Synbiotics containing nanoprebiotics: a novel therapeutic strategy to restore gut dysbiosis

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Research Article

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Abstract

**Background:** In our previous study, it was demonstrated that nanoprebiotics, phthalyl pullulan nanoparticles (PPNs), a new formulation enhanced the antimicrobial activity of probiotics *Lactobacillus plantarum* (LP) *in vitro* by intracellular stimulation more than backbone prebiotics generally used by far. In this study, we aimed to investigate whether this combination may exert a distinguished effect as synbiotics *in vivo*. To accomplish this goal, the synbiotics combination of LP, pullulan, and PPNs were treated to a dysbiosis-induced mouse model and assessed their restoring effect using pathogen *Escherichia coli* K99 (EC) challenge.

**Results:** The experiment group fed with synbiotics containing PPNs more suppressed the infection of EC in mice and reinforced the gut barrier by proving the decreased serum endotoxin level. Also, the synbiotics containing PPNs increased microbial diversity as a representative parameter of healthy status compared with other groups, including a normal control group. Furthermore, distinct from treated probiotics alone, the synbiotics showed additive effects to enrich several well-known beneficial bacteria such as *Lactobacillus, Bifidobacterium*, and several butyrate-producing bacteria including *Faecalibacterium*.

**Conclusion:** Our results indicated that synbiotics containing PPNs are very effective at restoring gut dysbiosis and suppressing pathogenic infection with an increase in microbial diversity, suggesting that the synbiotics with nanoprebiotics have the potential to be a novel strategy for curing gut dysbiosis and infectious diseases.

**Background**

Gut microbiota has gained much attraction in recent years to function as a key organ in regulating host homeostasis. Indeed, several researches have substantiated gut dysbiosis is highly associated with various diseases such as inflammatory bowel disease (IBD), obesity, and cancer [1–3]. Therefore, maintaining or restoring gut microbiota in a balanced state is important for host health. As dysbiosis is mainly concomitant to thrive or/and infection of harmful microbes, the ability of gut microbiota to suppress enteric pathogens is a pivotal factor [4]. To this, several strategic therapies such as probiotics, prebiotics, and synbiotics are being widely studied [5].

Probiotics are living microorganisms that provide health benefits to the host when adequate amounts are administered [6]. They are generally safe and have potent to prevent and cure dysbiosis owing to their producing antibacterial peptides such as bacteriocins and their ability to enhance the intestinal barrier functions [7–9]. In the case of prebiotics, they stimulate the growth of probiotics or other beneficial microorganisms in the GI tract, decreasing pathogens and providing favorable effects to the host [10]. Synbiotics, a combination of probiotics and prebiotics, are also designed to make a synergistic effect on pathogen suppression [11]. Shortly, they all aim to make it easier for gut microbiota to control pathogens by strengthening their antimicrobial ability.
Our previous studies have demonstrated nanoprebiotics (NPs) whose backbones were inulin (PIN), dextran (PDN), starch (PSN), and pullulan (PPN) increased antimicrobial ability of probiotics in vitro [12–15]. They enhanced the expression of bacteriocin biosynthetic genes and activated the defense system of the probiotics by internalizing themselves into the probiotics. The probiotics showed much higher antimicrobial activity against both Gram-positive and Gram-negative pathogens when treated with NPs. In the case of PDN, an in vivo feeding experiment was conducted to investigate the effects on gut microbiota when fed with the NPs and their probiotics partner [13]. The synbiotics suppressed pathogenic infection, which implied that the synbiotics had potent as preventive drugs against gut dysbiosis. However, their effects were only explored under eubiosis condition. Also, the mixing effect of both NPs and their backbone polysaccharide as partners of synbiotics was not investigated.

In this study, we aimed to check if synbiotics containing NPs are effective at recovering from dysbiosis in terms of restoring the gut barrier and suppressing pathogenic infection. Accordingly, PPNs were prepared using pullulan and a newly designed synbiotics combination (probiotics: LP, prebiotics: pullulan and PPNs) was treated to antibiotics-induced gut dysbiosis murine model and evaluated its gut-restoring effects by measuring the amount of invading pathogen, alteration of the gut microbiome, and other host physiological changes after challenging pathogen EC.

Methods

Materials

Pullulan used in this study was purchased from Shandong Freda Biotechnology Co., Ltd. (Shandong, China), and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). For bacterial cultures, lysogeny broth (LB), LB agar, De Man, Rogosa, and Sharpe agar (MRS) broth, Brain Heart Infusion (BHI) broth, MacConkey sorbitol agar and Oxford agar were purchased from BD Difco (Sparks, MD, USA).

SCFA production of synbiotics

To determine its availability of pullulan and PPNs by LP, in vitro SCFA profiles of LP when fermenting pullulan and PPNs were investigated. Gas chromatography (GC) according to a previously described method with a slight modification was conducted [16]. Briefly, a 5.0 mL aliquot of cultured supernatant was mixed with 1.0 mL 25% HPO3 and 0.2 mL 2% pivalic acid (internal standard), and the mixture was analyzed using an Agilent 7890B GC system (Agilent Technologies, Santa Clara, CA, USA) with a FID detector. The inlet and detector temperature were maintained at 220 °C. Aliquots (1 μl) were injected with a split ratio of 10:1 into a 30 m × 0.25 mm × 0.25μm Nukol fused-silica capillary column (Cat. No: 24107, Supelco, Sigma-Aldrich, St. Louis, MO, USA) with helium carrier gas set to a flow rate of 1 mL/ min. The oven temperature was held constant at the 80 °C for 1 min, and thereafter increased at 20 °C /min to a
temperature of 180 °C and held for 1 min, and increased at 10 °C /min to a final temperature of 200 °C. The total run time per sample was about 14 min.

