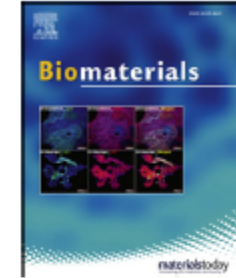




Contents lists available at ScienceDirect

Biomaterials

journal homepage: [www.elsevier.com/locate/biomaterials](http://www.elsevier.com/locate/biomaterials)



## Novel production of natural bacteriocin via internalization of dextran nanoparticles into probiotics

Whee-Soo Kim<sup>a</sup>, Geon Goo Han<sup>a</sup>, Liang Hong<sup>a</sup>, Sang-Kee Kang<sup>b</sup>, Mohammadreza Shokouhimehr<sup>c</sup>, Yun-Jaie Choi<sup>a,d,\*\*</sup>, Chong-Su Cho<sup>a,d,\*</sup>

<sup>a</sup> Department of Agricultural Biotechnology, Seoul National University, Seoul 08826, Republic of Korea

<sup>b</sup> Institute of Green-Bio Science & Technology, Seoul National University, Pyeongchang, Gangwon-do 25354, Republic of Korea

<sup>c</sup> Department of Materials Science and Engineering, Research Institute of Advanced Materials, Seoul National University, Seoul 08826, Republic of Korea

<sup>d</sup> Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea



2022.05.19

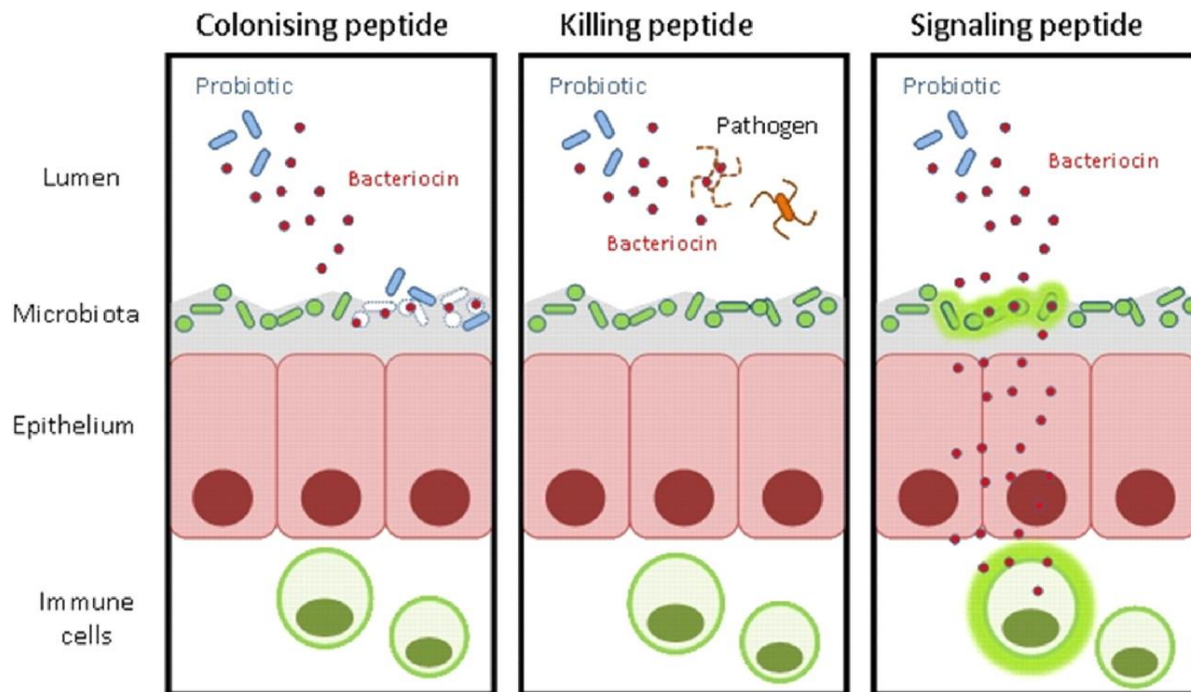
박재한

# Introduction

**Enteric pathogens** - a major cause of infections in the gastrointestinal track worldwide.

Concern on using **antibiotics** are increasing.

Use of **probiotics** as alternatives to **antibiotics** has been growing



Probiotics produce **antimicrobial molecules** (e.g., lactic acid and **bacteriocins**) and enzymes

- Inhibit the colonization of pathogens
- modulate the immune system
- enhance nutrient absorption

Prebiotics treatments enhance probiotics producing bacteriocins

# Introduction

---

## Prebiotics

- generally defined as indigestible food ingredients
- induce the growth or activity of beneficial microorganisms in the gastrointestinal tract
- provide favorable health effects to the host
- Indigestible polysaccharides
- inulin, pullulan and dextran

