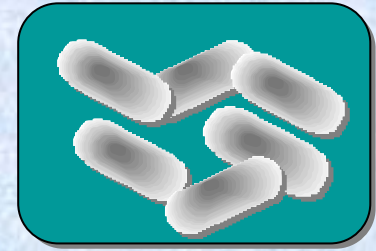




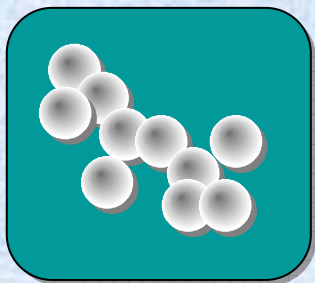
**The annual meeting of Taiwan
Lactic acid bacteria (TLAB)
2006**



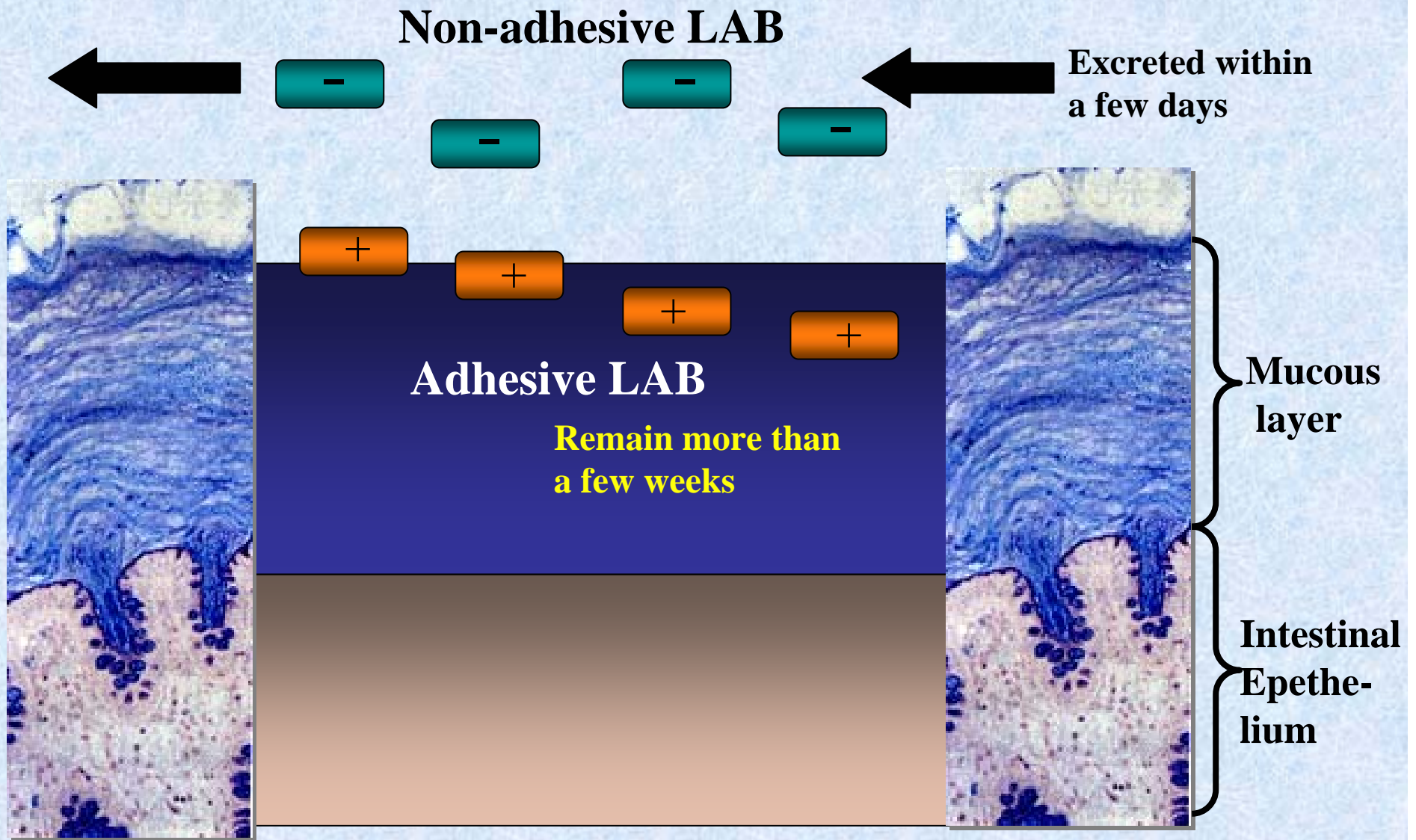
**The Search for Probiotic Lactic Acid Bacteria (LAB)
and Their Application in Human**

