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Swine-derived probiotic *Lactobacillus plantarum* modulates porcine intestinal endogenous HDP synthesis

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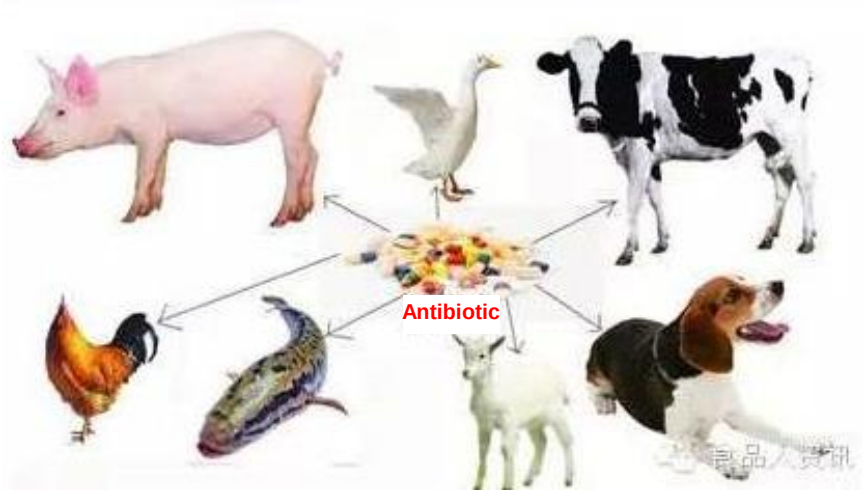
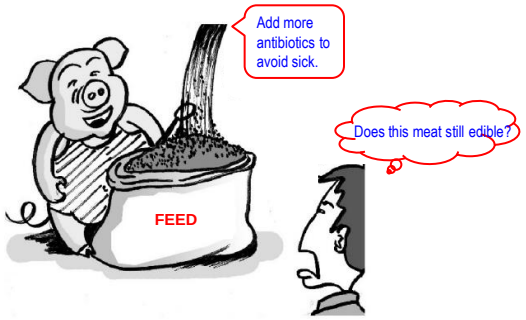
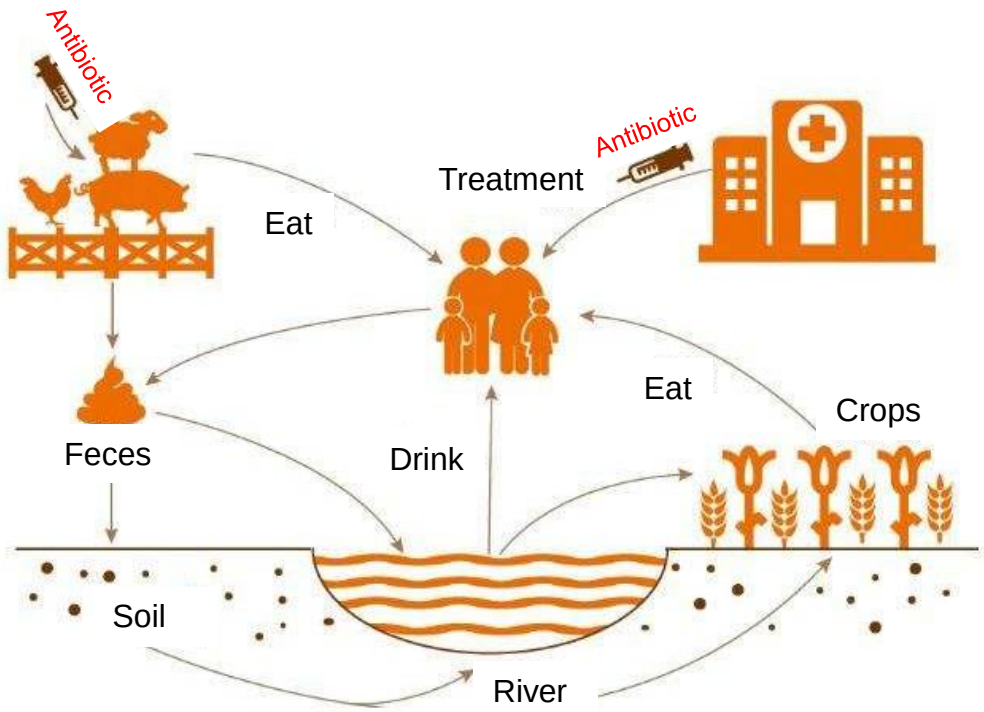
ANTIBIOTICS

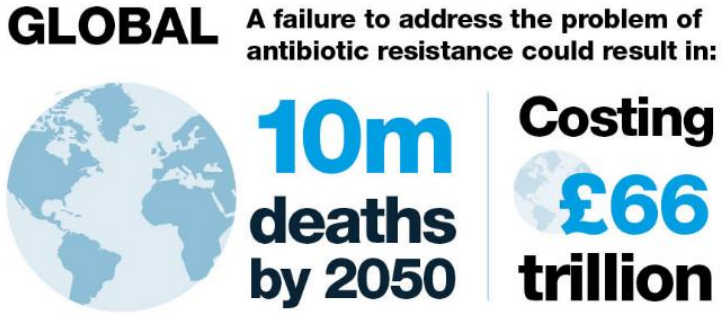
Antibiotics has greatly affected the development of breeding industry.

