動物來源之乳酸桿菌預防沙門氏菌感染之研究

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Probiotic strain characteristics

- Strain safety (GRAS)
- Acid and bile stability
- Adherence to human intestinal cells
- Colonization in the human intestinal tract
- Production of antimicrobial substances
- Antagonism against pathogenic bacteria
- Other functions: Immunomodulation of host, Cholesterol lowering, and Anticancer effect, etc.

Pathogenic bacteria

uropathogenic *Escherichia coli*
shiga toxin-producing *Escherichia coli* O157:H7
enerohemorrhagic *Escherichia coli*
enterotoxigenic *E. coli*
enteropathogenic *E. coli*
Listeria monocytogenes
Clostridium difficile
Staphylococcus aureus
Bacillus cereus
Shigella sonnei
Salmonella typhimurium
Salmonella typi
Salmonella choleraesuis
Salmonella enteritidis
Helicobacter pylori

Probiotic strains

- *Lactobacillus rhamnosus*
- *Lactobacillus reuteri*
- *Lactobacillus delbrueckii* subsp. *lactis*
- *Lactococcus lactis* subsp. *lactis* ATCC11454
- *Lactobacillus johnsonii* strain CNCM I-1225
- *Lactobacillus lactis* NRRL B-5628
- *Lactobacillus acidophilus*
- *Lactobacillus casei*
- *Lactobacillus salivarius*
- *Lactobacillus fermentum* ME-3
- *Bifidobacterium adolescentis* 1027
- *Enterococcus faecium* strain 68

Mechanisms of the antimicrobial effect

- Production of H_2O_2
- Production of acids
- Production of biosurfactants
- Production of bacteriocin
- Competition with the pathogenic bacteria for adherence to intestinal epithelium
- Immunomodulation

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2. Wen Hsin Lin, Chin-Fa Hwang, Li-Wei Chen and Hau-Yang Tsen*. 2005. Viable counts andcharacteristic evaluation for commercial lactic acid bacteria products. Food Microbiol. 23:78~81. (SCI)
3. Cheng-Chih Tsai, Ming-Hui Chen, Tseng-Huang Liu, Chin-Gin Chau, Li-Tung Chang, Chin-Chuan Tsai and Hau-Yang Tsen*. 2004. Evaluation of the toxicity of *Lactobacillus acidophilus* LAP5 in a 28-Day feeding study in Wistar rats. Journal of Food Safety. 24(4):268~280. (SCI)
4. Cheng-Chih Tsai, Tseng-Huang Liu, Ming-Hui Chen, Chin-Chuan Tsai, and Hau-Yang Tsen*. 2004. Safety evaluation for an *Enterococcus faecium* strain TM39 *in vitro* and *in vivo*. Food and Chemical Toxicology. 42(10):1601~1609. (SCI)
5. Cheng-Chih Tsai, Li-Fang Huang, Chia-Chen Lin and Hau-Yang Tsen*. 2004. Antagonistic activity against *Helicobacter pylori* infection *in vitro* by a strain of *Enterococcus faecium* TM39. International Journal of Food Microbiology. 96(1):1~12. (SCI)
6. **Other LAB works in our laboratory: Development of DNA probes, PCR primers and DNA biochip for *Bifidobacterium* spp. and *Lactobacillus* spp., etc.**

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Antagonistic activity against *Salmonella* infection in vitro and in vivo for two *Lactobacillus* strains from swine and poultry

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Method

- Isolation and identification of LAB
- Resistance of LAB to the simulated gastrointestinal conditions
- Inhibition of the LAB on the growth of pathogenic bacteria
- Adhesion of LAB to the Int-407 and Caco-2 cell lines
- Adhesion of LAB to the intestinal epithelial cells from swine, poultry, and BALB/c mouse
- Antagonistic effect of LAB strains against Salmonella invasion to Int-407 cells
- Feeding mice with LAB and challenging with Salmonella cells

Table 1
Adhesion of the lactic acid bacteria cells to the Int-407 and Caco-2 cell line

Strain	Adherence to the epithelial cells[a]	
	Int-407	Caco-2
L. acidophilus LAP5	390±26	253±61
L. fermentum LF33	383±90	157±25

[a] Ten of the epithelial cells were used to calculate the average number of the adhering LAB cells per epithelial cell.