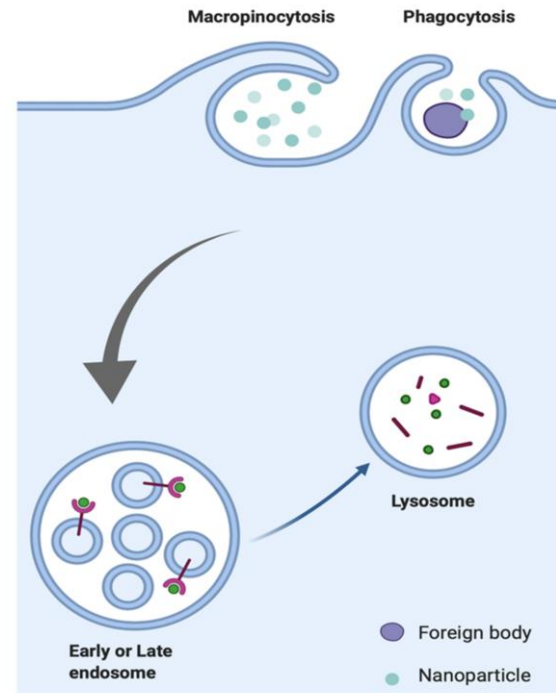
# Introduction

## Mammalian cells

- polymeric nanoparticle makes easier endocytosis
- Better overcoming cellular barriers

## Microorganisms

- Little size, cell wall
- Internalization of the polymeric nanoparticles is still in an early stage
- producing natural antimicrobial peptides by polymeric nanoparticles as new type of prebiotics will be very challenging

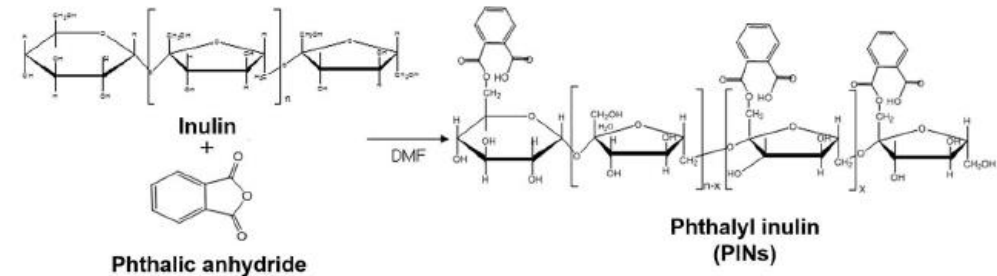


OPEN

## A new way of producing pediocin in *Pediococcus acidilactici* through intracellular stimulation by internalized inulin nanoparticles

Received: 30 May 2017  
Accepted: 15 February 2018  
Published online: 12 April 2018

Whee-Soo Kim<sup>1</sup>, Jun-Yeong Lee<sup>1</sup>, Bijay Singh<sup>2,3</sup>, Sushila Maharjan<sup>2,3</sup>, Liang Hong<sup>1</sup>, Sang-Mok Lee<sup>1</sup>, Lian-Hua Cui<sup>5</sup>, Ki-June Lee<sup>1</sup>, GiRak Kim<sup>1</sup>, Cheol-Heui Yun<sup>1,2</sup>, Sang-Kee Kang<sup>4</sup>, Yun-Jaie Choi<sup>1,2</sup> & Chong-Su Cho<sup>1,2</sup>



## Previous study

- Synthesized phthalyl inulin nanoparticles (PINs) as prebiotics
- demonstrated that PINs were able to be internalized by *Pediococcus acidilactici* (PA)
- internalization increased pediocin biosynthetic genes
- higher antimicrobial activity against on both Gram-negative and Gram-positive pathogens

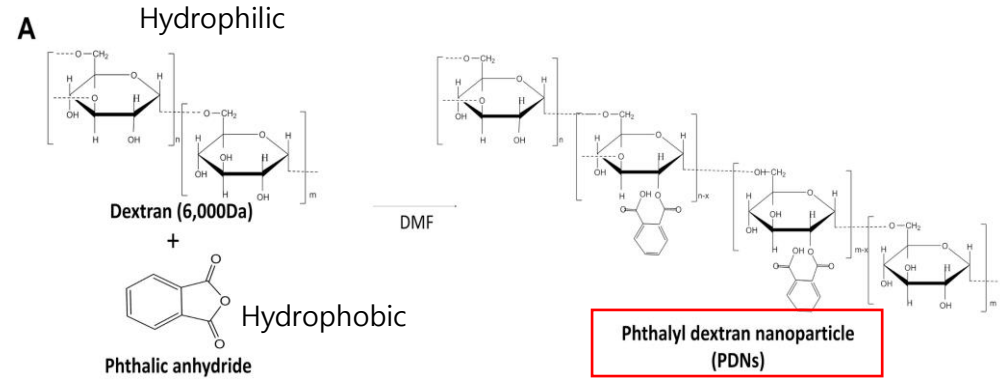
# Figure 1. Chemical reaction scheme for the synthesis of PDNs