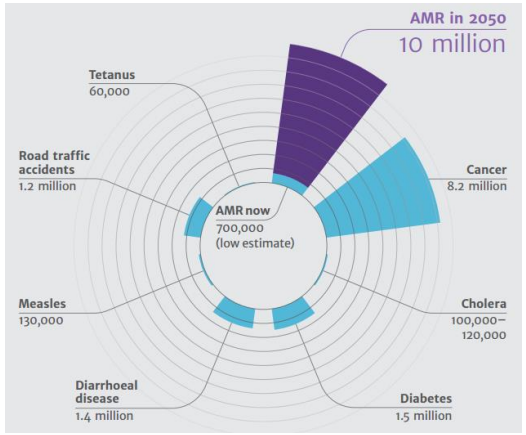


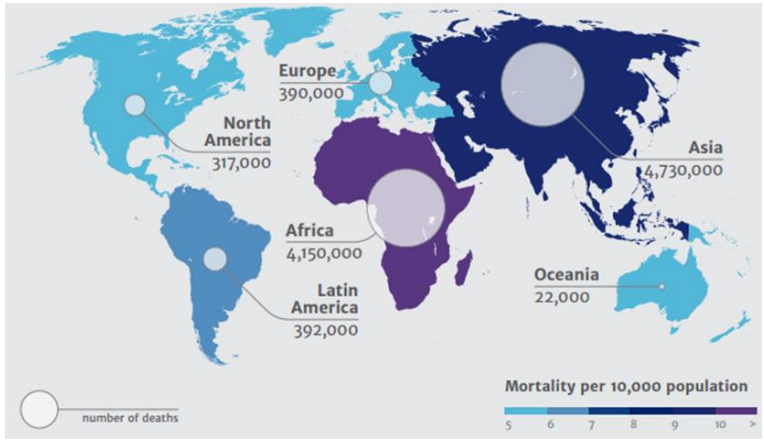


Antibiotic Resistance









Review on Antimicrobial Resistance Jim O'Neill. 2014

Antibiotic resistance poses a major public health risk.

Antibiotic Prohibition

The road of global antibiotic prohibition

1996 Sweden

1998 Denmark

2006 EU

2007 Mexico

2011 New ZealandSouth Korea

2013 United States

2019 .....

Antibiotic Prohibition in China

Announcement (2428) Banned colistin sulfate **2016.11.1**

Announcement (2638) Banned olaquindox, arsanilic acid, and roxarsone **2019.5.1**

Announcement (194) Banned all kinks of growth-promoting medicine additives **2020.1.1**

Antibiotic Alternatives

Antibiotic Alternatives are urgently needed!

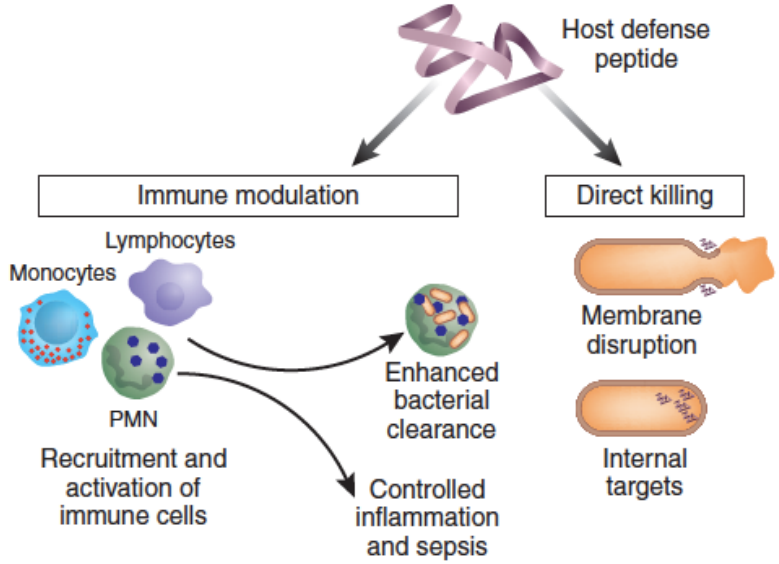
- ✓ Probiotic/Prebiotic/Synbiotic
- ✓ Phytochemicals/Chinese herbs
- ✓ Enzyme Preparation
- ✓ Acid preparation
- ✓ Essential oil
- ✓ Antimicrobial peptide
- ✓ Bacteriophages
- ✓ ……

Lactobacillus plantarum ZLP001

- ***Lactobacillus plantarum* ZLP001** was isolated from a healthy piglet in our laboratory, identified by the China Center of Industrial Culture Collection (Beijing, China), and preserved in the China General Microbiological Culture Collection Center (CGMCC No. 7370).
- **Exert beneficial effects on growth performance and antioxidant status in weaning piglets (Wang et al., 2011, 2012)**
- **Show protective effect on ETEC - induced intestine epithelial cell injury (Wang et al., 2018)**
- **Have syngestic effect combined with fructo-oligosaccharide (Wang et al., 2019)**

Host Defense Peptides (HDPs)

- HDPs have both direct antimicrobial killing and immune modulation activities.



