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Prebiotic Effects of Poly-Gamma-Glutamate on Bacterial Flora in Murine Gut

Hee-Eun Jin[1], Jae-Chul Choi[1,2], Yong Taik Lim[3], and Moon-Hee Sung[1,2*]

[1]*Department of Bio and Fermentation Convergence Technology, Kookmin University, Seoul 02707, Republic of Korea*
[2]*BioLeaders Corporation, Daejeon 34028, Republic of Korea*
[3]*SKKU Advanced Institute of Nanotechnology, School of Chemical Engineering, Sungkyunkwan University, Suwon 16419, Republic of Korea*

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**Corresponding author*
Phone: +82-2-910-5347;
Fax: +82-2-910-5739;
E-mail: smoonhee@kookmin.ac.kr

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Prebiotics improve the growth or activities of specific microbial genera and species in the gut microbiota in order to confer health benefits to the host. In this study, we investigated the effect of poly-gamma-glutamate (γ-PGA) as a prebiotic on the gut microbiota of mice and the organ distributions of γ-PGA in mice. Pyrosequencing analysis for 16S rRNA genes of bacteria indicated that oral administration of γ-PGA increased the abundance of Lactobacillales while reducing the abundance of Clostridiales in murine guts. It is suggested that oral administration of γ-PGA can be helpful for modulating the gut microbiota as a prebiotic.

Keywords: Poly-γ-glutamate, gut microbiota, prebiotics, organ distributions

Poly-gamma-glutamate (γ-PGA) is an anionic polymer of glutamic acid bonded by γ-amide linkages [1]. It is a natural biopolymer obtained by the fermentation of *Bacillus* species from traditional fermentation foods. The specific applications of γ-PGA have continued to be developed during the last 10 years, and the research for potential industrial and scientific benefits is being carried out. The gut microbiome can be altered by prebiotics by inducing the growth of probiotic microorganisms and providing beneficial effects [2, 3]. The human microbiome and its relationship with diseases, such as irritable bowel syndrome [4], polyposis or colorectal cancer [5], type II diabetes [6], and obesity [7], have been proven. The composition of the microbial community impacts directly or indirectly the host's conditions, and can contribute to health or illness [8]. This microbial colonization processing promotes health benefits with other factors that modify the microbial composition [9]. The aim of this study was to evaluate the effect of γ-PGA as a prebiotic on the gut microbiome. The composition of fecal microbiota was analyzed after oral administration of γ-PGA.

Five-week-old female C57BL/6 mice were purchased from Jung Ang Lab Animal Inc. (Korea) and were housed under air-uncontrolled conventional conditions. Analysis for organ distribution was carried out using γ-PGA-Bz-DTPA-Gd, synthesized as previously described [10], and using ^{123}I-labeling of tyrosinamide conjugated γ-PGA as described elsewhere [11, 12]. To evaluate γ-PGA in organs, the organs were collected after oral administration and γ-PGA was detected using ICP-MS (PerkinElmer, USA). When using γ-PGA-Bz-DTPA-Gd, were collected the organs at the time point of 0, 1, 2, 4, 8, 12, 16, and 24 h. For oral administration of ^{123}I-labeling of tyrosinamide-conjugated γ-PGA, the organs were collected at time point of 2, 4, 8, 12, and 24 h, respectively. The number of mice per group was five. γ-PGA would stay in the large intestine for the longest time, seen through evaluation of organ distribution using Gd (Fig. 1) and radioiodine (Fig. 2).

We confirmed that γ-PGA affected the composition change of the gut microbiota in mice. A 2,000 kDa γ-PGA (average molecular mass: 2,250 kDa; polydispersity: 2.4) and a 2 kDa γ-PGA (average molecular mass: 2.1 kDa; polydispersity: 1.2) were extracted as previously described [13] from culture broth of *Bacillus subtilis* chungkookjang used in this study. The experiment was conducted to evaluate the change of microbial community, by oral administration of γ-PGA, in

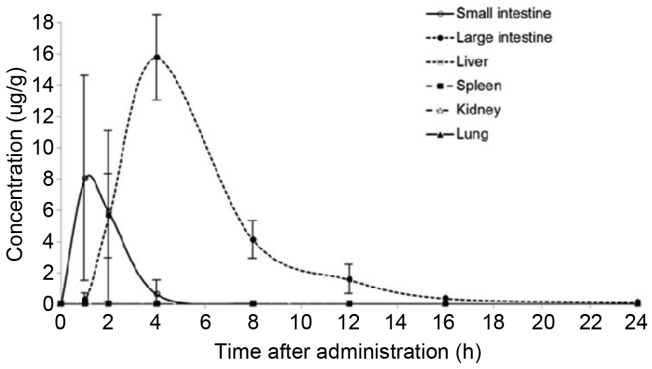

Fig. 1. Organ distribution in mice following oral administration of Gd-labled γ-PGA.