**Synthesis of PPNs**

Phthalyl pullulan was synthesized according to the fine-tuned method described in the previous study [15]. In brief, 1.0 g of pullulan was dissolved in 10ml of dimethylformamide (DMF), and 0.1 mol-% dimethylamino pyridine (DMAP) per pullulan sugar residue was added to the solution as a catalyst. Subsequently, phthalic anhydride was added to the above solution at a 9:1 (phthalic anhydride: pullulan) molar ratio. The reaction was performed at 54 °C for 48 h under nitrogen. The produced phthalyl pullulan was dialyzed first in DMF to remove unreacted phthalic anhydride, and then in distilled water at 4 °C for 24 h to form self-assembled phthalyl pullulan nanoparticles (PPNs). The unreacted pullulan was removed after ultra-centrifugation. Finally, the PPNs were freeze-dried and stored at −20 °C until further use. The surface topography of the PPNs was analyzed using a field-emission scanning electron microscopy (FE-SEM) with 55VP-SEM (Carl Zeiss, Oberkochen, Germany).

**In vitro evaluation of antimicrobial activity**

To determine the effects of PPNs on antimicrobial activity of LP, the co-cultivation assay and agar diffusion test with slight modification were conducted using EC and LM as pathogens [17, 18]. LP, EC, and LM were cultured in MRS, LB, and BHI broth respectively under 37 °C with shaking (255 rpm) for 24 h before being used in subsequent experiments or being stored at -70 °C in 15% (v/v) glycerol.

For the co-cultivation assay, 2.0 x 10^6 CFU/ml of EC or LM were co-cultured with 2.0 x 10^5 CFU/ml of LP treated with or without 0.5% (w/v) PPNs or pullulan in MRS broth for 8 h at 37 °C under aerobic conditions in a shaking incubator (250 rpm). The co-cultured samples were spread on the MacConkey or Oxford agar and incubated for 24 h at 37 °C, and the numbers of EC or LM colonies were counted, respectively. For agar diffusion assay, 100ul of EC or LM stock (2.0 x 10^8 CFU/ml) were spread onto LB or BHI agar. A paper disc was placed on the pathogen-spread plate, then 120 ul of 8 h-cultured media of LP treated with or without (0.5% w/v) PPNs or pullulan was dropped onto the paper disc. After drying at room temperature, the plate was cultured overnight at 37 °C. The zones of inhibition were used as a direct measurement of antimicrobial activity.

**Animal experimental procedures and measurements**

The synbiotic feeding study was performed using four-week-old BALB/c female mice under international ethical guidelines. The Institutional Animal Care and Use Committee (IACUC) at Seoul National University approved the animal experiments (SNU-180904-2-1). Mice were housed at a controlled temperature (22 ± 2 °C) on a 12 h light/dark cycle. Animals were fed standard mouse chow ad libitum and were provided
distilled water at all times. After 7 days of acclimation, mice were randomly allocated into six groups (6 BALB/c mice per group) (Fig. 2A). The control group (C) continued to be fed as before without any antibiotics, synbiotics, and pathogen challenge during the overall experimental period. The other groups (T1~T5) were administered with an antibiotics cocktail (ampicillin: gentamicin: neomycin: vancomycin=2:2:2:1, total 20 mg/mice) for three days at the beginning of the experiment to induce dysbiosis in their gut according to previously described methods with modification [19, 20].

Fecal samples were spread onto LB agar and numerated colonies to simply check antibiotics effect on gut microbiota (Fig. S2). Consequently, the groups were fed with pro-/synbiotics in saline solution via oral gavage for 2 weeks as follows: 1) T1 (No pro-/synbiotic); 2) T2 (LP $10^8$ CFU/mice); 3) T3 (pullulan (0.5 wt.-%) -treated LP (LP/P) $10^8$ CFU/mice); 4) T4 (PPNs (0.5 wt.-%) -treated LP (LP/PPNs) $10^8$ CFU/mice); 5) T5 (pullulan (0.5 wt.-%) and PPNs (0.5 wt.-%) -treated LP (LP/P/PPNs) $10^8$ CFU/mice). From the 11th day of feeding pro-/synbiotics, EC ($10^9$ CFU/mice) was administered with 0.2ml of 1% NaHCO$_3$ (treated 30min before EC administration) to the mice from the T1 to T5 groups via oral gavage for three days.

The body weights and food intakes of mice were monitored daily over the entire experimental period. At the end of the experiment, mice were sacrificed by CO$_2$. Then, ceca, colons, intestinal contents, and sera samples were collected for further analysis. The length of colons and the weight of ceca samples were measured immediately after dissection.

Viable CFU counts of lactobacilli and coliform bacteria were enumerated using intestinal contents (10mg/ml) spread onto MRS and MacConkey sorbitol agar respectively and incubated for 20 h at 37 °C.

**In vivo gut permeability assay**

To evaluate the pro-/synbiotic effect on gut barrier integrity, FITC-dextran assay and serum endotoxin level detection kit were used [21].

On the day of sacrifice, mice were subjected to fasting for 4 h, followed by intragastric injection with FITC-dextran tracer (0.6 mg/g body weight; Cat. No. 46944, Sigma-Aldrich) dissolved in 0.1 mL PBS. After 3 h, sera samples were collected to measure the intensity value of FITC fluorescence at an excitation wavelength of 490 nm and an emission wavelength of 530 nm using a Hitachi F-4500 fluorescence spectrophotometer. The samples were diluted in PBS to plot a standard curve. The measurement has been conducted a minimum of three times per sample.

Serum endotoxin levels were detected using the Pierce™ Chromogenic Endotoxin Quant Kit (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer’s protocol. 50 μl of diluted serum samples were tested by a colorimetric method.

**DNA extraction and sequencing**
The bacterial gDNA of intestinal samples were extracted to identify and estimate specific bacteria or their community by quantitative PCR (qPCR) and high-throughput sequencing of the bacterial 16S ribosomal RNA (16S rRNA) genes.

DNA was extracted according to the manufacturer's protocol from 50 mg of each intestinal content sample using the AccuPrep® Stool DNA extraction kit (Bioneer, Daejeon, Republic of Korea), followed by storage at -20 °C until further analysis. For species-specific qPCR, the primers used were designed based on the sequences, and qPCR was performed as previously described [22]. The primers used to detect LAB (Lactobacillus, Bifidobacterium) and E. coli were mentioned in Table S1.