B : SEM

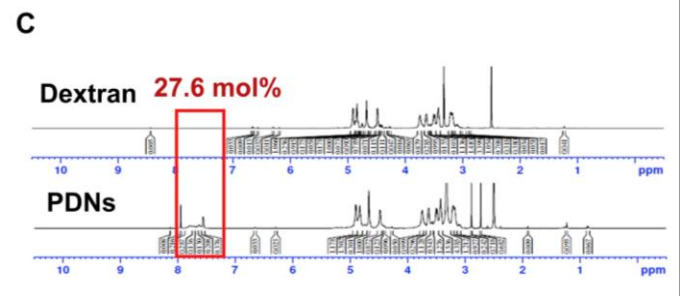
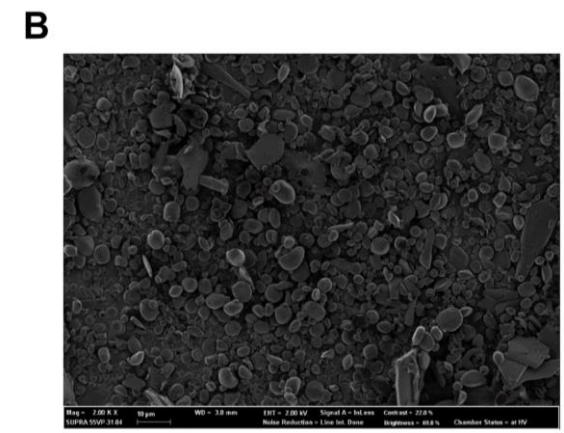
C : <sup>1</sup>H nuclear magnetic resonance (NMR) spectroscopy

D : Dynamic light scattering(DLS), Electrophoretic light scattering (ELS) spectrophotometer

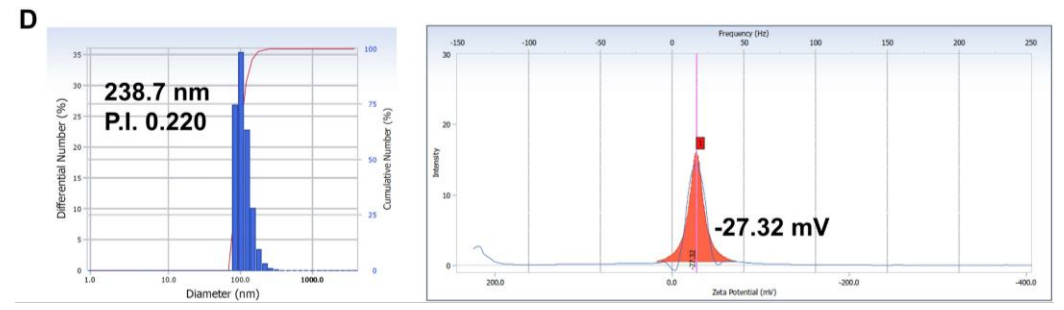
Dextran(1g) added to 5ml dimethyl formamide  
 |  
 0.2ml of 5% sodium acetate added as catalyst  
 |  
 Phthalic anhydride added to solution at 2:1 M  
 |  
 40C 24h with nitrogen  
 |  
 Freeze dry  
 Store -20C



\*DMF : Dimethyl formamide (극성 유기용매)