the gut of mice. For this, 400 μg of either 2,000 kDa or 2 kDa γ-PGA per day was orally administrated for 2 weeks, and fecal samples were collected at day 14, for three mice per group. The bacterial DNA was extracted using a FastDNA Spin Kit (MP Biomedicals, USA) and the quantity of amplicon DNA was determined with a Quant-iT PicoGreen dsDNA Assay kit (Thermo Fisher Scientific, USA) after amplifying the bacterial 16S rRNA genes in all samples. Equal concentrations of purified products were pooled together, and we removed short fragments (non-target products) with the Ampure beads kit (Agencourt Bioscience, USA). Mixed ampicons were pooled and the sequencing was carried out at Chunlab. Inc. (Korea), with the Illumina MiSeq Sequencing system (illumina, USA) according to the manufacturer's instructions. Each sequence was identified using the EzTaxon database. We used the CLcommunity software provided by ChunLab (http://www.chunlab.com) to assess the species richness and diversity. With this software, the rarefaction technique is used to visualize the rate of increase in the number of operational taxonomic units (OTUs). Rarefaction means the species richness of a sample. OTUs are defined as a cluster of reads with 97% sequence similarity, using the CD-HIT program.

The microbial compositions were analyzed in fecal samples of mice administrated with 400 μg each of 2,000 kDa or 2 kDa γ-PGA daily. We found that the bacterial community of mice administrated each γ-PGA showed different aspects compared with that of control group under order level (Figs. 3A and 3C). The relative abundance of Lactobacillales increased dramatically in both γ-PGA-treated mice, from 8% to 42% and 38%, whereas Clostridiales decreased from 43% to 15% and 8% by 2,000 kDa and 2 kDa γ-PGA, respectively. Interestingly, 2,000 kDa and 2 kDa γ-PGA-treated groups showed different aspects under the species level (Figs. 3B and 3D). The populations of *Lactobacillus intestinalis* increased from 0.9% to 23% by 2,000 γ-PGA, and from 0.3% to 30% in 2 kDa γ-PGA-treated mice. In particular, it was found that a decreased *Clostridium* numbers corresponded with an increased level of *Lactobacillus animalis*, which had inhibitory activity against *Clostridium perfringens* [14]. The populations of EF602759, HQ765871, and EF406368 that belonged to the same order (Bacteroidales) showed different relative abundances between the mice administrated high- and low-molecular-weight γ-PGAs.

Lactobacillus spp. secrete lactic acid and produce compounds that can decrease the in vivo colonization of pathogenic *Escherichia coli* [15]. Moreover, *Lactobacillus* spp.

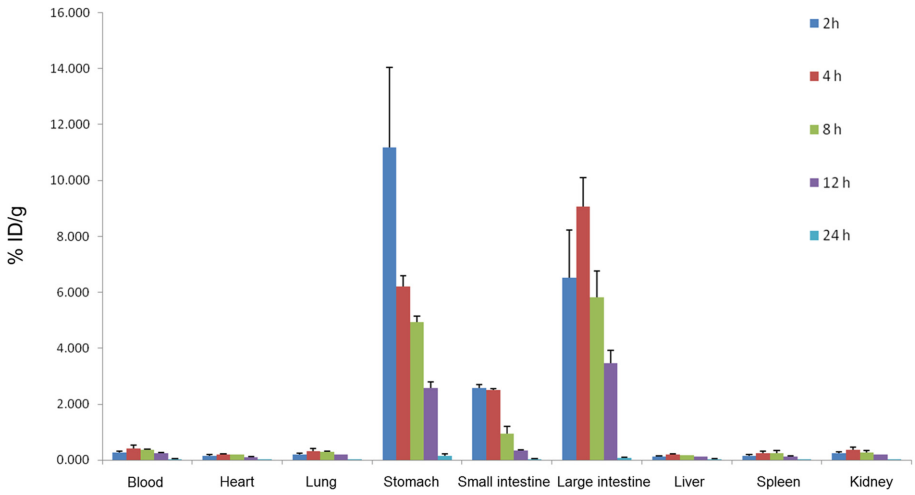

Fig. 2. Organ distribution in mice following oral administration of ^{123}I-labeled tyrosinamide-conjugated γ-PGA, expressed as the percentage of the injected dose per gram tissue (%ID/g).