To explore the microbial community of intestinal samples, the V4 region of the bacterial 16S rRNA gene was amplified using Takara Ex-Taq polymerase (Takara Bio, Shiga, Japan) and universal primers (F: 5’ GTGCCAGCMGCCGCGGTAA-3’, R: 5’-GGACTACHVGGGTWTCTAAT-3’). The amplification program consisted as follows: 1 cycle of 94 °C for 3 min, 40 cycles of 94 °C for 45 sec, 55 °C for 1 min, and 72 °C for 1.5 min, and 1 cycle of 72 °C for 10 min. The amplicons were separated by agarose gel electrophoresis (100 V, 45 min) and purified using a QIAquick Gel Extraction Kit (Qiagen, CA, USA).

The NEB Next Ultra DNA Library Prep Kit for Illumina (New England Biolabs, MA, USA) was used to construct DNA libraries with some modifications to the manufacturer's instructions. The size selection steps for the adaptor-ligated DNAs and the cleanup steps were replaced by PCR products using a QIAquick PCR Purification Kit (Qiagen, CA, USA). The adaptor and index primers were added to the amplicons using the NEBNext Multiplex Oligos for Illumina Kit (New England Biolabs, MA, USA). The construction of the DNA libraries was confirmed by agarose gel electrophoresis, and the libraries were purified using a QIAquick Gel Extraction Kit. The components of the libraries were then sequenced using an Illumina MiSeq 2 × 250 bp paired-end sequencing platform (Macrogen, Daejeon, Republic of Korea). The 16S rRNA gene sequences determined in this study were deposited in the NCBI SRA database with accession number SRX7944600.

**Microbial community analysis**

The microbial community was analyzed by using Quantitative Insights Into Microbial Ecology (QIIME) version 1.9.1 software and several in-house Perl scripts. Briefly, raw sequence reads were checked for their quality by FastQC V0.11.8 and trimmed by FASTX-Toolkit v.0.0.13 before the poor quality region. Then, trimmed paired-end reads were merged using FLASH v1.2.11 and demultiplexed. The sequence reads were then clustered into operational taxonomic unit (OTU) tables by subsampled open-reference OTU picking at a 97 % level of sequence similarity with the GreenGenes 13_8 database as the reference. The OTU picking method was usearch61, and the value of parameter percent_subsample was 0.1 [23]. The representative sequences were aligned using the PyNAST program, which were taxonomically assigned using the lust consensus taxonomy assigner [24].
The microbial diversity of the samples (alpha diversity) was determined using the abundance-based coverage estimator (ACE), Chao1, observed OTUs, phylogenetic diversity (PD), Shannon, and Simpson indices. These indices were calculated from 85,000 sequence reads through rarefaction with 10 iterations. Principal coordinates analysis (PCA) was performed at the phylum and genus level based on weighted and unweighted UniFrac distances, and the effect of probiotics, PPNs, pullulan, or their synbiotics were evaluated using Adonis statistical tests using the R vegan package. The abundance of the microbial taxa was expressed as a percentage of the total 16S rRNA gene sequences.

**Prediction of the functions of the microbial communities**

The Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) version 1.0.0 (http://picrust.github.io/picrust/) was used to predict the functional profile of the microbial communities based on the 16S rRNA gene sequences obtained [25]. The metagenomes were predicted using the precalculated Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologs, classified in a hierarchy using the KEGG pathway metadata. Linear discriminant analysis (LDA) was performed using LefSe by P<0.05 and LDA>3.0 using Galaxy (https://huttenhower.sph.harvard.edu/galaxy/) [26].

**Statistical analysis**

One-way ANOVA and post hoc Tukey's HSD test for multiple mean comparisons were used to find significant differences among groups. One-way ANOVA and simple linear regression were performed using the R statistics and corrplot packages version 3.0.3 (R Foundation for Statistical Computing, Vienna, Austria) respectively. The significance was assumed at *P < 0.05, **P < 0.01, ***P < 0.001.

To explore the relationship between serum endotoxin level and intestinal microbiota, simple linear regression analysis was performed, which assessed its significance by Pearson's correlation coefficient (r) and P values.

**Results**

**Synthesis and characterization of phthalyl pullulan nanoparticles (PPNs)**

The chemical reaction scheme of phthalyl pullulan is shown in Fig. 1A. The content of phthalate groups in the phthalyl pullulan was confirmed by measurement of $^1$H-NMR spectroscopy and was estimated by determining the ratio of protons of phthalic acid to sugar protons as the same method previously reported [15]. Also, the morphologies of the PPNs observed by SEM were spherical shapes as shown in Fig. 1B. The internalization of PPNs was confirmed by a confocal laser scattering microscope (CLSM). It was found that the PPNs were internalized into LP as shown in Fig. 1C.
To examine any internal changes in LP by PPNs, LP was treated with PPNs or pullulan, and its commonly secreted SCFAs such as acetate (C2) (Fig. S1A), propionate (C3) (Fig. S1B), and butyrate (C4) (Fig. S1C) were analyzed in vitro. It was found that the total SCFA amount in the culture medium of LP was not changed when treated PPNs whereas a significant increase was observed when treated pullulan (Fig. 1D).

The antimicrobial activities of the PPNs-internalized LP against pathogens EC and *Listeria monocytogenes* (LM) were checked by co-culture assay and agar diffusion test. The results showed that the antimicrobial activity of the PPNs-internalized LP was much higher than that of untreated LP or pullulan itself by co-culture assay and agar diffusion test (Fig. 1E, F).

**Physiological changes in host**

The mouse feeding experiment was performed to evaluate the effect of synbiotics against dysbiosis as mentioned in Fig. 2A. The effect of antibiotics treatment to mimic dysbiosis was determined by the extent of decrease of colonies on LB agar compared to that of a saline-treated group (Fig. S2).

Groups fed diets supplemented with PPNs increased body weight at the end of the feeding experiment compared with other groups (Fig. 2B). In particular, the highest body weights were obtained for the T5 groups which were supplemented with LP/P/PPNs combination, while the body weights of the T1 group were decreased on the endpoint. In the case of feed intake, the values were higher in the PPNs-supplemented group (T4 and T5) than in the others (Fig. 2C).

There were also significant changes in colon length and cecum weight by pro-/synbiotics treatment (Fig. 2D, E). The T4 group had the longest colon length and all groups supplemented with synbiotics (T3, T4, and T5) had a longer colon length compared with the T1 group (Fig. 2F), showing that the pattern of the result was similar to that of the body weight. Likewise, a noticeable increase in values of the cecum weights was only observed in the T1 group, to whom only EC was administered, whereas others fed with probiotics or synbiotics showed lower cecum weight similar to that of the C group.