Phythalyl group contents in PDN

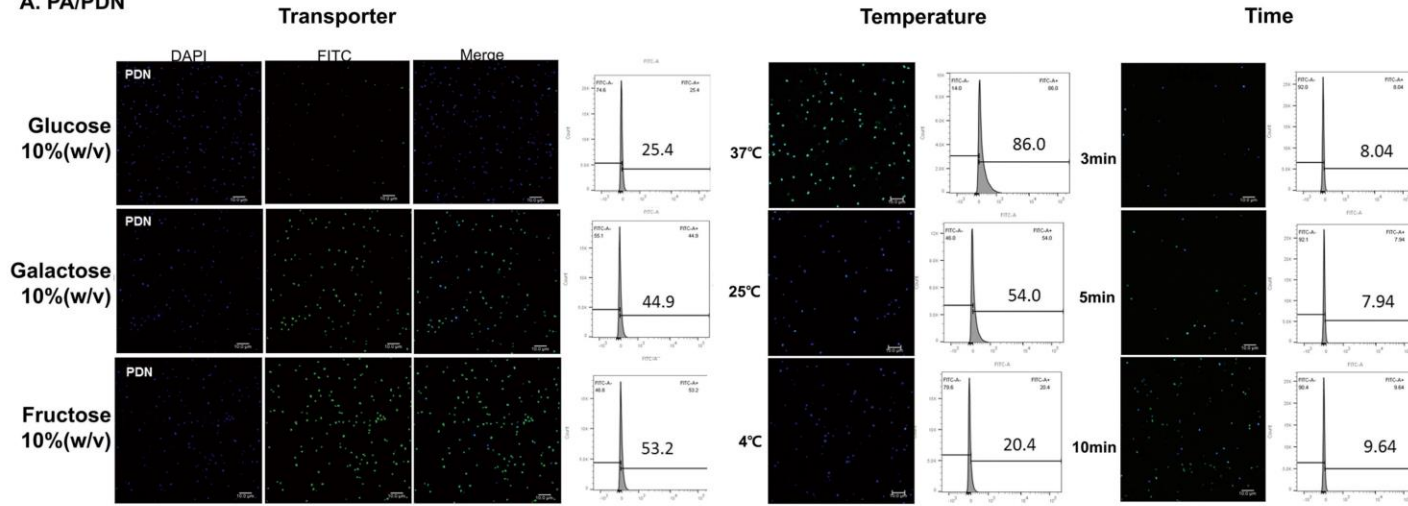


✓ PDN synthesis and characterization

# Figure 2. Analysis of the internalization of dextran and PDNs by PA.

A, B : FITC labeled PDN, dextran – confocal laser microscopy, FACS

A. PA/PDN

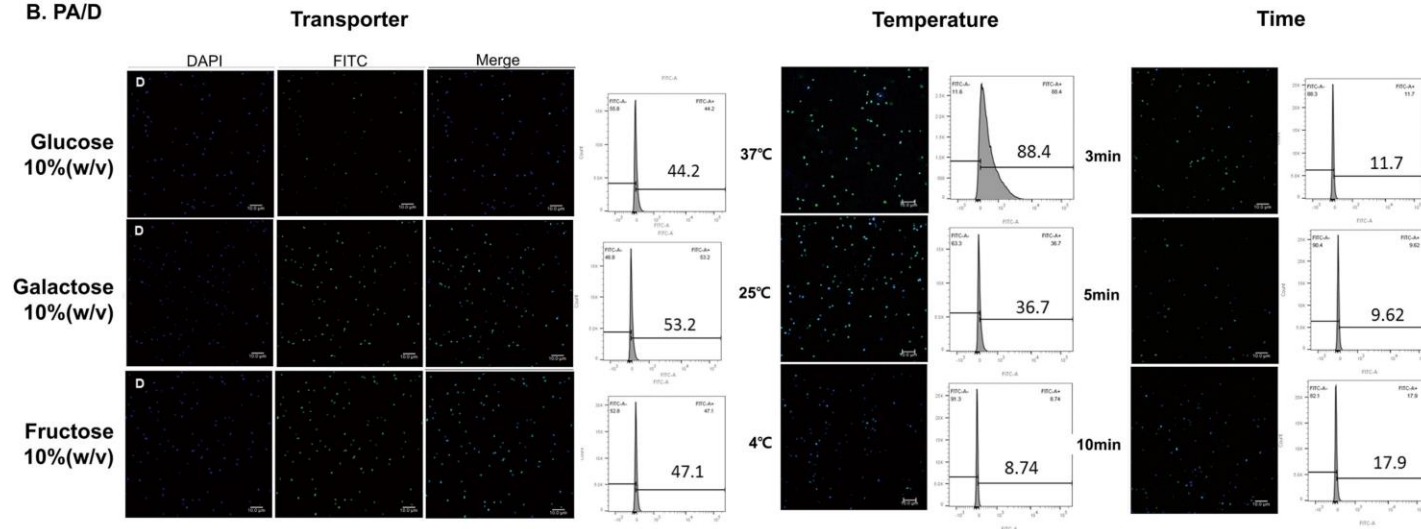


\*PA : *Pediococcus acidilactici*, PDN : phthalyl dextran nanoparticle

Transporter blocking agent 10%, 10min pretreat - 0.5% D, PDN 2h  
Co-Incubate in MRS broth, wash, confocal laser microscopy

- ✓ Might be recognized by glucose transporter
- ✓ Temperature (Energy) dependent
- ✓ Internalization increase with time

B. PA/D



- ✓ Passive diffusion

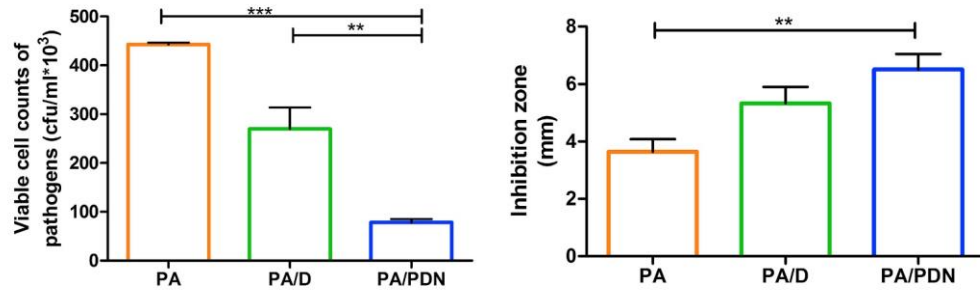
# Figure 3. Antibacterial activity of PA after internalization of PDNs

A~D : co-culture assay, agar diffusion test

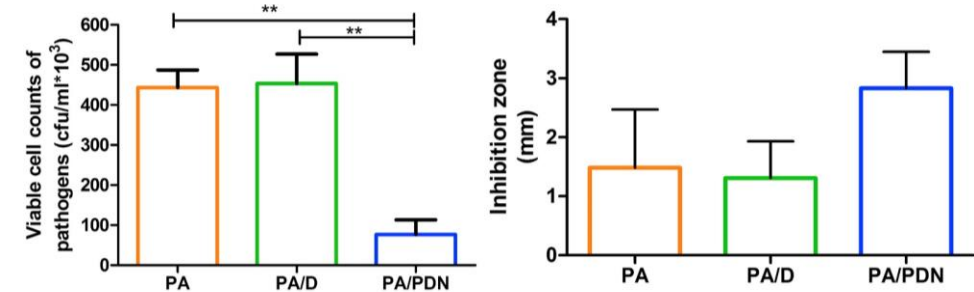
\* Coculture assay :  $1 \times 10^6$  CFU coculture with PA(treated with/wo PDN or dextran)