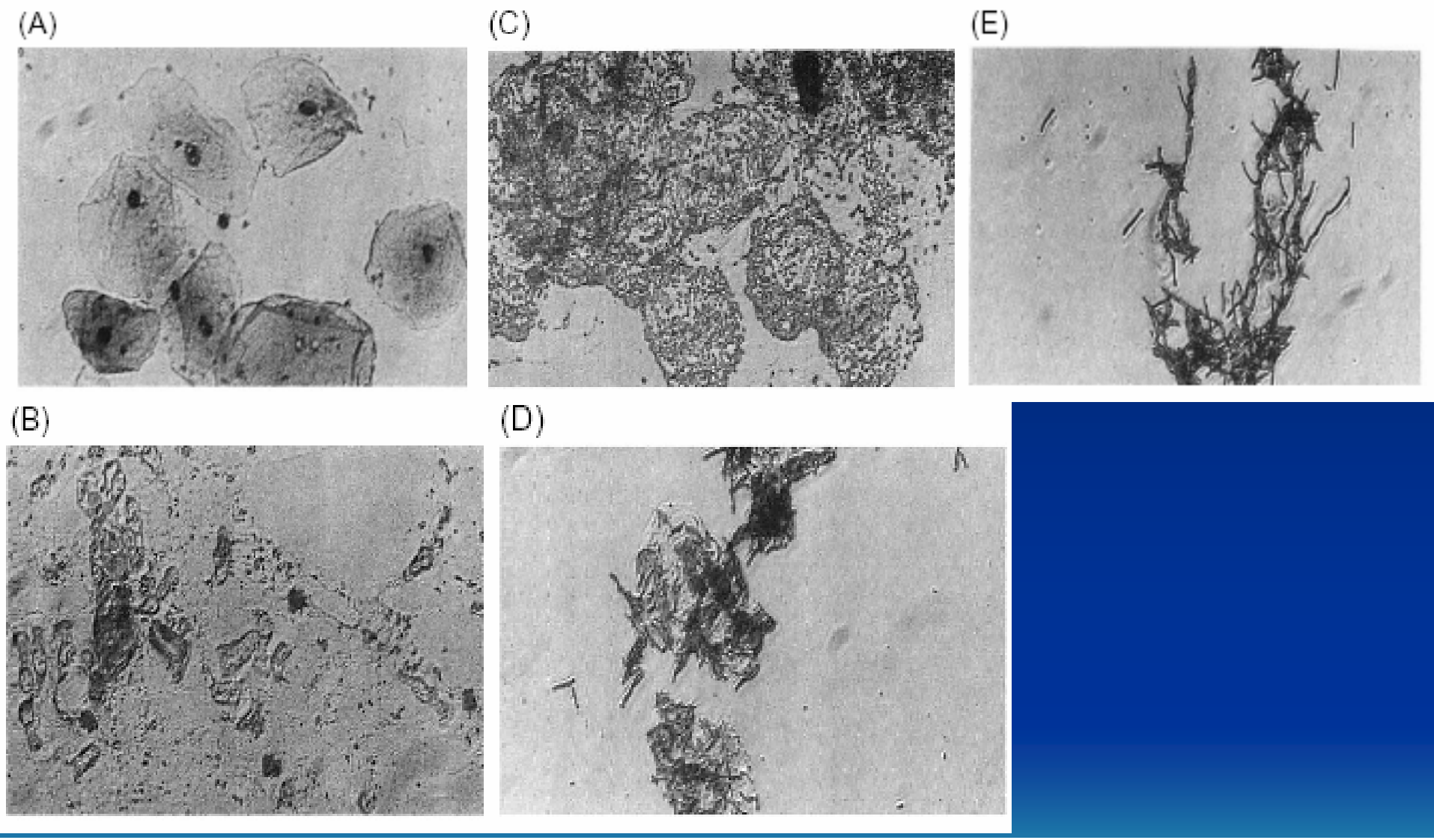


Fig. 1. Adhesion of lactic acid bacteria to the epithelial cells from poultry and swine intestine. (A) Negative control 0.01 M PBS to poultry, (B) negative control 0.01 M PBS to swine, (C) *L. fermentum* LF33 to poultry, (D) *L. acidophilus* LAP5 to poultry, (E) *L. acidophilus* LAP5 to swine intestinal epithelium cell.

Table 2

Effect of the lactic acid bacteria cultures and their spent culture supernatants (SCS) on the *Salmonella* invasion to the Int-407 cells[a]

LAB samples coincubated with salmonellae	*Salmonella* invasion (log CFU/ml)[b]
Control PBS (pH 7.2)	6.0±0.5
MRS broth (Control)	5.8±0.2
L. acidophilus LAP5:	
Cell culture	2.7±0.3*
Neutralized cell culture	3.5±0.2*
SCS	2.9±0.4*
Neutralized SCS	4.2±0.2*
L. fermentum LF33:	
Cell culture	4.3±0.9*
Neutralized cell culture	4.1±0.3*
SCS	3.9±0.6*
Neutralized SCS	6.0±0.9

[a] Experimental conditions are described in Materials and methods. The inoculum level of *S. enterica* serovar Typhimurium I50 was 7.6±0.2 (log CFU/ml).

[b] Intracellular *Salmonella* numbers in the suspension of the lysed cells (see Materials and methods).

* Value indicates a significant difference ($p<0.05$) from the control PBS.