To assess gut barrier restoration by pro-/synbiotics, the levels of serum endotoxin were measured. The T1 group fed only with EC, showed the highest values whereas the levels were significantly lower in the T4 and T5 groups fed with LP/PPNs and LP/P/PPNs respectively (Fig. 2G). Likewise, when injecting fluorescein isocyanate (FITC)-dextran into the guts of mice to determine intestinal permeability, a similar tendency was observed that the T1 group showed the highest level of FITC-dextran in serum while significantly lower in synbiotics-fed groups including T4 and T5 (Fig. S3).

**Effects of synbiotics on the gut microbiota**

To determine the effects of PPNs on gut microbiota, both culture-in-/dependent analysis were conducted using intestinal contents and their genomic DNA (gDNA).
Firstly, viable cells of coliform bacteria including EC and lactic acid bacteria (LAB) were enumerated by plating intestinal contents onto MacConkey agar and MRS agar respectively. The mean value of viable coliform bacteria of the T1 group was approximately $6 \log_{10} (CFU/mg \text{ of intestinal content})$ whereas other groups treated pro-/synbiotics were under $4 \log_{10} (CFU/mg \text{ of intestinal content})$ (Fig. 3A). Interestingly, the groups treated PPNs (T4 and T5) showed much lower coliform bacteria than T2 and T3.

On the other hand, the LAB amount of groups tended to be contrary to the result of coliform (Fig. 3B). The T1 group (2-3 $\log_{10} \text{ CFU/mg}$) was lowest among groups whereas the values of pro-/synbiotics groups (T2, T3, T4 and T5) were all over $4 \log_{10} (CFU/mg)$ (Fig. 3B). Also, the values of those groups were significantly increased than that of the C group.

To cross-check the results of culture-dependent analysis and investigate overall changes in the gut microbial community by pro-/synbiotics, gDNA-based analysis such as quantitative PCR (qPCR) and 16S rRNA sequencing was performed. By qPCR, similar results were observed in the levels of enteropathogenic *Escherichia coli* (intimin) (Fig. S4A) and LABs (*Lactobacillus* spp., Fig. S4B; *Bifidobacterium* spp., Fig. S4C).

Meanwhile, microbial community dynamics were explored based on 16S rRNA sequencing. Observed OTUs (operational taxonomic units), a microbial richness index, were higher in the order of T1, C, T2, T3, T4 and T5 (Fig. 3C). Other alpha diversity indices such as Shannon (diversity index; Fig. S5A) and Simpson (evenness index; Fig. S5B) had similar patterns that the lowest in the T1 and highest in the T5 were commonly observed. To examine the effect of PPNs as synbiotic partners on the microbial richness, the groups were reorganized by PPNs treatment (C, T1, T2, T3 vs. T4, T5). Interestingly, observed OTUs were higher when treating PPNs (Fig. S5C).

Principal coordinate analysis (PCoA) based on unweighted (Fig. 3D; $R^2=0.28$, $P<0.001$) and weighted (Fig. 3E; $R^2=0.85$, $P<0.0001$) UniFrac distances revealed that the gut microbiota was altered by synbiotics treatment, indicating their significant influence by Adonis test. In the PCoA plot, samples were clustered into three distinct groups (C vs. T1 vs T2, T3, T4 and T5). The samples of T2, T3, T4 and T5 were placed between the C and T1 samples, each of which is also distinguished from each other.

Next, the relative abundance of the microbial taxa in each group was compared and it was found that several phyla and genera appeared to be at quite different levels. At the phylum level, all groups shared the following 13 phyla: *Actinobacteria, Bacteroidetes, Cyanobacteria, Deferribacteres, Euryarchaeota, Firmicutes, Fusobacteria, Lentisphaerae, Proteobacteria, Spirochaetes, TM7, Tenericutes*, and *Verrucomicrobia* (Table S2). Three dominant phyla, containing more than 95% of total 16S rRNA gene sequences, were *Bacteroidetes, Firmicutes*, and *Proteobacteria* at C and T1 group, while *Bacteroidetes, Firmicutes*, and *Tenericutes* at T2, T3, T4, and T5 group (Fig. 3F). In particular, *Proteobacteria* was more abundant in the T1 group than C group, while it was significantly reduced in T2, T3, T4, and T5 groups (Fig. S6).
At the genus level, the gut microbiota of the six groups shared 102 genera (Table S2). Three dominant genera containing more than 55% of total 16S rRNA gene sequences were as follows: 1) C group: an unclassified genus of family *S24-7*, *Helicobacter*, and *Odoribacter*; 2) T1 group: unclassified genera of family *Enterobacteriaceae*, *Erysipelotrichaceae*, and, *Lachnospiraceae*; 3) T2 group: *Oscillospira*, unclassified genera of family *Lachnospiraceae*, *Rikenellaceae*; 4) T3 and T4 group: *Oscillospira*, the unclassified genus of family *Lachnospiraceae*, and unclassified genus of family *Ruminococcaceae*; 4) T5 group: unclassified genera of family *Lachnospiraceae*, *Rikenellaceae*, and *Ruminococcaceae* (Fig. S7A).

In particular, *Lactobacillus* was more abundant in T4 and T5 than other groups (Fig. S7B). Likewise, *Bidobacterium* was significantly more abundant in T4 fed with LP/PPN than other groups, which is similar to the results of qPCR (Fig. S7C). Also, *Faecalibacterium* and unclassified genus of *Veillonellaceae* family showed significantly higher abundance in the T5 group than others (Fig. S7D, E).

Taken together, supplementing synbiotics, especially LP/PPNs or LP/P/PPNs, modulated gut microbiota in increasing microbial richness, diversity. Concurrently, the relative abundances of several bacteria were different among groups.

