論 文 の 内 容 の 要 旨

論文題目　　Antibiotics shapes population-level diversity in the human gut
microbiome
（抗生物質はヒト腸内細菌叢の集団レベルでの多様性を形成する）

氏名　　西嶋　傑

Introduction

Over 100 trillion microbes colonize human gut and they have profound influences on host's physiology. Recent studies using NGS-based metagenomic approaches have suggested their high ecological diversity across countries, suggesting that factors such as dietary intake, host's genetics and/or host's life style significantly affects the gut microbiomes. However, little is known about the factors and extent of their contribution to the population-level diversity because the studies were limited mostly between a few cohorts with different countries. To explore the association of the factors with human gut microbiomes, I conducted a large-scale association study of the epidemiological data on dietary intake and antibiotic use with metagenomic data of 861 human gut microbiomes from 12 countries, including Japan.

Methods

Fecal DNA from 104 Japanese individuals was prepared with enzymatic methodand sequenced using 454 GS FLX (Roche), Ion PGM/Proton (Life Technologies), and MiSeq (Illumina). Metagenomic data of gut microbiomes of 757 individuals from other 11 countries were downloaded from NCBI and EBI. Evaluation of the microbial composition was performed by mapping the metagenomic reads to a reference genome dataset comprised of 6,149 microbial genomes using Bowitie2 with $\geq$ 95% identity. Epidemiological data of dietary intake in the 12 countries were collected from Food and Agriculture Organization Corporate Statistical Database (FAOSTAT) and antibiotics usages in 11 countries were obtained from several papers.

Results

The multi-dimensional scaling (MDS) plot and hierarchical clustering of the microbial compositions showed that each country had a tendency to form distinct clusters, which had little relation with geographical and genetic closeness (Fig. 1a). A permutation test confirmed significantly higher similarity of the microbial composition between individuals within a

country than those between different countries ($P < 0.01$; Fig. 1b). These results suggested that the gut microbiome structure is significantly diverse across these countries.
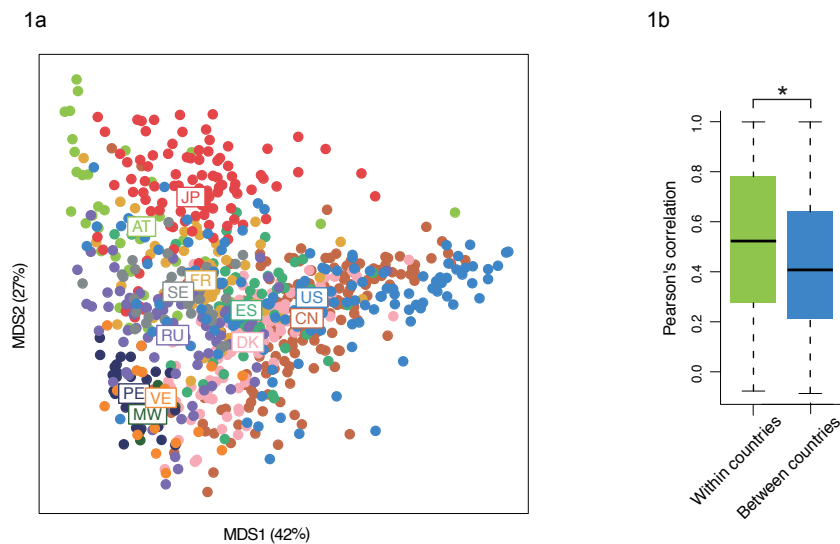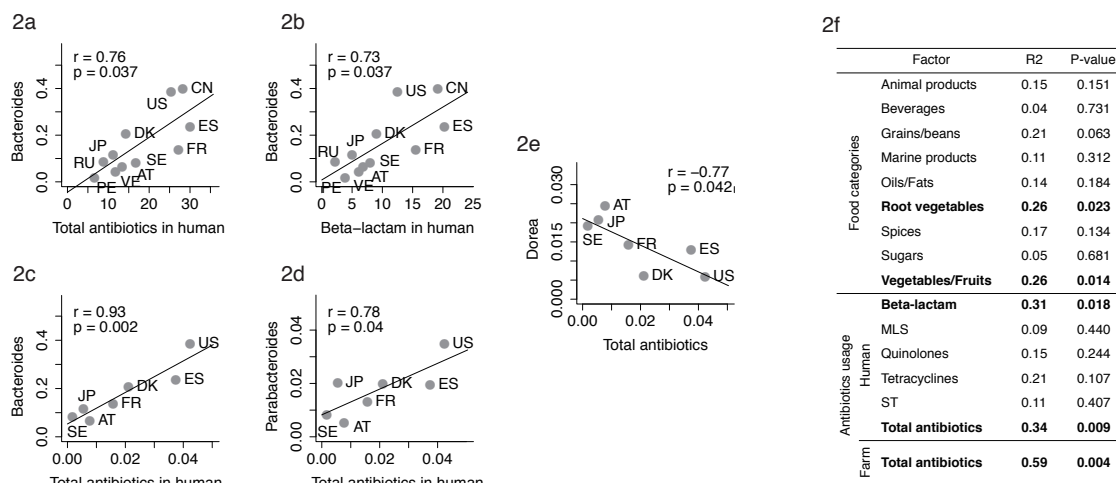

Fig. 1. Population-level diversity in human gut microbiomes from 12 countries. a, MDS plot of microbial compositions. b, Comparison of Pearson's correlation of microbial compositions in individuals within and between countries.