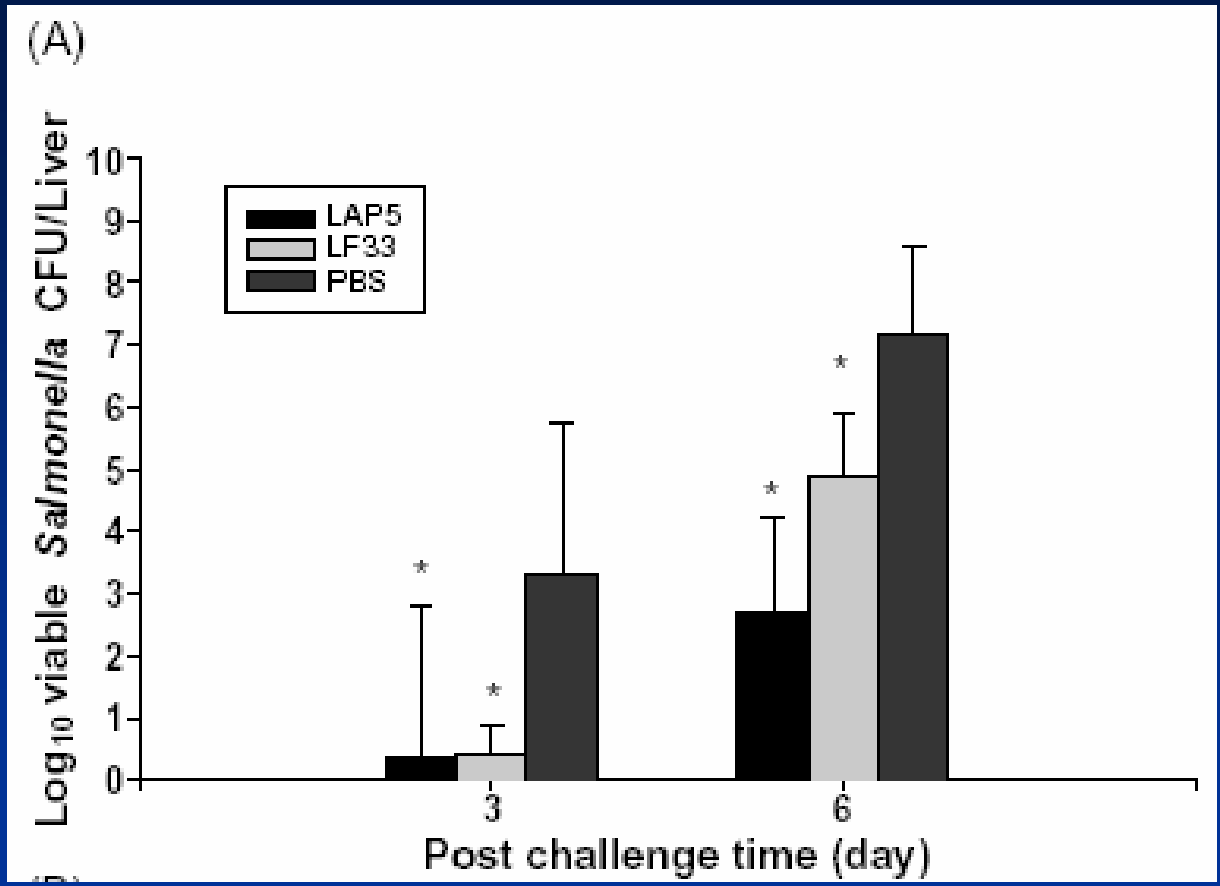