**Predicted effects of synbiotics on the gut metagenome**

To predict the functions of the gut metagenome of each group, the abundances of Kyoto Encyclopedia of Genes and Genome (KEGG) pathways were predicted by PICRUSt software and assessed its prediction accuracy by the Nearest Sequenced Taxon Index (NSTI) scores. The average NSTI score of C, T1, T2, T3, T4, and T5 were 0.18 (±0.02), 0.07 (±0.03), 0.12 (±0.01), 0.17 (±0.01), 0.15 (±0.01), and 0.15 (±0.01), respectively, which were in similar with other mammal microbiota studies [27, 28]. Subsequently, linear discriminant analysis (LDA) effect size (LEfSe) analysis was performed to determine KEGG pathways whose abundances were different among groups.

The effects of synbiotics on the KEGG were predicted by comparing T1 and T5 groups. It was found that several significantly different KEGG pathways were identified between the two groups (Fig. 4A). For example, ‘Metabolism’, ‘Amino acid metabolism’, ‘Replication and repair’, ‘Cellular processes’ were predicted at significantly higher levels in the T5 group whereas ‘Infectious diseases’, ‘Lipopolysaccharide biosynthesis’, ‘Bacterial secretion system’, ‘Membrane transport’ were predicted at significantly higher levels in T1 group. A similar result was observed when compared with T1 and T4 (Fig. S8A).

The effects of PPNs on the intestinal microbiome were also predicted by comparing T2 and T4 groups (Fig. 4B). T4 groups showed higher levels within ‘Membrane transport’, ‘Transporters’, ‘ABC transporters’, ‘Carbohydrate metabolism’, ‘Transcription factors’, ‘Transcription’ whereas T2 groups showed higher levels within ‘Lipopolysaccharide biosynthesis’, ‘Pore’s ion channels’, ‘Folding sorting and degradation’, ‘Glycan biosynthesis and metabolism’ in T2 group, respectively. The results suggest that the administration of PPNs with probiotics may upregulate gene expression of the gut microbiome to induce the internalization of outer PPNs. Also, the PPNs are suggested to downregulate the biosynthesis of glycan such as lipopolysaccharide (LPS).
Discussion

In this study, we report perturbation in gut microbiota and host physiology associated with 2-weeks of supplementation with synbiotics including PPNs. Distinct from the case of PDN conducted under eubiosis condition, synbiotics including PPNs were administered into *in vivo* antibiotics-induced dysbiosis murine model [13]. Additionally, pullulan was also used as a synbiotic partner to investigate whether it could make additive benefits to host health. After that, the abilities of these pro-/synbiotics to restore gut from dysbiosis so that improving susceptibility against pathogen were evaluated by how effectively they suppressed infection of pathogen EC.

Firstly, PPNs were prepared and examined their ability to increase antimicrobial ability of LP *in vitro* as in a previous study [15]. Besides, it was also observed that pullulan can be fermented by LP to produce SCFAs which are crucial for intestinal health. Especially, the butyrate is well-known to regulate the intestinal barrier function, and propionate is also demonstrated its function to cure DSS-induced intestinal dysfunction [29, 30]. Although the contents of propionate and butyrate were also slightly increased when treated PPNs, the total amounts of SCFAs were not significantly changed. It was implied that PPNs would function as particles themselves when internalized into probiotics, not as carbon sources to microbial fermentation.

Meanwhile, there were significant changes in the microbial richness, diversity, composition in the gut and improvements in host physiological indices following the synbiotics-supplementing trial, which supported the dysbiosis-restoring effect of the synbiotics. The increases in body weight and feed intake of synbiotics especially LP/PPNs and LP/P/PPNs showed the synbiotics effect to recover the pathogen-suppressing activity of gut microbiota, as losses in weight and appetite are both common signs of inflammation triggered by pathogenic *Escherichia coli* [31]. The results suggest that synbiotics containing PPNs may prevent weight loss and intestinal inflammation which is provoked by pathogenic infection. Meanwhile, the length of the colon and the weight of the cecum are usually used to determine whether the gut is in an abnormal state, as they are the main reservoirs of intestinal microbes. It was reported that the colon became shorter when it is damaged by pathogen invasion or inflammation [32]. In the case of the cecum, its swelling was observed in the antibiotics administered mice and this observation is similar to the case of germ-free mice [33, 34]. In this study, pathogen EC infection induced decreases in colon length and increases in cecum weights, which were all returned to normal state by synbiotics treatment. The results suggested that the synbiotics with nanoprebiotics may play an important role in curing gut disruption such as strengthening the gut barrier. Indeed, the gut permeability was improved, thus regulating the influx of endotoxin into the blood circulation by supplementing synbiotics especially LP/PPNs and LP/P/PPNs. As endotoxin is widely known to provoke endotoxemia, causing inflammation and various diseases, the result means that synbiotics, including nanoprebiotics, may have the potential to be a novel therapeutics to treat endotoxemia or its associated diseases [35, 36].

In terms of the microbiome, there were also several interesting findings suggesting that the synbiotics can be also useful in rebuilding disrupted gut microbiota. Notably, the viable cell counts of challenged
pathogens were monitored to determine the degrees of recovering gut robustness by pro-/synbiotics. Expectedly, synbiotics with nanoprebiotics showed a distinguished ability to inhibit more pathogens than probiotics alone or synbiotics without nanoprebiotics. Also, the synbiotics increased the number of beneficial bacteria such as LAB in the gut. The results of gene expression levels detecting enteropathogenic *Escherichia coli* (e.g., intimin), *Lactobacillus* spp., and *Bifidobacterium* spp., by qPCR of bacterial gDNA using intestinal contents showed similar results with their counterparts of viable cell counts, supporting that the synbiotics may provide the benefits of both suppressing pathogen and abounding commensal beneficial bacteria. Besides, the synbiotics influenced not only changes in certain species but also overall microbial communities of gut microbiota. The microbial richness and diversity indices were consistently the highest in the microbiota of synbiotics LP/P/PPN-treated group whereas the lowest in that of pathogen only infected group. Emerging data have demonstrated that the higher the diversity of gut microbiota, the healthier it is [37–39]. In the PCoA plot based on weighted UniFrac distances, distinct clusters were observed by pathogen infection and pro-/synbiotics treatment. Considering these results, it can be seen that the synbiotics with nanoprebiotics cause an apparent shift in the gut microbiota in a positive way, which has offset the adverse effects of dysbiosis and pathogen invasion, rather improving key indices even more than normal state.