November 3, 2006

Taiwan University, Taipei, Taiwan



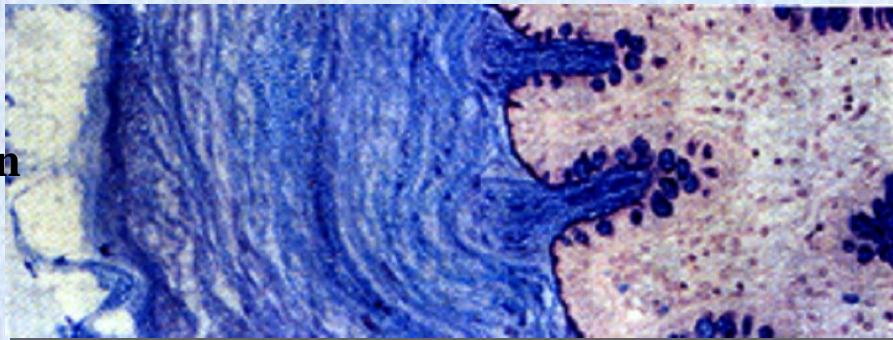
**Tadao Saito (Prof.)
Graduate School of
Agricultural Science
Tohoku University**



Adhesive LAB can survive in intestine more than non-adhesive LAB!

Adhesive activity is one of the important characteristics of Probiotics.

lumen



Mucous layer

epithelium

Human colonic mucin (HCM)

NeuAc

α 2-6

GalNAc α 1-3Gal β 1-3GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3GalNAc α 1---The(Ser)

A-antigen !

α 1-2
Fuc

+ -OSO₃⁻

Terminal

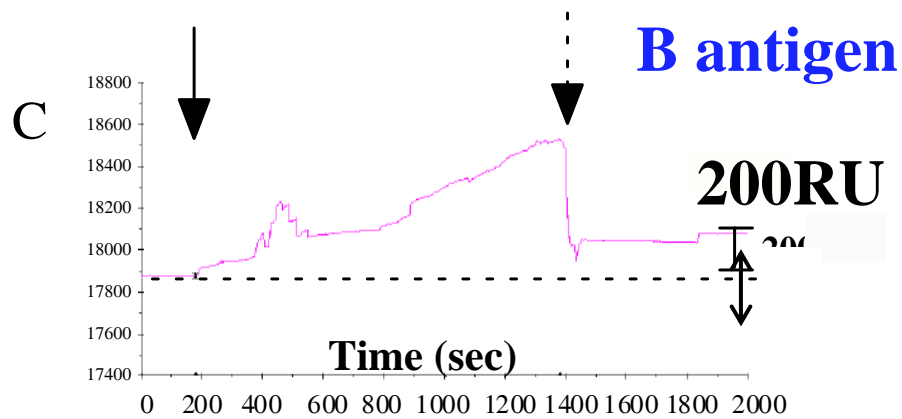
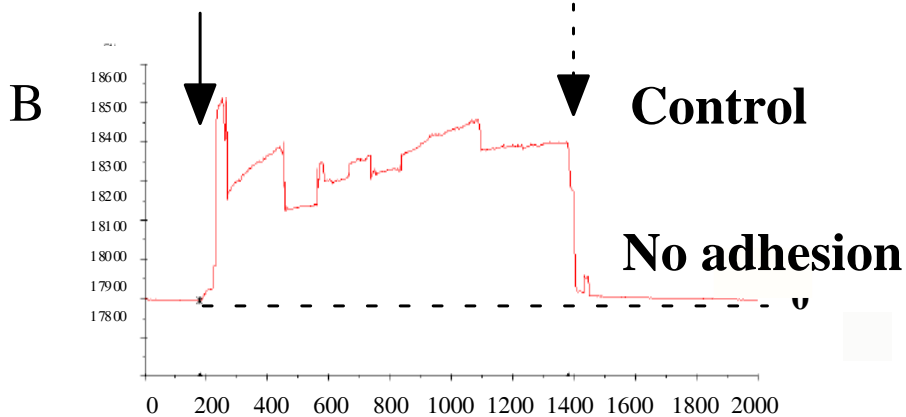
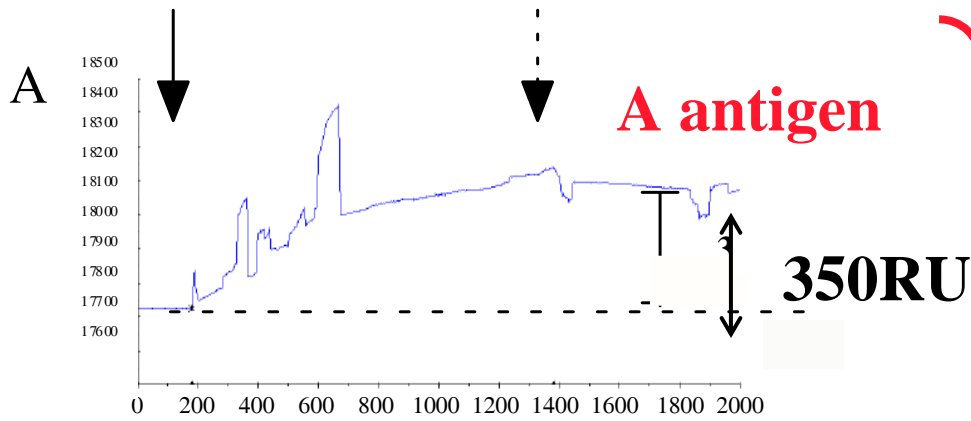
Skeleton