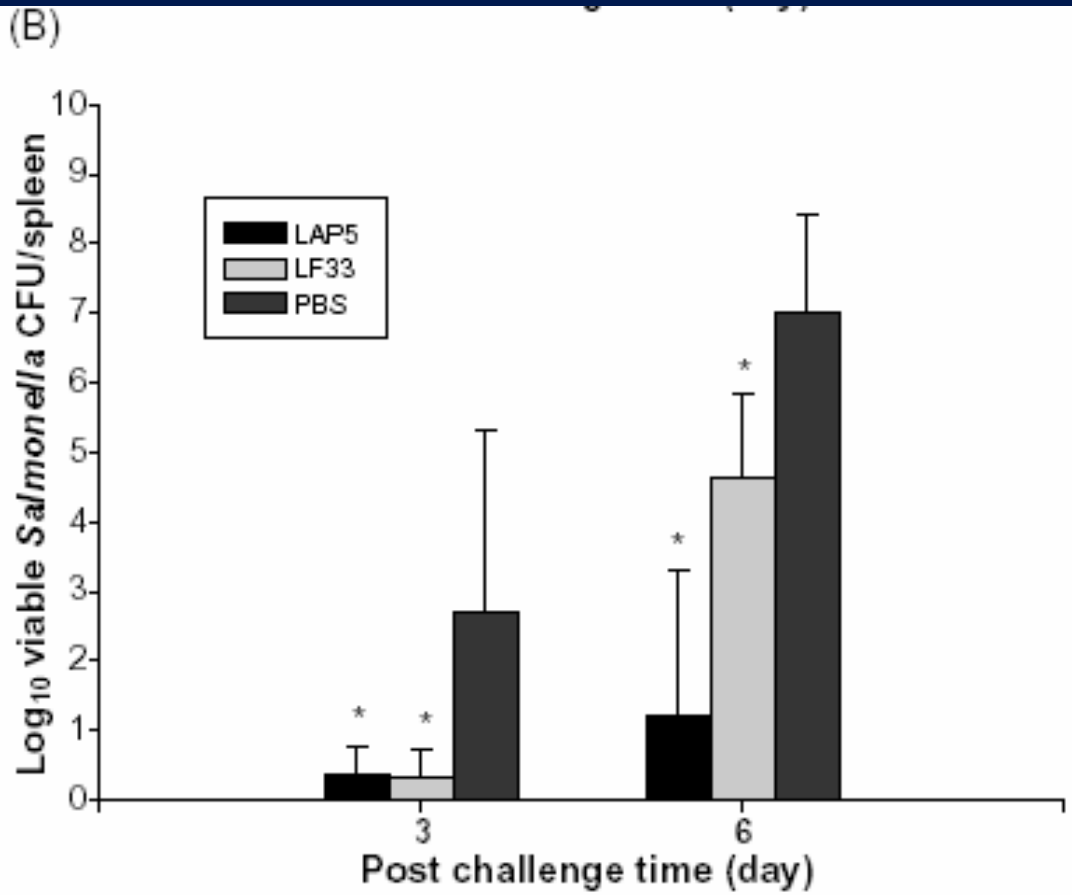