Besides externally invading pathogens, there were numerous commensal bacteria with opportunistic pathogenicity already distributed in the intestine [40]. For example, *Proteobacteria*, which was significantly decreased when treated synbiotics, is the phylum containing many disease-provoking bacteria such as *Escherichia, Vibrio, Helicobacter, Salmonella* and other notable bacteria [41]. As pathogen EC used in this study is also one of the species belonging to *Proteobacteria*, the drop in this phylum by synbiotics suggests that the synbiotics may have restrained outgrowth of pathogens from the gut.

At the genus level, various genera were also affected by synbiotics (Fig. S7; Table S2). Interestingly, distinct from treating probiotics alone, pullulan and PPNs showed additive effects to enrich several commensal beneficial bacteria. For example, both *Lactobacillus* (Fig. S7B) and *Bifidobacterium* (Fig. S7C), which are widely used due to their probiotic properties, increased their relative abundances in the microbiota, which corresponded to the results of qPCR [42]. Moreover, several butyrate-producing bacteria such as *Faecalibacterium* (Fig. S7D), the unclassified genus of family *Veillonellaceae* (Fig. S7E), *Coprococcus* and *Ruminococcus* were increased when treated with synbiotics especially LP/P/PPNs (Table S2). They have attracted attention due to their distinguished ability to produce butyrate having important roles in gut homeostasis such as energy source for colonocytes and immunomodulatory factor [43]. In particular, *Faecalibacterium* is a genus that has been reported as a representative biomarker of healthy microbiota [44]. One of the genus in the family *Veillonellaceae, Veillonella*, was reported for their abundance in a physically active state [45]. Likewise, *Coprococcus* and *Ruminococcus* were reported as beneficial bacteria due to their anti-cancer effect against colon cancer and decreased levels in various microbiota-related diseases such as IBD respectively [46, 47].
Based on PICRUSt analysis, several differences with significance were demonstrated although the findings should be interpreted with caution. In mice fed with synbiotics LP/PPNs or LP/P/PPNs, the genes related to nutrient metabolism and normal cellular processes were prevalent whereas the pathways for bacterial invasion and its associated disease were scarce. Furthermore, the gene regarding LPS biosynthesis was also predicted to be upregulated by pathogen EC infection. Meanwhile, there were intriguing functional differences by nanoprebiotics (PPNs) as a synbiotic partner. For example, compared to probiotics alone, pathways representing membrane transport or transcription were upregulated whereas the pathway for LPS biosynthesis was downregulated when treating with nanoprebiotics. Also, distinct from when using backbone polymer (pullulan) as prebiotics, nanoprebiotics upregulated pathways representing glycan synthesis and various carbohydrate metabolisms. These results suggest that synbiotics with nanoprebiotics may recover gut microbiota from damages by mainly promoting metabolism regarding various nutrients and inhibiting LPS biosynthesis.

Since LPS is a representative component of endotoxin originating from gram-negative bacteria such as *Proteobacteria*, we chose to examine whether there were bacteria whose relative abundance in the gut were correlated with serum endotoxin level (Fig. S9) [48]. Expectedly, *Proteobacteria* (r = 0.45, P = 0.007; Fig. S9A) was the only phylum positively correlated with serum endotoxin level, whereas no phylum was negatively correlated. Additionally, several genera such as *Cronobacter* (r = 0.62, P < 0.001; Fig. S9B), unclassified genus of *Enterobacteriaceae* family (r = 0.60, P < 0.001; Fig. S9C), *Enterobacter* (r = 0.59, P < 0.001; Fig. S9D), *Streptococcus* (r = 0.49, P = 0.003; Fig. S9E) were positively correlated. *Cronobacter* has been known to provoke severe illnesses like necrotizing enterocolitis (NEC), meningitis, and septicemia in people with vulnerable gut, such as neonates, infants, and the elderly [49]. *Streptococcus* is significantly widely studied as a pathogen provoking various diseases [50]. Several genera belonging to *Enterobacteriaceae* including *Enterobacter* were regarded as fatal pathogens because of their resistance to antibiotics and relation with diseases [51]. On the other hand, beneficial bacteria such as *Lactobacillus* (r = -0.34, P = 0.046) (Fig. S9F), which was increased by the synbiotics, was negatively correlated with serum endotoxin level. Therefore, it is possible that the synbiotics LP/P/PPNs modulated gut microbiota into mainly inhibiting LPS-producing bacteria, thus contributing to the alleviation of endotoxin influx into the host circulation system.

**Conclusions**

Overall, both physiological and microbiological findings suggest that synbiotics with nanoprebiotics have distinguished effects on restoring dysbiosis than probiotics or synbiotics only with backbone polysaccharide. As of now, more studies should be required to clarify the effects on the gut microbiota and host when only treated nanoprebiotics without a probiotics partner. Also, to be used as a microbiome drug, there should be a process of verifying that these novel synbiotics can act as effectively as this study in various enteropathic disease models. Nevertheless, the results suggest that nanoprebiotics may have the potential to be a novel agent for the synbiotics partner, thereby widely applicable to improve susceptibility to invasive pathogens and to cure diseases associated with dysbiosis such as IBD.
Declarations

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Data and materials availability: All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

References

1. Guinane CM, Cotter PD. Role of the gut microbiota in health and chronic gastrointestinal disease: understanding a hidden metabolic organ. Therap Adv Gastroenterol. 2013;6:295–308.
2. Aron-Wisnewsky J, Prifti E, Belda E, Ichou F, Kayser BD, Dao MC, Verger EO, Hedjazi L, Bouillot JL, Chevallier JM, et al. Major microbiota dysbiosis in severe obesity: fate after bariatric surgery. Gut. 2019;68:70–82.
3. Fan X, Jin Y, Chen G, Ma X, Zhang L. Gut Microbiota Dysbiosis Drives the Development of Colorectal Cancer. Digestion 2020:1–8.
4. Ducatelle R, Eeckhaut V, Haesebrouck F, Van Immerseel F. A review on prebiotics and probiotics for the control of dysbiosis: present status and future perspectives. Animal. 2015;9:43–8.
5. da Silva TF, Casarotti SN, de Oliveira GLV, Penna ALB. The impact of probiotics, prebiotics, and synbiotics on the biochemical, clinical, and immunological markers, as well as on the gut microbiota of obese hosts. Crit Rev Food Sci Nutr. 2021;61:337–55.
6. Hotel ACP, Cordoba A. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Prevention. 2001;5:1–10.
7. Daba GM, Elkhateeb WA. Bacteriocins of lactic acid bacteria as biotechnological tools in food and pharmaceuticals: Current applications and future prospects. Biocatalysis and Agricultural Biotechnology 2020:101750.
8. Ohland CL, Macnaughton WK. Probiotic bacteria and intestinal epithelial barrier function. Am J Physiol Gastrointest Liver Physiol. 2010;298:G807–19.