Core

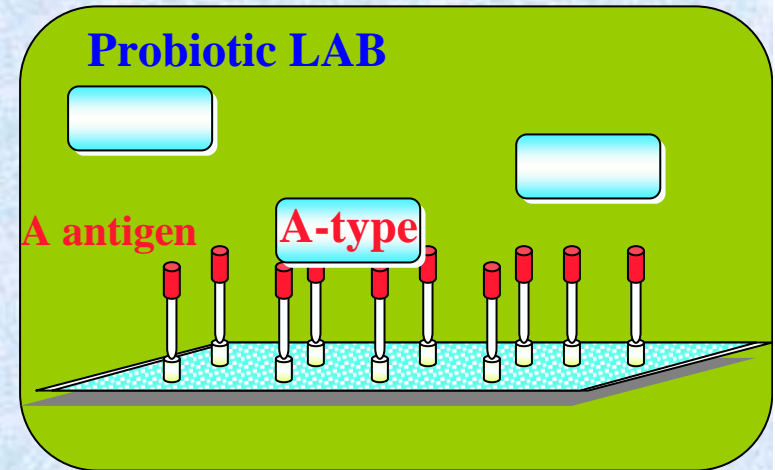
Chemical structure of sugar chain combined to human colonic mucin

(Podolsky *et al.*, *J. Biol. Chem.*, 260, 8262 (1985))

Resonance unit (RU)



BIACORE sensorgram

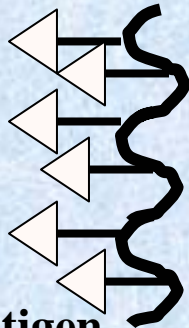


The existense of LAB which can recognize A-type antigen more stronger than B-type antigen was first isolated.