(Hancock, R. E., & Sahl, H. G. 2006. **Nature Biotech**. 24, 1551-7.)

- Nutrients can regulate the expression of HDPs.

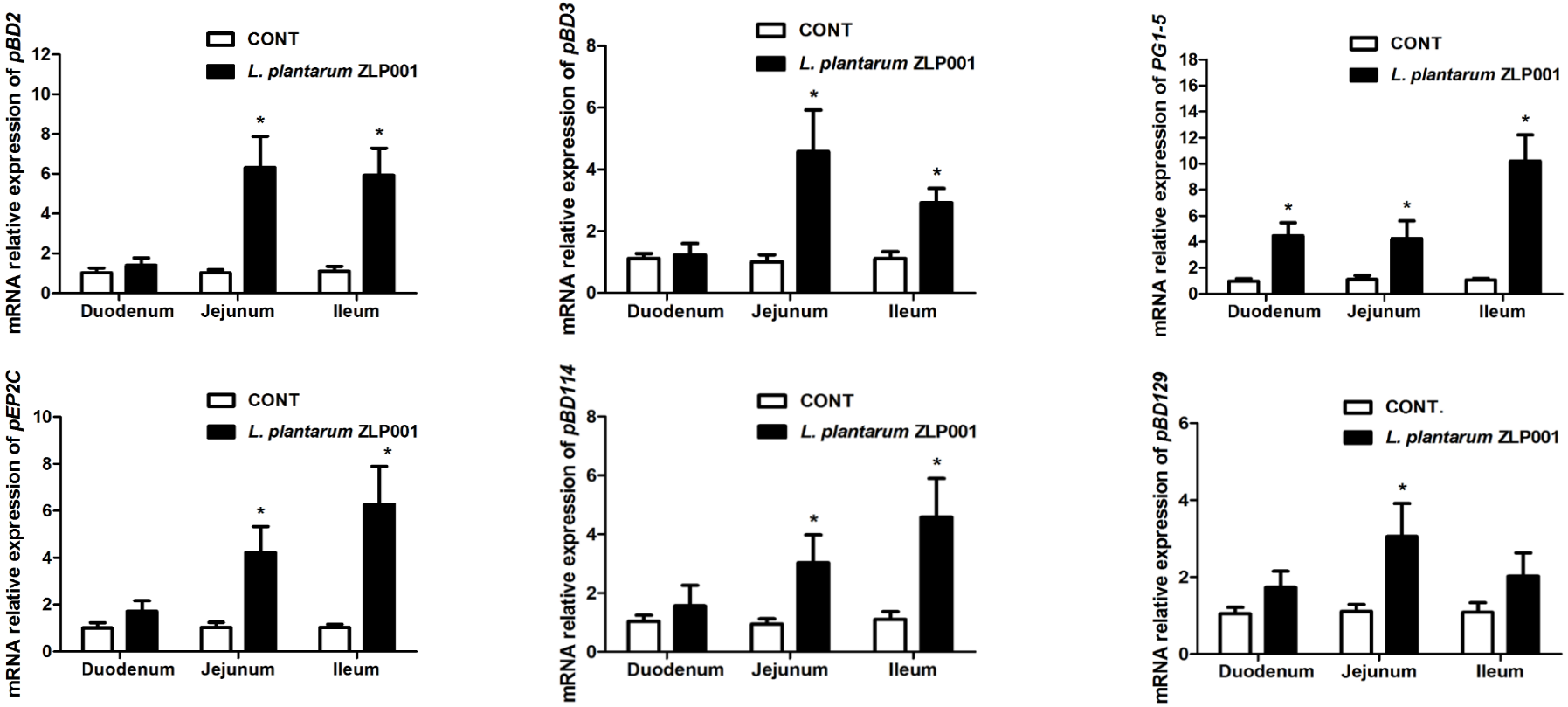
(Lyu, W. T. 2015. **Curr Protein Pept Sci**. 16, 672-9.)