9. Liu Q, Yu Z, Tian F, Zhao J, Zhang H, Zhai Q, Chen W. Surface components and metabolites of probiotics for regulation of intestinal epithelial barrier. Microb Cell Fact. 2020;19:23.

10. Wang S, Xiao Y, Tian F, Zhao J, Zhang H, Zhai Q, Chen W. Rational use of prebiotics for gut microbiota alterations: Specific bacterial phyotypes and related mechanisms. Journal of Functional Foods. 2020;66:103838.

11. Zheng HJ, Guo J, Wang Q, Wang L, Wang Y, Zhang F, Huang WJ, Zhang W, Liu WJ, Wang Y. Probiotics, prebiotics, and synbiotics for the improvement of metabolic profiles in patients with chronic kidney disease: A systematic review and meta-analysis of randomized controlled trials. Crit Rev Food Sci Nutr. 2021;61:577–98.

12. Kim W-S, Lee J-Y, Singh B, Maharjan S, Hong L, Lee S-M, Cui L-H, Lee K-J, Kim G, Yun C-H. A new way of producing pediocin in Pediococcus acidilactici through intracellular stimulation by internalized inulin nanoparticles. Scientific reports. 2018;8:1–14.

13. Kim W-S, Han GG, Hong L, Kang S-K, Shokouhimehr M, Choi Y-J, Cho C-S. Novel production of natural bacteriocin via internalization of dextran nanoparticles into probiotics. Biomaterials. 2019;218:119360.

14. Hong L, Cho CS, Kim WS, Choi YJ, Kang SK. Phthalyl starch nanoparticles as prebiotics enhanced nisin production in Lactococcus lactis through the induction of mild stress in probiotics. Journal of Applied Microbiology. 2020.

15. Hong L, Kim W-S, Lee S-M, Kang S-K, Choi Y-J, Cho C-S. Pullulan nanoparticles as prebiotics enhance the antibacterial properties of Lactobacillus plantarum through the induction of mild stress in probiotics. Frontiers in microbiology. 2019;10:142.

16. Erwin E, Marco G, Emery E. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. J Dairy Sci. 1961;44:1768–71.

17. Ditu LM, Chifiriuc MC, Bezirtzoglou E, Voltsi C, Bleotu C, Pelinescu D, Mihaescu G, Lazar V. Modulation of virulence and antibiotic susceptibility of enteropathogenic Escherichia coli strains by Enterococcus faecium probiotic strain culture fractions. Anaerobe. 2011;17:448–51.

18. Driscoll AJ, Bhat N, Karron RA, O’Brien KL, Murdoch DR. Disk diffusion bioassays for the detection of antibiotic activity in body fluids: applications for the pneumonia etiology research for child health project. Clin Infect Dis. 2012;54:159–64.

19. Samuelson DR, Shellito JE, Maffei VJ, Tague ED, Campagna SR, Blanchard EE, Luo M, Taylor CM, Ronis MJJ, Molina PE, Welsh DA. Alcohol-associated intestinal dysbiosis impairs pulmonary host defense against Klebsiella pneumoniae. PLoS Pathog. 2017;13:e1006426.

20. Bayer F, Ascher S, Pontarollo G, Reinhardt C. Antibiotic Treatment Protocols and Germ-Free Mouse Models in Vascular Research. Front Immunol. 2019;10:2174.

21. Chelakkot C, Ghim J, Rajasekaran N, Choi JS, Kim JH, Jang MH, Shin YK, Suh PG, Ryu SH. Intestinal Epithelial Cell-Specific Deletion of PLD2 Alleviates DSS-Induced Colitis by Regulating Occludin. Sci...
22. Brown K, Godovannyi A, Ma C, Zhang Y, Ahmadi-Vand Z, Dai C, Gorzelak MA, Chan Y, Chan JM, Lochner A. Prolonged antibiotic treatment induces a diabetogenic intestinal microbiome that accelerates diabetes in NOD mice. ISME J. 2016;10:321–32.

23. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010;26:2460–1.

24. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol. 2006;72:5069–72.

25. Douglas GM, Beiko RG, Langille MG. Predicting the functional potential of the microbiome from marker genes using PICRUSt. In: Microbiome Analysis. Springer; 2018. pp. 169–77.

26. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12:R60.

27. Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Thurber RLV, Knight R. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature biotechnology. 2013;31:814–21.

28. Kieler IN, Osto M, Hugentobler L, Puetz L, Gilbert MTP, Hansen T, Pedersen O, Reusch CE, Zini E, Lutz TA. Diabetic cats have decreased gut microbial diversity and a lack of butyrate producing bacteria. Scientific reports. 2019;9:1–13.

29. Bach Knudsen KE, Laerke HN, Hedemann MS, Nielsen TS, Ingerslev AK, Gundelund Nielsen DS, Theil PK, Purup S, Hald S, Schioldan AG, et al: Impact of Diet-Modulated Butyrate Production on Intestinal Barrier Function and Inflammation. Nutrients 2018, 10.

30. Tong LC, Wang Y, Wang ZB, Liu WY, Sun S, Li L, Su DF, Zhang LC. Propionate Ameliorates Dextran Sodium Sulfate-Induced Colitis by Improving Intestinal Barrier Function and Reducing Inflammation and Oxidative Stress. Front Pharmacol. 2016;7:253.

31. Greenhill C. Gut microbiota: Proteins released by E. coli in the gut influence host appetite control. Nat Rev Endocrinol. 2016;12:4.