Uchida, Saito et al. Biosci. Biotechnol. Biochem.,68, 1004-1010, 2004

Existence of LAB which can recognize each blood type antigen

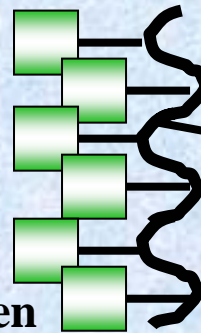
A-type LAB
lectin



A-antigen
GalNAc-

Blood type A
(40%)

B-type LAB
lectin

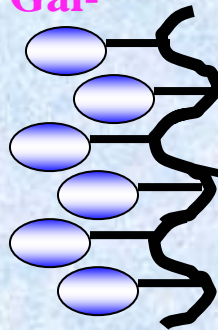


B-antigen

Gal-

Blood type B
(20%)

O-type LAB
lectin



H-antigen

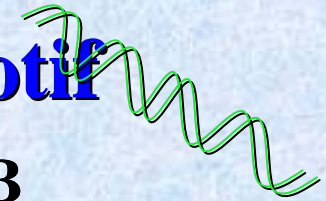
Fuc-

Blood type O
(30%)

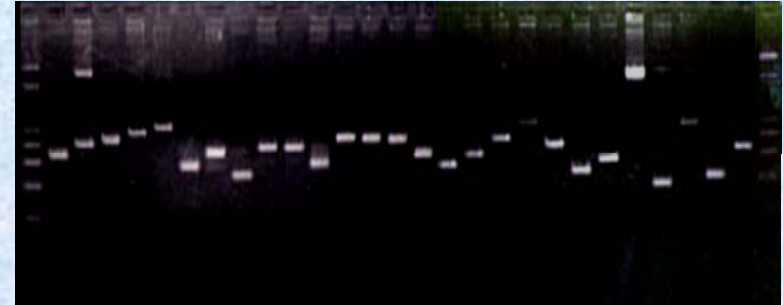
Discovery of immunostimulatory ODN-motif



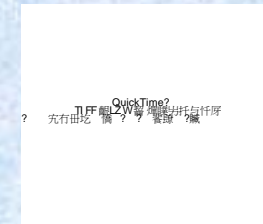
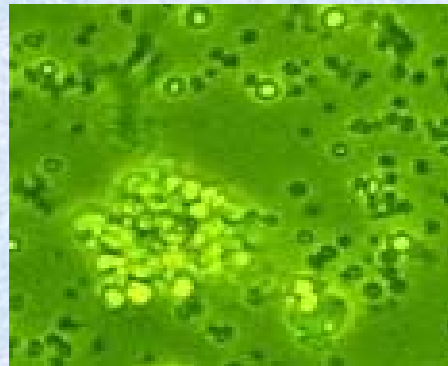
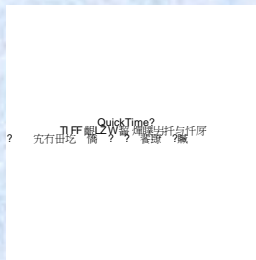
genome DNA from immunobiotic LAB