Does *L. plantarum* ZLP001 affect HDPs expression of weaning piglets？

Objective

Evaluate the ability of *L. plantarum* ZLP001 to regulate the expression of porcine HDPs and explore the potential signaling pathway

L. plantarum ZLP001 regulate the HDPs expression *in vivo*

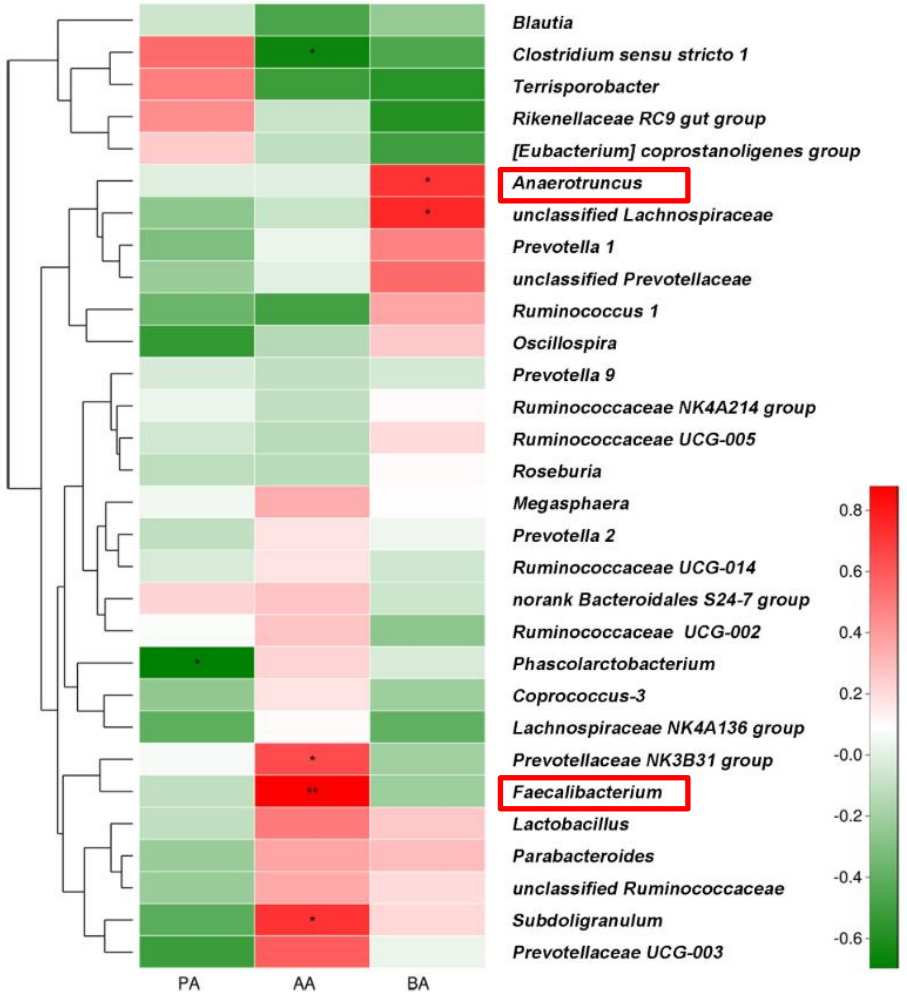


Relative mRNA expression of porcine HDPs in the duodena, jejuna, and ilea of piglets supplemented with *L. plantarum* ZLP001 for 4 weeks as determined by RT-qPCR. mRNA expression was standardized to that of GAPDH. Relative fold changes versus levels in non-stimulated controls were calculated by the ΔΔCt method. Data are the mean ± SEM of three independent experiments. *P < 0.05 versus non-treated control group. White and black bars represent control and *L. plantarum* ZLP001 treatment, respectively.

L. plantarum ZLP001 increase butyrate-producing bacteria

Effects of *L. plantarum* ZLP001 on SCFA concentration (mmol kg^{-1}) in piglet feces.

Short chain fatty acid	Acetic acid (AA)	Propionic acid (PA)	Butyric acid (BA)
Control	28.3	14.7	8.2
L.plantarum ZLP001	30.3	13.8	9.3
SEM	1.26	0.52	0.48
P-value	0.276	0.117	0.068