32. Kajiya M, Silva MJ, Sato K, Ouhara K, Kawai T. Hydrogen mediates suppression of colon inflammation induced by dextran sodium sulfate. Biochem Biophys Res Commun. 2009;386:11–5.

33. Nameda S, Miura NN, Adachi Y, Ohno N. Antibiotics protect against septic shock in mice administered beta-glucan and indomethacin. Microbiol Immunol. 2007;51:851–9.

34. Okada Y, Setoyama H, Matsumoto S, Imaoka A, Nanno M, Kawaguchi M, Umesaki Y. Effects of fecal microorganisms and their chloroform-resistant variants derived from mice, rats, and humans on immunological and physiological characteristics of the intestines of ex-germfree mice. Infect Immun. 1994;62:5442–6.

35. Thorn J. The inflammatory response in humans after inhalation of bacterial endotoxin: a review. Inflamm Res. 2001;50:254–61.
36. Moludi J, Maleki V, Jafari-Vayghyan H, Vaghef-Mehrabany E, Alizadeh M. Metabolic endotoxemia and cardiovascular disease: A systematic review about potential roles of prebiotics and probiotics. Clin Exp Pharmacol Physiol. 2020;47:927–39.

37. Backhed F, Fraser CM, Ringel Y, Sanders ME, Sartor RB, Sherman PM, Versalovic J, Young V, Finlay BB. Defining a healthy human gut microbiome: current concepts, future directions, and clinical applications. Cell Host Microbe. 2012;12:611–22.

38. Claesson MJ, Jeffery IB, Conde S, Power SE, O’Connor EM, Cusack S, Harris HM, Coakley M, Lakshminarayanan B, O’Sullivan O, et al. Gut microbiota composition correlates with diet and health in the elderly. Nature. 2012;488:178–84.

39. Jacouton E, Chain F, Sokol H, Langella P, Bermudez-Humaran LG. Probiotic Strain Lactobacillus casei BL23 Prevents Colitis-Associated Colorectal Cancer. Front Immunol. 2017;8:1553.

40. Bhat MI, Sowmya K, Kapila S, Kapila R. Escherichia coli K12: An evolving opportunistic commensal gut microbe distorts barrier integrity in human intestinal cells. Microb Pathog. 2019;133:103545.

41. Litvak Y, Byndloss MX, Tsolis RM, Baumer AJ. Dysbiotic Proteobacteria expansion: a microbial signature of epithelial dysfunction. Curr Opin Microbiol. 2017;39:1–6.

42. Suez J, Zmora N, Elinav E. Probiotics in the next-generation sequencing era. Gut Microbes. 2020;11:77–93.

43. Zeng X, Gao X, Peng Y, Wu Q, Zhu J, Tan C, Xia G, You C, Xu R, Pan S. Higher risk of stroke is correlated with increased opportunistic pathogen load and reduced levels of butyrate-producing bacteria in the gut. Front Cell Infect Microbiol. 2019;9:4.

44. Cheema ZM. The role of Faecalibacterium prausnitzii in health and disease. Catalyst: Facets of Biochemistry Biomedical Sciences. 2019;3:11–7.

45. Scheiman J, Luber JM, Chavkin TA, MacDonald T, Tung A, Pham L-D, Wibowo MC, Wurth RC, Punthambaker S, Tierney BT. Meta-omics analysis of elite athletes identifies a performance-enhancing microbe that functions via lactate metabolism. Nature medicine. 2019;25:1104–9.

46. Ai D, Pan H, Li X, Gao Y, Liu G, Xia LC. Identifying Gut Microbiota Associated With Colorectal Cancer Using a Zero-Inflated Lognormal Model. Front Microbiol. 2019;10:826.

47. Nagao-Kitamoto H, Kamada N. Host-microbial Cross-talk in Inflammatory Bowel Disease. Immune Netw. 2017;17:1–12.

48. Rizzatti G, Lopetuso LR, Gibiino G, Binda C, Gasbarrini A. Proteobacteria: A Common Factor in Human Diseases. Biomed Res Int. 2017;2017:9351507.

49. Holý O, Forsythe S. Cronobacter spp. as emerging causes of healthcare-associated infection. J Hosp Infect. 2014;86:169–77.

50. Wu C, Liang B, Gong Y, Zhang L, Zou Y, Ge J. Streptococcus acidominimus causing invasive disease in humans: a case series. J Med Case Rep. 2014;8:57.

51. Davin-Regli A, Lavigne JP, Pages JM. Enterobacter spp.: Update on Taxonomy, Clinical Aspects, and Emerging Antimicrobial Resistance. Clin Microbiol Rev 2019, 32.
Synthesis and characterization of phthalyl pullulan nanoparticles (PPNs). Chemical reaction scheme for the synthesis of PPNs (A). Morphologies of PPNs were observed by SEM (Magnification:100.00k, Scale bar=200nm) (B). Analysis of the internalization of PPNs by LP and observed by CLSM (C). SCFA amount in the culture medium of LP and treated with pullulan or PPNs (D). Antimicrobial activity of PPNs treated LP against EC (E) and LM (F).
Figure 2

In vivo experiment schedule and physiological changes of dysbiosis-induced mice upon pro-/synbiotics after pathogen EC infection. Experiment schedule and group information (A). Body weight change before and after the EC challenge (B). Average daily feed intake per mice (C). Colon length (D) and cecum weight (E) after the trial, and their representative picture after dissection (F). Serum endotoxin level per group (G).
Figure 3

Effects of pro-/synbiotics on the gut microbiota of murine dysbiosis model with pathogen EC infection. The viable cell counts of coliform bacteria (A) and lactic acid bacteria (LAB) (B) in intestinal contents. Microbial richness (observed OTUs) per group (C). Principal coordinate analysis (PCoA) plot based on unweighted (D) and weighted (E) UniFrac distances. The overall compositions of the gut microbiota at the phylum level (F).
Figure 4

Metagenomic prediction of gut microbiota of dysbiosis-induced mice upon pro-/synbiotics after pathogen EC infection. Microbial functions were predicted using PICRUSt at the third level of the KEGG pathway. LEfSe analysis were represented as histogram determining synbiotics LP/P/PPN effect (A) and nanoprebiotics PPN effect (B).

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