana and PNG but not in US are shown by brown. The genus included in top 20 in Ghana and US but not in PNG is shown by green. (B) Comparison of relative abundance of fecal microbiome among Ghana, PNG, and US. Relative abundance of the top 10 genera in Ghana is shown.

**Figure 3. Comparison of alpha diversity of fecal microbiome between females and males.**

Shannon diversity of fecal microbiome was compared between females and males in our Ghana cohort. No significant difference was observed by Wilcoxon rank sum test.
Figure 4. Comparison of alpha diversity of fecal microbiome among Ghana, PNG, and US cohorts.

Shannon diversity of fecal microbiome was compared among Ghana, PNG, and US cohorts. Our Ghana cohort showed significantly lower alpha diversity compared to PNG and US \((p < 0.001 \text{ [****] by Kruskal Wallis test with Benjamini, Krieger and Yekutieli FDR correction})\).
Table 1. Top 20 abundant genera in fecal microbiome of healthy Ghanaian adults

| Phylum        | Class          | Order          | Family       | Genus                      | Mean rel. abundance<sup>1</sup> | PNG<sup>2</sup> | US<sup>3</sup> |
|---------------|----------------|----------------|--------------|----------------------------|----------------------------------|----------------|---------------|
| **Firmicutes**| Clostridiales  | Ruminococcaceae| Ruminococcus | Faecalibacterium            | 20% 2% 6%                        |                |               |
|               |                |                | UCG-014      | Closipucelus              | 3% 0.4% 0.1%                     |                |               |
|               |                |                | UCG-002      | Coprostanolesgenes group  | 2% 0.2% 0.4%                     |                |               |
|               |                |                | Clostridium  | Closipucelus sensu strictum| 2% 2% 0.3%                       |                |               |
|               |                | Lachnospiraceae| Agathobacter  |                            | 2% 2% 4%                         |                |               |
|               | Negativicutes  | Selenomonadales| Veillonellaceae| Dialister                 | 2% <0.1% 0.2%                    |                |               |
|               | Bacilli        | Lactobacillales| Streptococcaceae| Streptococcus        | 1% 23% <0.1%                     |                |               |
| **Proteobacteria**| Gammaproteobacteria | Enterobacteriales| Enterobacteriaceae | Escherichia-Shigella | 7% 0.3% <0.1%                    |                |               |
|               |                |                | Klebsiella    |                            | 3% <0.1% <0.1%                   |                |               |
|               |                |                | Enterobacteriaceae Unclassified |                  | 2% 1% <0.1%                     |                |               |
|               | Bacteroides    | Prevotellaceae  | Prevotella    |                            | 4% 2% 1%                         |                |               |
|               | Bacteroides    | Bacteroides    | Bacteroides   |                            | 3% 0.3% 2%                       |                |               |
|               | Coriobacteriales| Coriobacteriaceae| Senegalimassilia |                            | 2% 0.3% <0.1%                    |                |               |
|               |                |                | Eggerthellaceae| Collinsella              | 1% 6% 5%                         |                |               |
| **Actinobacteria**|                |                | Bifidobacteriaceae| Bifidobacterium       | 1% 2% 7%                         |                |               |

<sup>1</sup> Mean relative genera abundance in our cohort in Ghana.
<sup>2</sup> Mean relative genera abundance in a cohort in Papua New Guinea (PNG) or United States (US) (MG-RAST accession number: mgp10381). nd, not described.
Figure 2

### Relative Abundance Comparison

**A**

- **Ghana**
  - Faecalibacterium
  - Subdoligranulum
  - Escherichia-Shigella
  - Prevotella 9
  - Ruminococcaceae UCG-014
  - Bacteroides
  - Klebsiella
  - Ruminococcaceae UCG-002
  - Achromobacter
  - Clostridium sensu stricto 1
  - Dialister
  - Agathobacter
  - [Eubacterium] coprostanoligenes group
  - Stenotrophomonas
  - Enterobacteriaceae unclassified
  - Megamonas
  - Senegalimassilia
  - Bifidobacterium
  - Streptococcus
  - Collinsella

- **PNG**
  - Faecalibacterium
  - Subdoligranulum
  - Escherichia-Shigella
  - Prevotella 9
  - Ruminococcaceae UCG-002
  - Klebsiella
  - Bacteroides
  - Clostridium sensu stricto 1
  - Dialister
  - Agathobacter
  - [Eubacterium] coprostanoligenes group
  - Stenotrophomonas
  - Enterobacteriaceae unclassified
  - Megamonas
  - Senegalimassilia
  - Bifidobacterium
  - Streptococcus
  - [Ruminococcus] torques group

- **USA**
  - Faecalibacterium
  - Subdoligranulum
  - Escherichia-Shigella
  - Prevotella 9
  - Ruminococcaceae UCG-002
  - Bacteroides
  - Klebsiella
  - Ruminococcaceae UCG-014
  - Achromobacter
  - [Eubacterium] coprostanoligenes group
  - [Eubacterium] hallii group
  - Anaerostipes
  - Faecalibacterium
  - Collinsella
  - Lachnospiraceae unclassified
  - Subdoligranulum
  - Calenibacterium
  - Blautia
  - Dorea
  - Clostridium sensu stricto 1
  - Prevotella 9
  - Faecalibacterium
  - Romboutsia
  - Bifidobacterium
  - Agathobacter
  - Erysipelotrichaceae UCG-003
  - [Eubacterium] hallii group
  - Lactobacillus
  - [Ruminococcus] torques group
  - [Eubacterium] coprostanoligenes group

**B**

- **Ghana**
- **PNG**
- **USA**

### Color Legend
- Faecalibacterium
- Subdoligranulum
- Escherichia-Shigella
- Prevotella 9
- Ruminococcaceae UCG-014
- Bacteroides
- Klebsiella
- Ruminococcaceae UCG-002
- Achromobacter
- Others
Figure 3
Figure 4