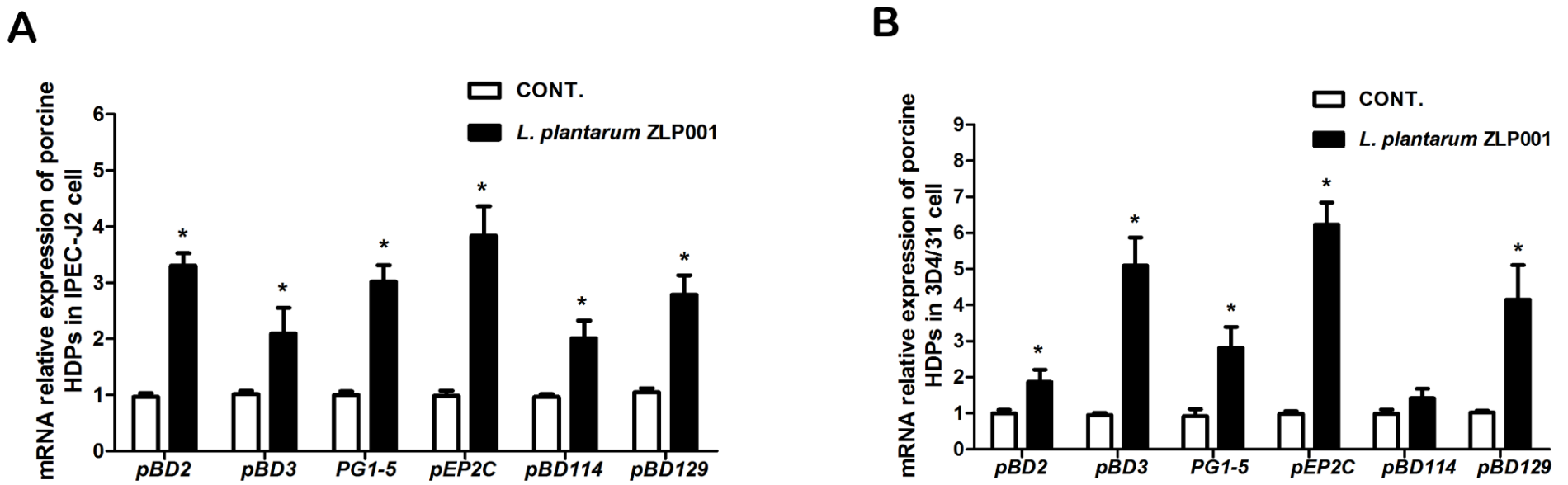


Correlations between relative generic abundance and SCFA concentrations in piglets feces

L. plantarum ZLP001 regulate the HDPs expression *in vitro*

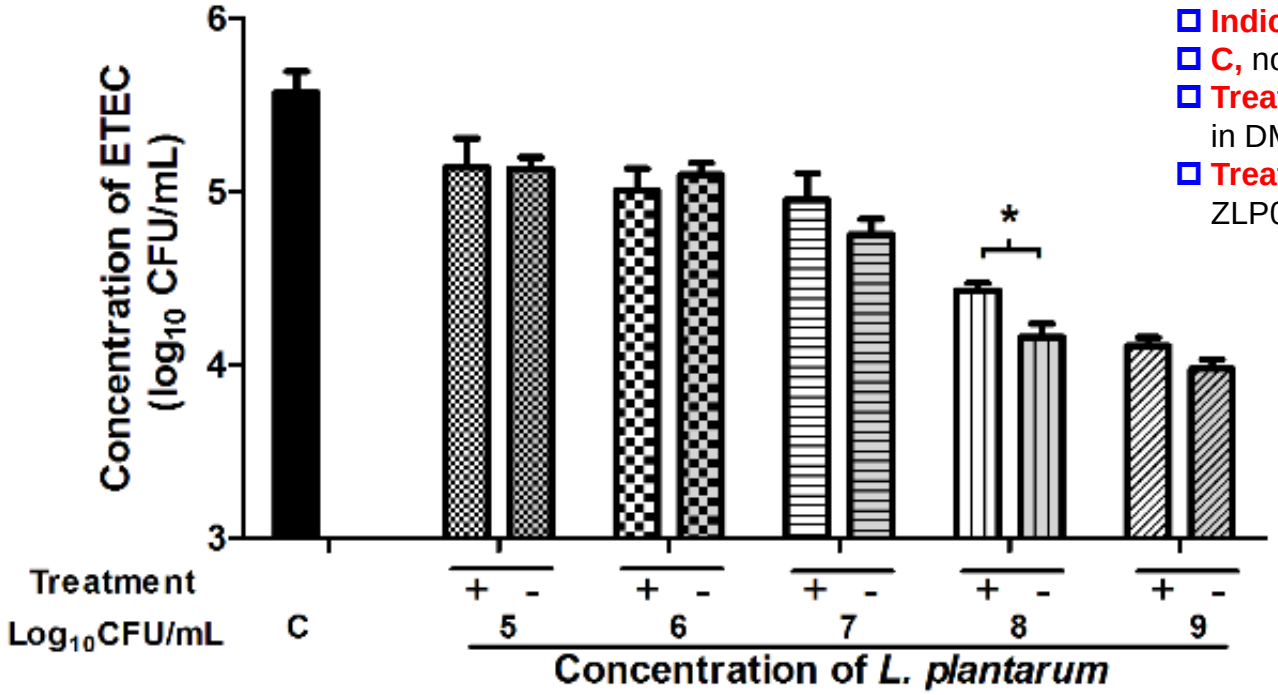
IPEC-J2 Cells

3D4/31



Relative mRNA expression and protein secretion of HDPs in different porcine cell lines after *L. plantarum* ZLP001 treatment. mRNA expression in (A) intestinal IPEC-J2 epithelial cells and (B) 3D4/31 lung alveolar macrophages as determined by RT-qPCR. Data are the mean ± SEM of three independent experiments. *$P < 0.05$ versus non-treated control group. White and black bars represent control and *L. plantarum* ZLP001 treatment, respectively.