\* Agar diffusion test : PA disc on spreaded pathogen

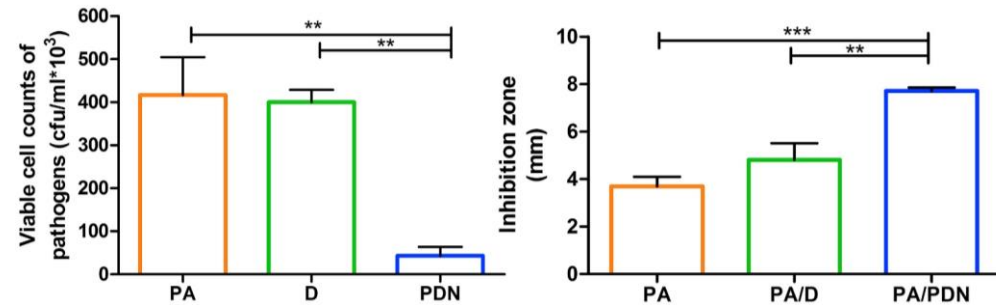
## A. *Salmonella Gallinarum*



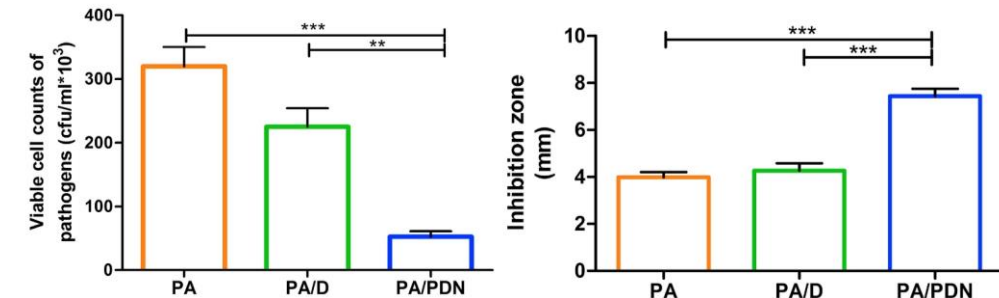
## B. *E.coli* K88



## C. *E.coli* O157:H7



## D. *Listeria monocytogenes*



✓ Internalization of PDNs to PA induce antimicrobial properties



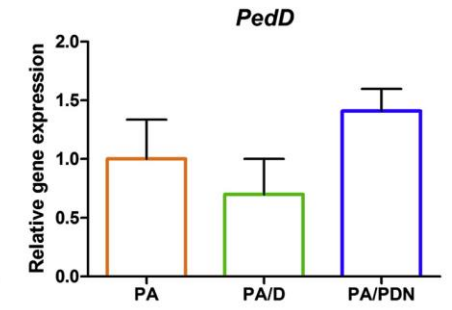
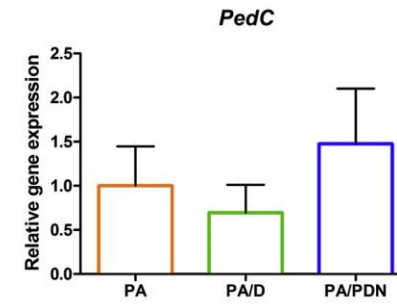
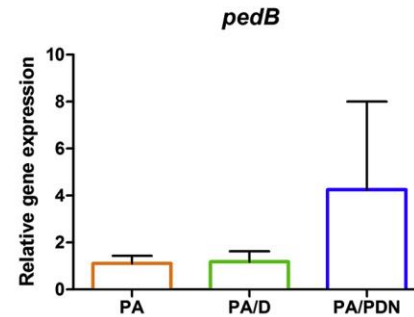
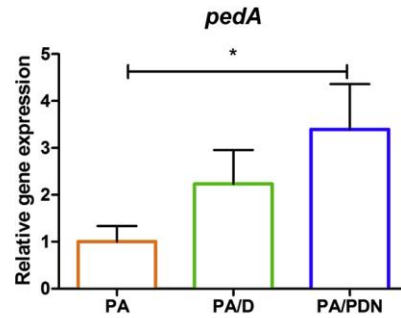
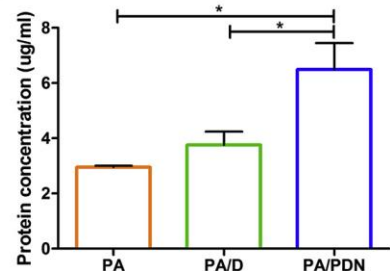
# Figure 4. Effects of PDNs internalization on PA pediocin production and stress response