I explored factors that are associated with the population-level diversity in the gut microbiomes across the 12 countries. Since diet is considered to be a major factor influencing microbial composition, I examined association of diet with human gut microbiomes. Correlation analysis revealed that major species *Prevotella* positively correlated with "Grains/beans" and "Root vegetables", and negatively with "Animal products" (Pearson's correlation = 0.78, 0.79 and -0.68 respectively). Unexpectedly, none of dietary factors showed a significant association with another major species *Bacteroides*. This shallow association of dietary intake with *Bacteroides* suggested the existence of factors other than diet that might have a large influence on this major genus, as well as the population-level diversity in human gut microbiomes.

I next examined antibiotic usage because it can significantly alter the gut microbiome composition. Correlation analysis with the antibiotic usage revealed significant positive correlations between *Bacteroides* with both total antibiotic usage and beta-lactam usage in human, and total antibiotic usage in farms (Fig. 2a-c). *Parabacteroides, Odoribacter*, *Parasutterella*, *Sutterella* and *Acetobacter* also showed significant positive correlations with total antibiotic usage in humans or in farms. In contrast, total antibiotic usage in farms showed a significant negative association with the abundance of *Dorea* (Fig. 2e). These strong association of antibiotics usage with human gut microbiomes were further supported by permutational

multivariate analysis of variance (PERMANOVA), where total antibiotic usage in humans and farms and beta-lactam usage in humans significantly contributed to the overall structure of the gut microbiome as much as dietary factors (Fig. 2f).

Fig. 2

	Factor	R2	P-value
Food categories	Animal products	0.15	0.151
	Beverages	0.04	0.731
	Grains/beans	0.21	0.063
	Marine products	0.11	0.312
	Oils/Fats	0.14	0.184
	Root vegetables	**0.26**	**0.023**
	Spices	0.17	0.134
	Sugars	0.05	0.681
	Vegetables/Fruits	**0.26**	**0.014**
Antibiotics usage Human	**Beta-lactam**	**0.31**	**0.018**
	MLS	0.09	0.440
	Quinolones	0.15	0.244
	Tetracyclines	0.21	0.107
	ST	0.11	0.407
	Total antibiotics	**0.34**	**0.009**
Farm	**Total antibiotics**	**0.59**	**0.004**

Fig. 2. Correlations between the genera and antibiotic usage. a-e, correlation of the genera with antibiotics usage in human and farms. f, PERMANOVA for dietary and antibiotic factors to gut microbiome variation.

Positive correlation between antibiotic usage in the country and the frequency of antibiotic resistant genes (ARs) in the gut microbiome of the nation was reported. To further explore the involvement of ARs in the association of microbial abundance with antibiotic usage, I compared the frequencies of ARs annotated in genomes between *Bacteroides*, four minor genera positively associated with antibiotic usage, and other genera having little association with antibiotic usage. The results showed that the positive-associated genera encoded more ARs, particularly resistance-nodulation-cell division (RND) efflux pump, than other genera (Fig. 3a and 3b), suggesting that the proliferation of the ARs underlies the positive correlation between these genera and antibiotic usage.

I also experimentally validated the positive correlation between the abundance of *Bacteroides* and beta-lactam usage by comparing the gut microbial compositions between mice treated and untreated with beta-lactam antibiotics. The 16S ribosomal RNA gene sequence (16S) analysis revealed that the antibiotic treatment increased the abundance of *Bacteroides* (Fig. 4).

Conclusion

The present study provides evidence for the strong impact of antibiotics on the human gut microbiome, particularly on the major species *Bacteroides* that is a determinant of gut microbial community types, leading to high population-level diversity. The strong association with antibiotic usage in farms implies the involvement of exposure of antibiotics from environments in perturbation of the human gut microbiome. Alteration of the gut microbiome by antibiotic treatment has been proven to link to the etiology of several diseases in mice, suggesting a connection between antibiotic usage and the prevalence of modern diseases in developed countries.
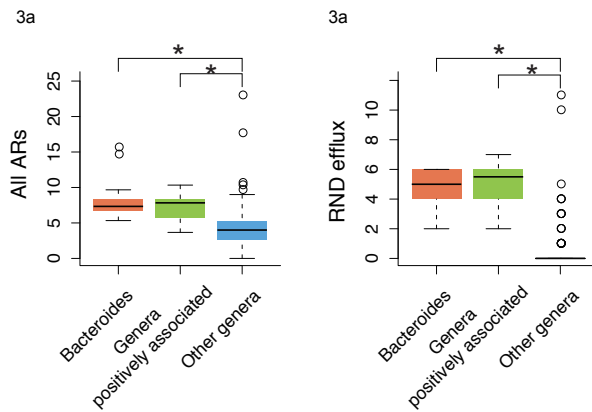
Fig. 3. Comparison of the number of ARs in genomes.
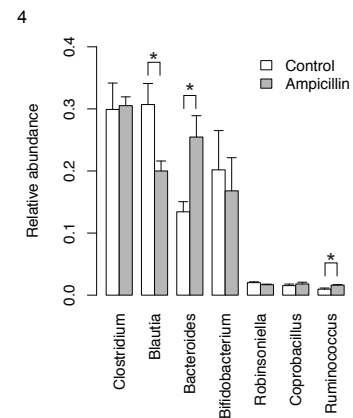
Fig. 4. Increase in the abundance of *Bacteroides* in mice treated with beta-lactam antibiotics.