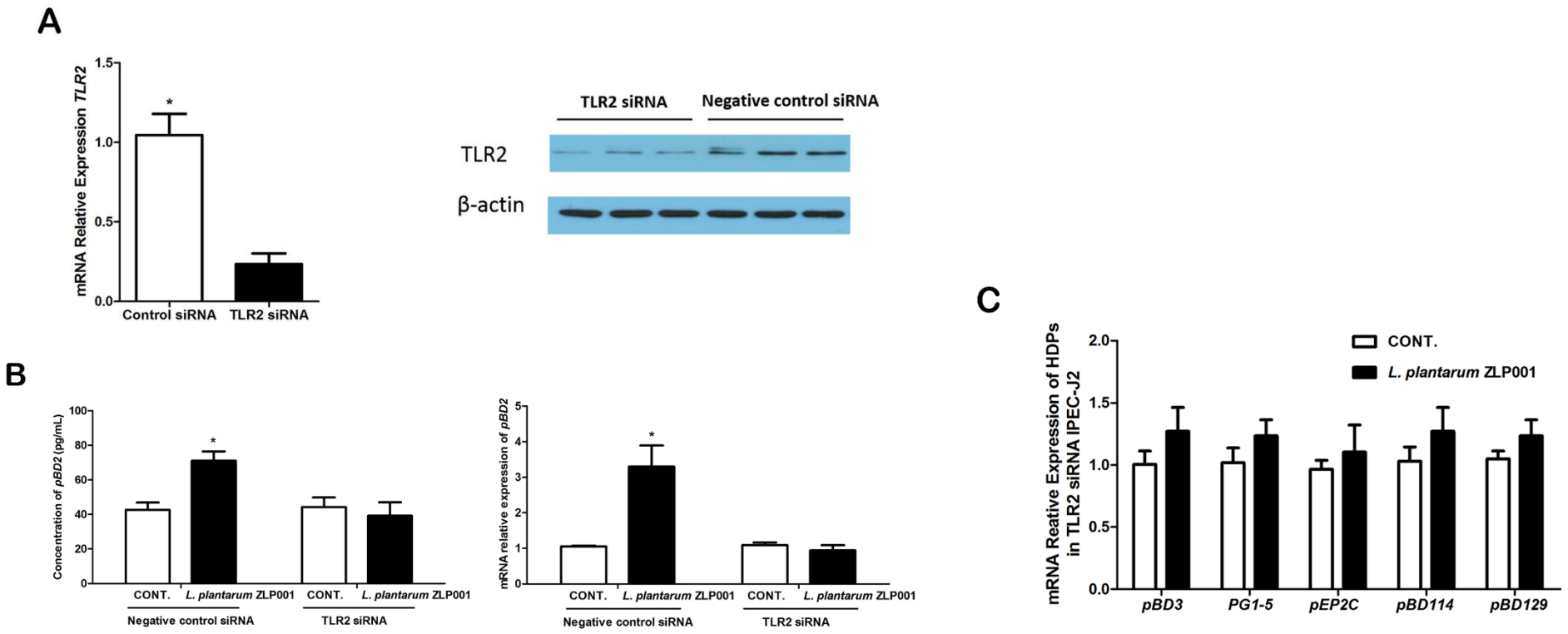
L. plantarum ZLP001 increase Antibacterial Activity of Cell-Culture Supernatant



- **Indicate bacteria,** ETEC (serotype O149:K91, K88ac)
- **C,** non-stimulated cell control (negative control)
- **Treatment +**, supernatant of *L. plantarum* ZLP001 culture in DMEM/F12 (positive control)
- **Treatment –,** cell-culture supernatant after *L. plantarum* ZLP001 incubation

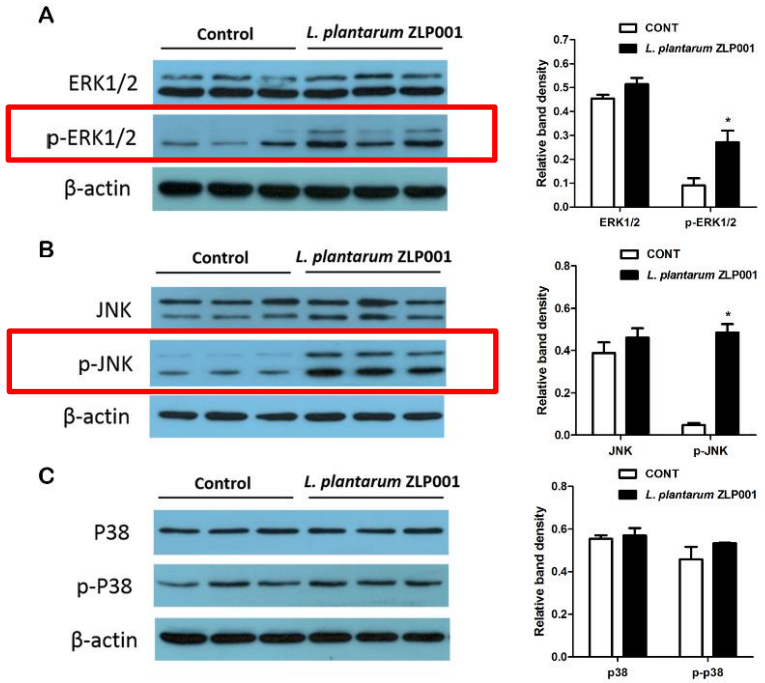
Antibacterial activity of IPEC-J2 cell-culture supernatants collected after incubation with *L. plantarum* ZLP001 at different concentrations (10^5, 10^6, 10^7, 10^8, and 10^9 CFU/mL) for 6 h or supernatants of *L. plantarum* alone incubated in DMEM/F12.
Values are expressed as the number of viable enterotoxigenic ETEC present after 2 h incubation in the supernatant in three independent experiments.