A : Bradford assay

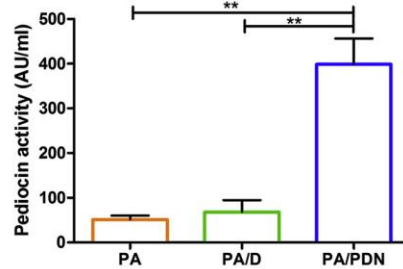
B : pediocin activity assay (supernatant diffusion method)

C, D : qRT PCR bacterial RNA

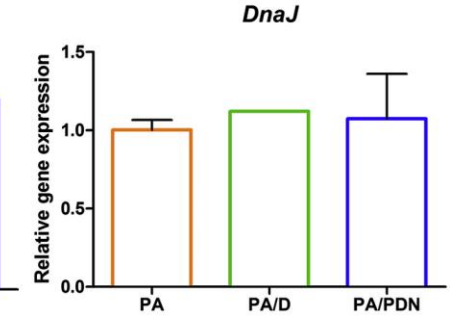
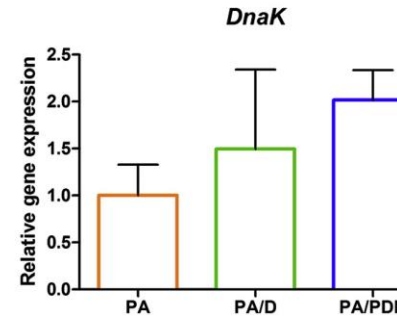
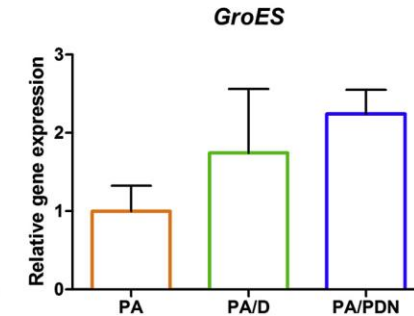
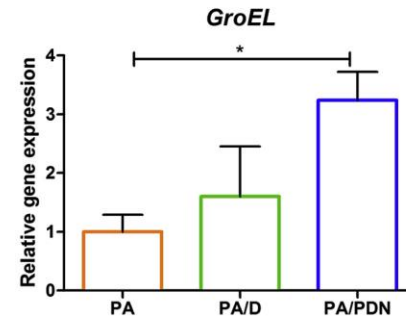
**A. Protein concentration (pediocin)**      **C. Pediocin gene expression**



**B. Pediocin activity**



**D. HSPs gene expression**



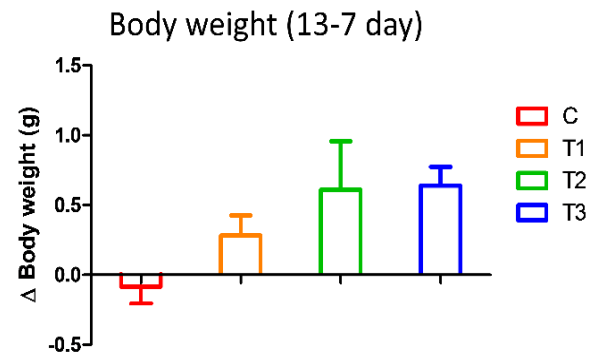
\*HSP : Heat shock protein, stress response

✓ Internalization of PDNs enhance antimicrobial ability through induction of pediocin production

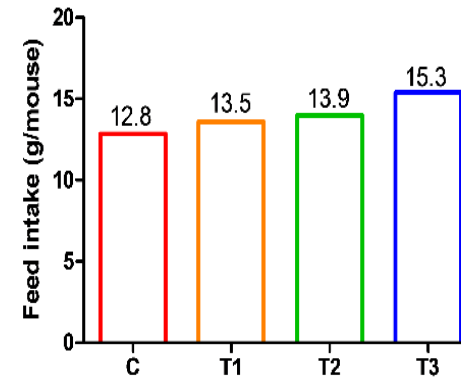
# Supplement Figure Physiological effects on mice

BALB/C male (n=10)  
|  
C : control  
T1 :  $10^8$  Pediococcus acidilactici  
T2 : dextran treated PA  
T3 : PDN treated PA  
Oral gavage, single dose  
| 7days  
E.coli O157:H7  $10^9$  CFU  
Oral gavage 3days  
|  
fecal sample daily | 6days, treatment