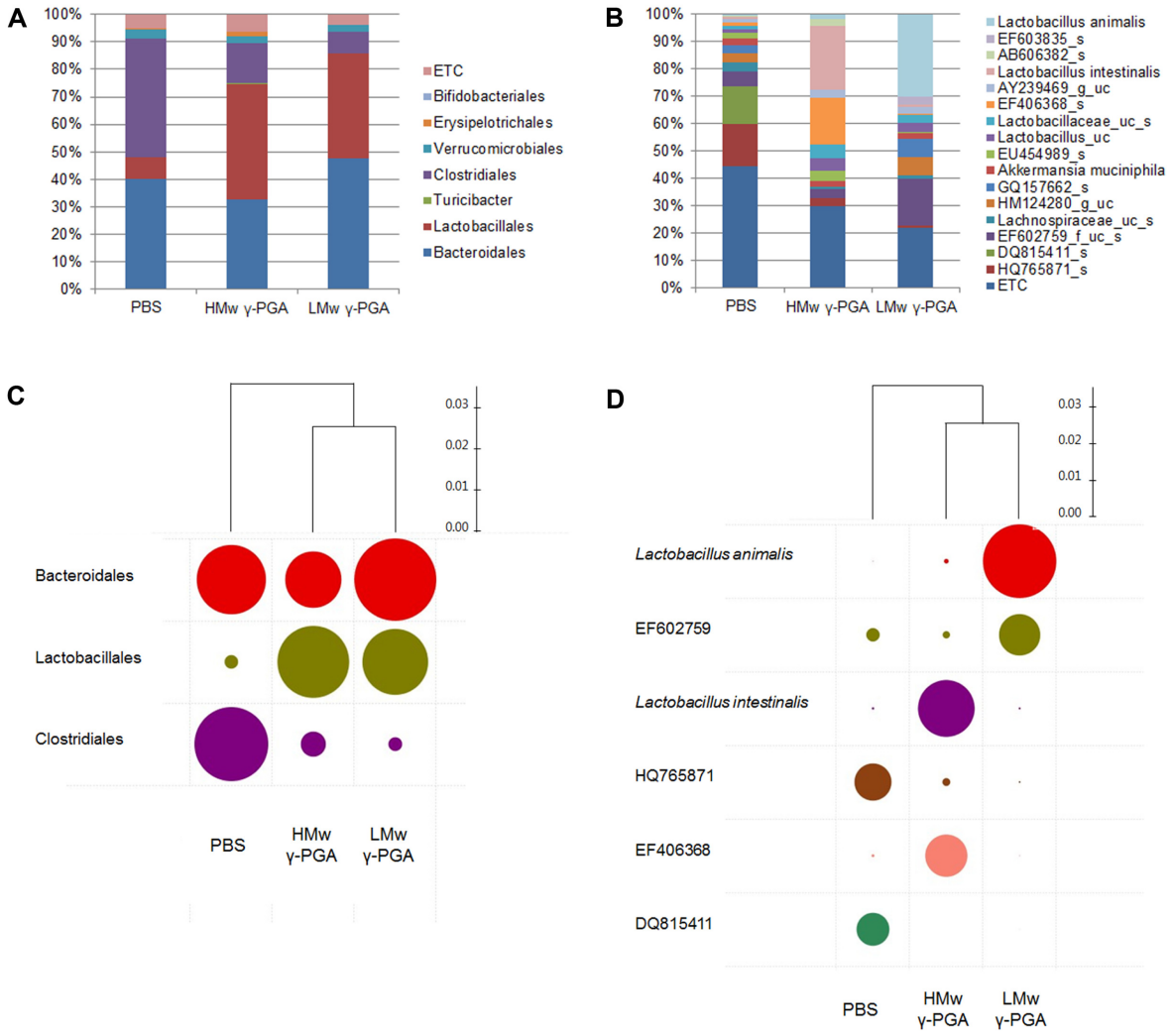

Fig. 3. Composition changes of the microbial community by administration of γ-PGA.
Bar graphs of the relative abundance of the major order (**A**) and species (**B**) classification of samples; the proportion with less than 2% occupancy is noted as ETC. Heat map circle type profiles and dendograms of the most abundant OTUs at the order (**C**) and species (**D**) levels in the gut microbiota. Display with a minimum ratio is 10% individual samples (HMw γ-PGA: 2,000 kDa γ-PGA; LMw γ-PGA: 2 kDa γ-PGA).

potentiate the antimicrobial activity by disrupting the bacterial outer membrane [16], and provide the maintenance of tight junctions in intestinal epithelia in order to protect against pathogen infiltration or intestinal injury [17]. Bruce-Keller AJ *et al*. [18] recently reported that the levels of *Clostridium* were elevated in subjects on a high-fat diet .

Prebiotics that can restore healthy gut microbiota by changing its composition are being developed as new therapeutic approaches to treat various diseases [3]. Because the gut microbiota plays an important role in maintaining physiological reactions of the host, new dietary treatments, such as γ-PGA, have been developed to modulate the gut immune response and restore intestinal homeostasis. The results of this study indicate that high-molecular-weight γ-PGA with its viscous characteristics can stabilize the gut microorganisms, and low-molecular-weight γ-PGA reaching the colon can be utilized as a prebiotics, able to increase the diversity of the microbial composition in gut. We expect that the effect of γ-PGAs as prebiotics could be an additional mechanism to verify the beneficial effect of γ-PGA on human health and diseases.

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