Fig. 2. *S. enterica* serovar Typhimurium I50 cells infected in the (A) liver and (B) spleen of the mice which were administrated daily with lactic acid bacteria or 0.01 M PBS (negative control) for 7 days prior to the oral administration of *Salmonella* cells. Experimental conditions were as described in Materials and methods. *Values with unlike superscripts indicate a significant difference from the control ($P<0.05$).



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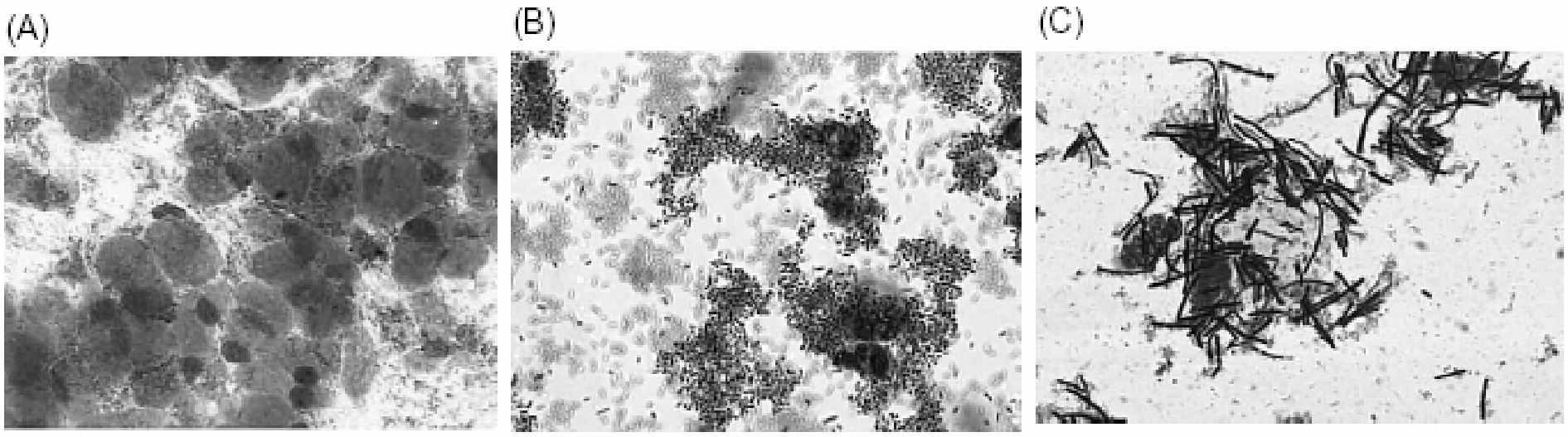

Fig. 3. Adhesion of LAB to the mouse intestinal cell (400×). (A) Negative control 0.01 M PBS, (B) *L. fermentum* LF33, (C) *L. acidophilus* LAP5.

Conclusion

As reported here, since strains LAP5 and LF33 were able to produce lactic acid, to inhibit the growth of pathogenic bacteria, to adhere to the intestinal epithelium cells of poultry, swine, mouse, and human intestinal cell lines Int-407 as well as Caco-2, both strains are with potential for use as probiotics against the infection of human and animals by salmonellae or other bacterial pathogens.