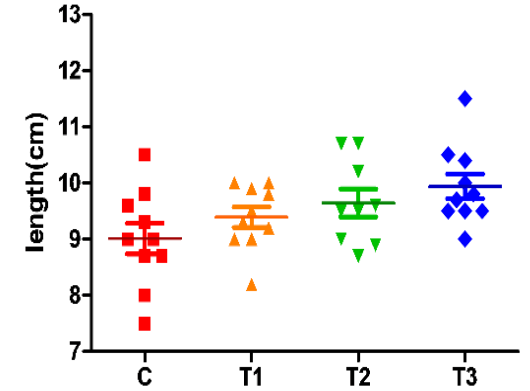
**A**



**B**



Colon length

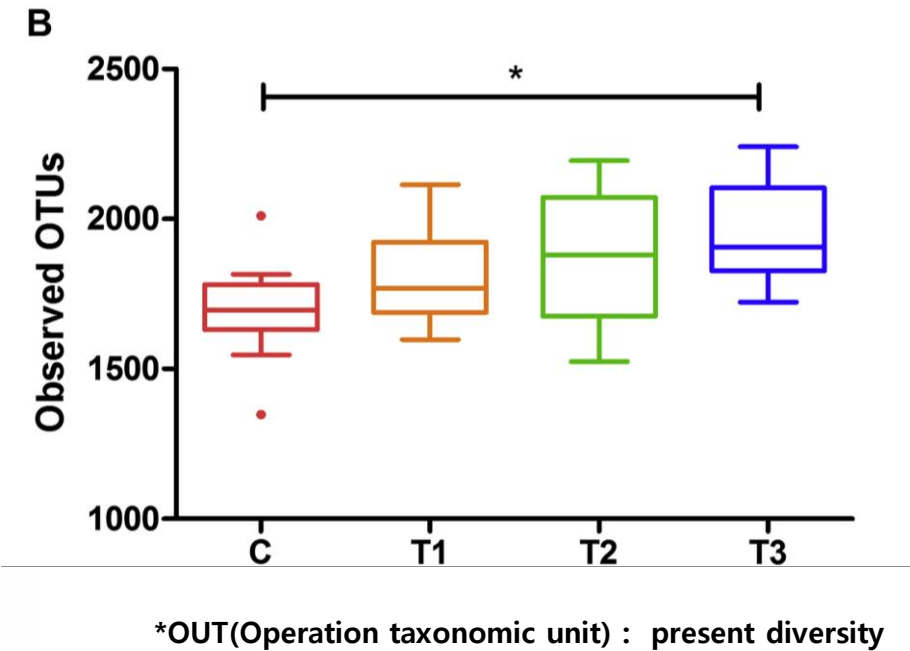
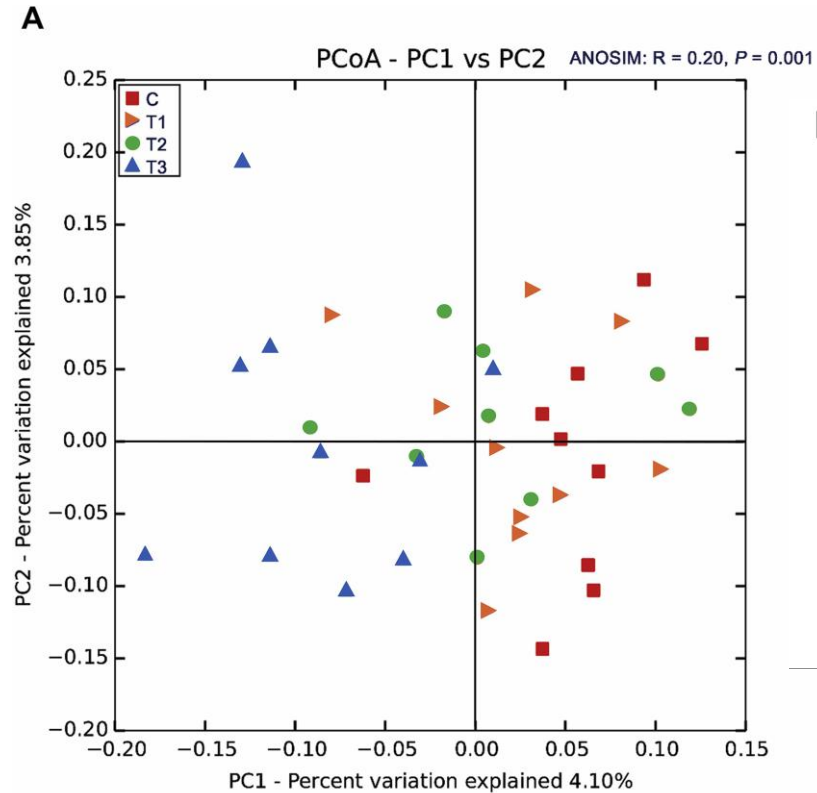


# Figure 5. Shift in the intestinal microflora of mice

A : 16s rRNA sequencing, PCoA based on unweighted UniFrac distances

B : OTU picking

BALB/C male (n=10)  
|  
C : control  
T1 :  $10^8$  *Pediococcus acidilactici*  
T2 : dextran treated PA  
T3 : PDN treated PA  
Oral gavage, single dose  
| 7days  
*E. coli* O157:H7  $10^9$  CFU  
Oral gavage 3days  
|  
fecal sample daily | 6days, treatment