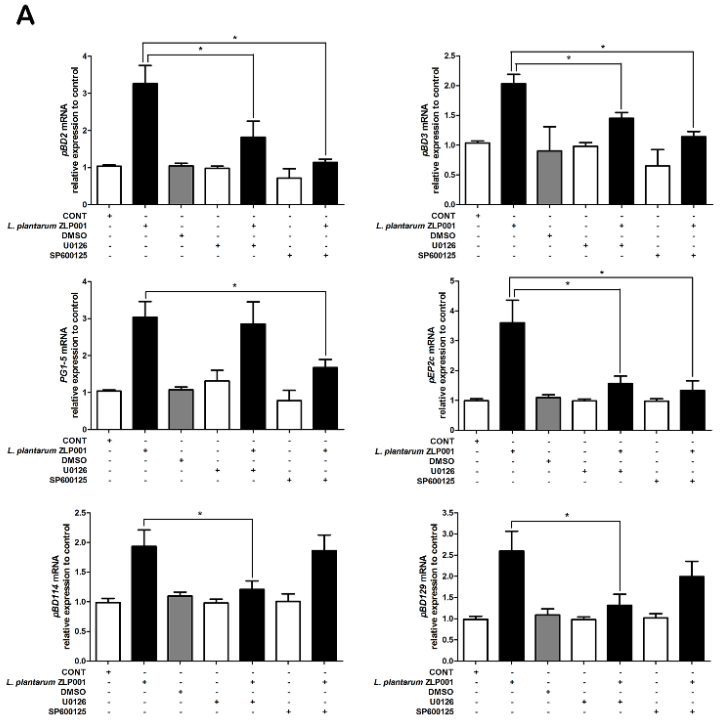
TLR2 is Required for *L. plantarum* ZLP001-induced HDP Upregulation

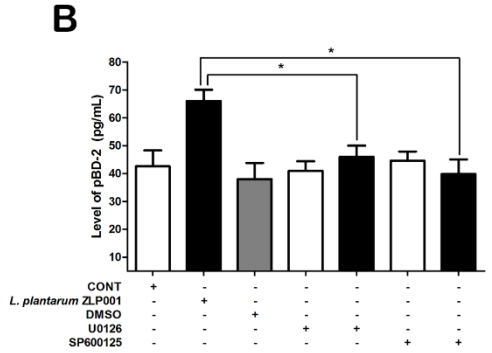


TLR2 is required for *L. plantarum* ZLP001-induced porcine HDP upregulation in IPEC-J2 cells. (A) *TLR2* gene and protein expression in TLR2 siRNA-transfected IPEC-J2 cells was determined using RT-qPCR and western blot analyses, respectively. (B) *L. plantarum* ZLP001 stimulates porcine pBD2 expression and secretion through TLR2 in IPEC-J2 cells. pBD2 expression and concentration were measured by RT-qPCR and ELISA, respectively. (C) TLR2 silencing suppresses porcine HDP expression induced by *L. plantarum* ZLP001 in IPEC-J2 cells.

L. plantarum ZLP001-induced HDP Expression is Regulated by MAPK Signaling



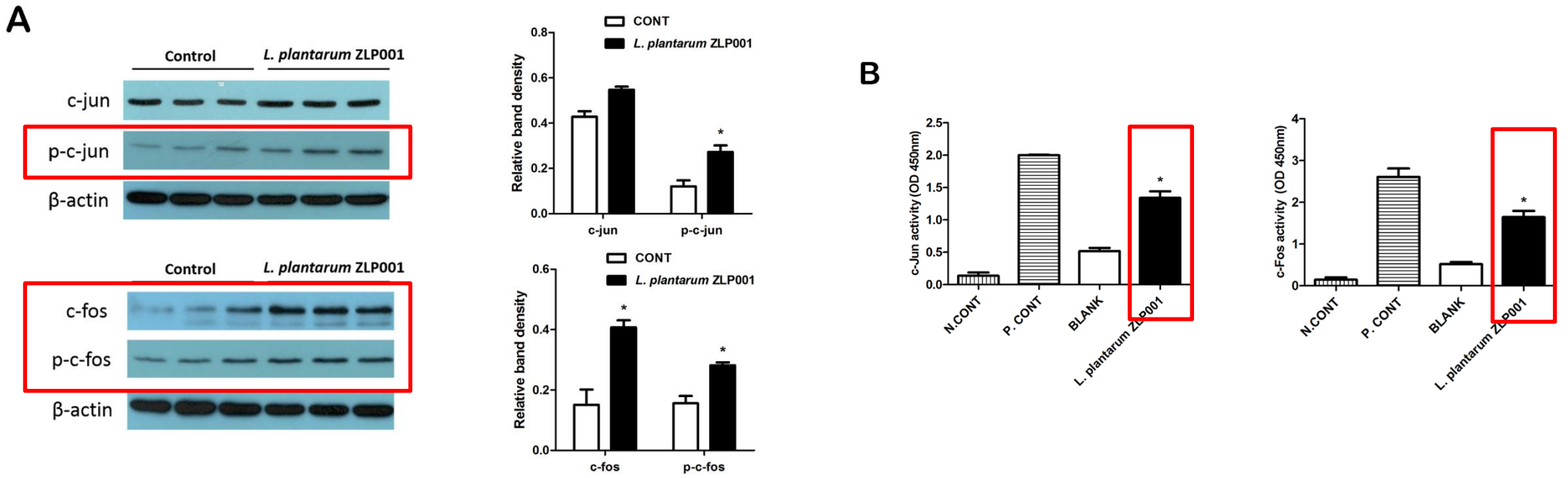




Role of MAPK signaling pathways in *L. plantarum* ZLP001-induced porcine HDP expression and secretion. IPEC-J2 cells were incubated with *L. plantarum* ZLP001 at 10^8 CFU/mL for 6 h, and protein expression and phosphorylation of ERK1/2 (A), ERK (B), and p38 (C) in whole cell lysates were assessed by western blot analysis.

Blocking the key proteins of MAPK signaling pathway affects porcine HDP expression and production. (A) Inhibition of ERK1/2 and JNK blocks porcine HDP mRNA expression. IPEC-J2 cells were pre-incubated with the specific ERK1/2 inhibitor U0126 (10 μM) and the specific JNK inhibitor SP600125 (10 μM) 1 h before incubation with 10^8 CFU/mL *L. plantarum* ZLP001 for 6 h. (B) Inhibition of ERK1/2 and JNK blocks porcine pBD2 production.

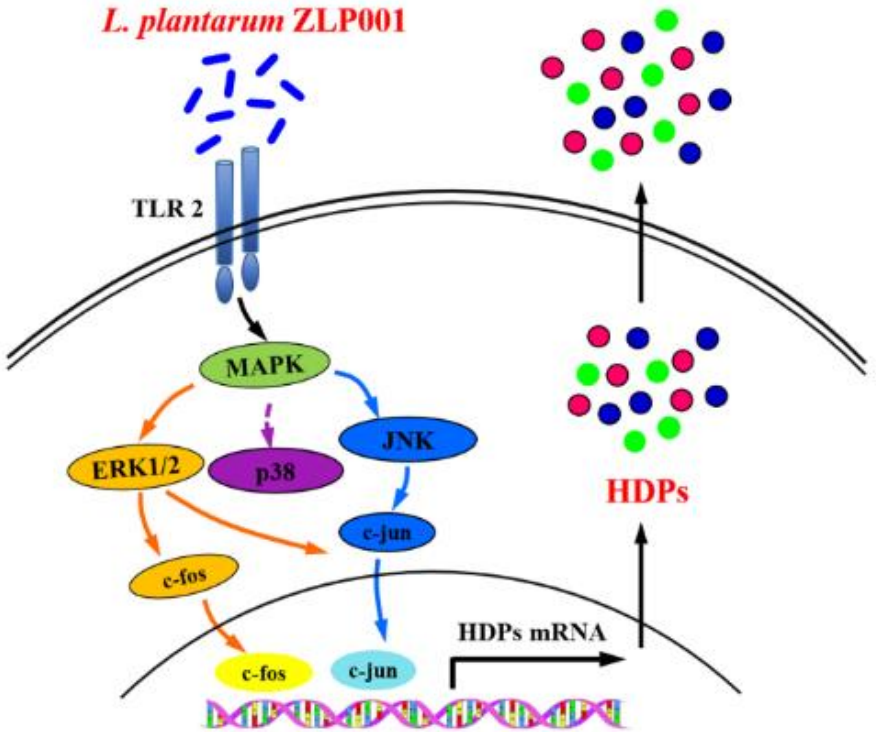
AP-1 Regulates *L. plantarum* ZLP001-induced HDP Upregulation



Role of transcription factor MAPK/AP-1 in *L. plantarum* ZLP001-induced HDP expression. (A) *L. plantarum* ZLP001 induces c-fos and c-jun protein expression. IPEC-J2 cells were incubated with 10^8 CFU/mL *L. plantarum* ZLP001 for 6 h, and protein expression and phosphorylation of c-jun and c-fos were assessed by western blot analysis in whole cell lysates. (B) *L. plantarum* ZLP001 increased AP-1 subunit c-jun and c-fos activities. c-jun and c-fos activities in nuclear extracts were assessed by TransAM assay. A nuclear extract provided from the supplier served as a positive control, and a negative control was incubated without nuclear extract.

Summary

***L. plantarum* ZLP001** induces porcine HDP expression *in vivo* and *in vitro*, and the induction seems to be regulated via TLR2 as well as the ERK1/2/JNK and c-jun/c-fos signaling pathways.



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