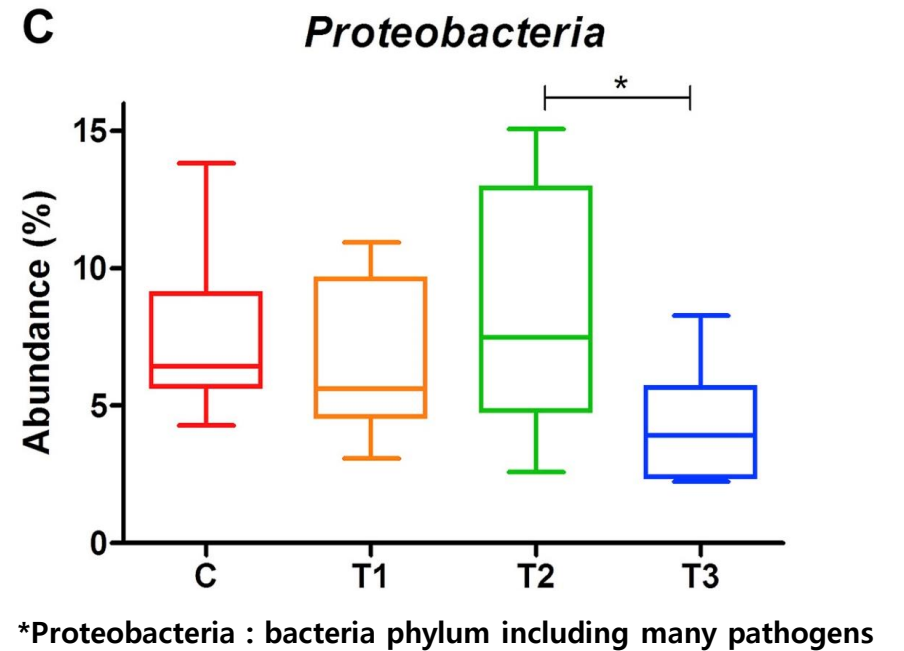
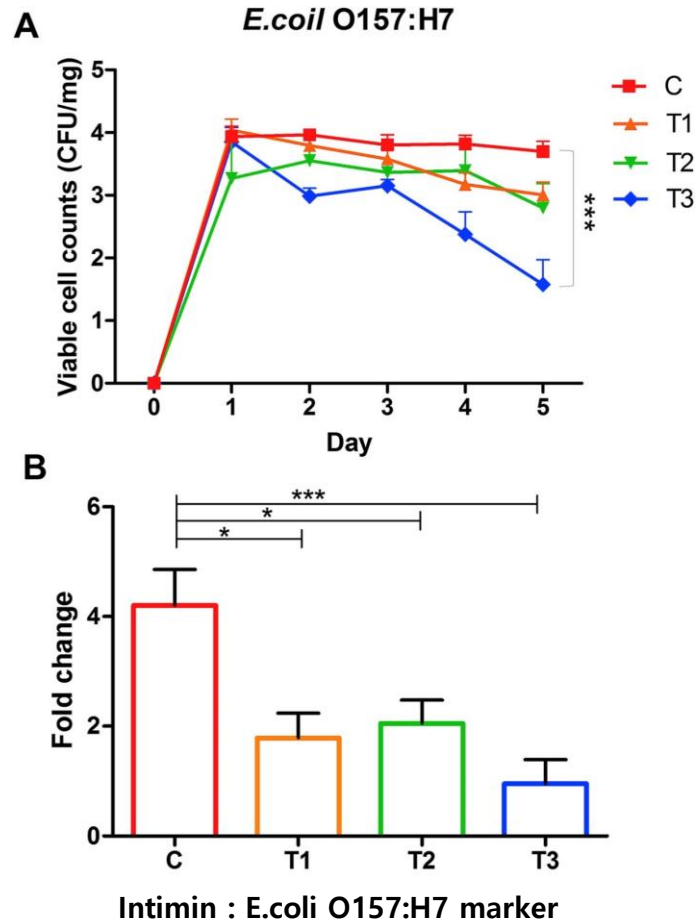


✓ Antimicrobial activity induced by PDN administration may prevent reduction in the diversity of microflora

# Figure 6. Microbial analysis in a murine model

- A : viable cell counts
- B : Fecal sample RNA qRT-PCR
- C : Fecal sample DNA sequencing

Daily fecal sample (10mg/ml)  
Spread on to agar plate  
  
Incubate 20h, 37C



✓ increased production of pediocin may have excluded pathogens from the intestines of mice

# Summary

---

- Confirm internalization of PDN into PA
- First study to report internalization of **dextran nanoparticles** by probiotics can enhance the production of antimicrobial peptides in vitro
- Internalization of PDNs into probiotics can modulate the metabolism of probiotics
- enhance antimicrobial activities against pathogens in vitro.
- Probiotics with enhanced antimicrobial activity could prevent pathogenic gut infections
- Change composition of the gut microbiome in vivo
- Study suggests the combination of prebiotic polymeric nanoparticles with probiotics can be used as an alternative to antibiotics

## Further study

---

- Study about how nanoparticles internalize into bacteria
- There are many previous studies about probiotics and synbiotics on inflammation associated disease
- There are few research about Nanoparticle internalization into probiotics
- This was a research that Nano particles may act better in modulating probiotics
- Introduction of Nano particle could enhance the immunomodulatory ability of probiotics