Digested & Cloning



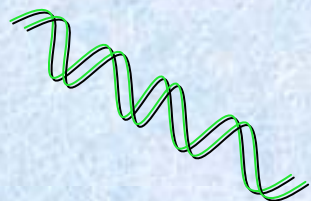
Mitogenic activity



Scintillation counter
LS1801, Beckman Coulter

Splenocytes or Peyer's patches cells from murine

**Immunostimulatory consensus sequence
including CpG or AT-motif**



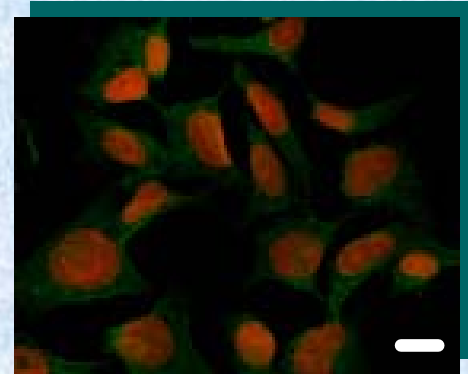
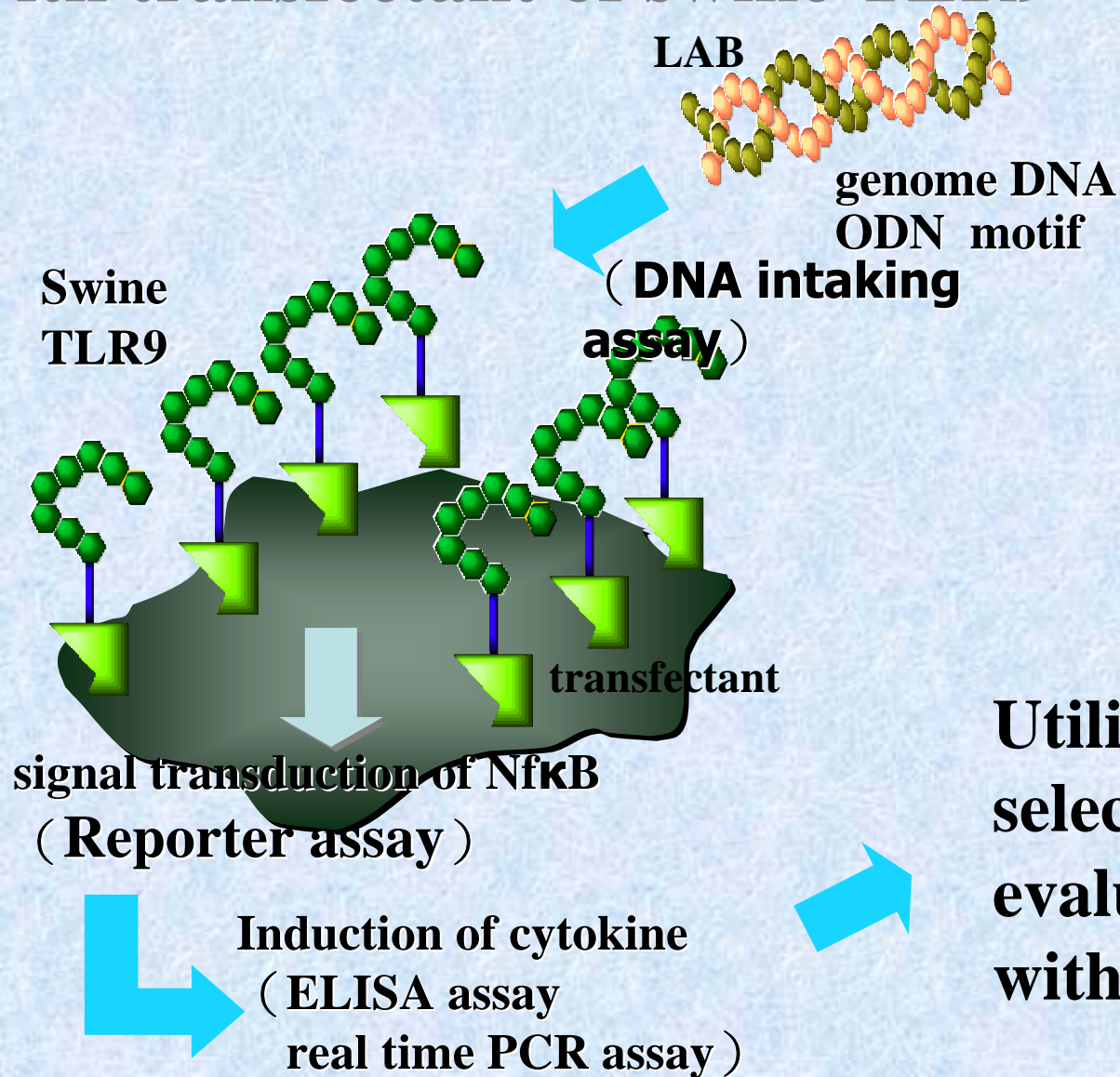
Immunostimulatory ODN from LAB

Name & Sequence	Reference & Source
ID35 ACTTTCGTTTCTGCGTCAA	Iliyan <i>et al.</i> Cell. Microbiol. 2004 <i>L. rhamnosus</i> GG genome
MsST CAGGACGTTGTATCACTGA	Kitazawa <i>et al.</i> submitted <i>S. thermophilus</i> ATCC19258 <i>lacZ</i> gene
^A AT5ACL TATAATTTTACCAACTAGC	Kitazawa <i>et al.</i> Int. J. Food. Microbiol. 2001 <i>L. gasseri</i> JCM1131 ^T genome
OLLB-7 CGGCACGCTCACGATTCTTG	Kitazawa <i>et al.</i> Int. J. Food. Microbiol. 2003 <i>L. bulgaricus</i> NIAIB6 genome
BL-7 GCGTCGGTTCGGTGCTCAC	Takahashi <i>et al.</i> submitted <i>B. longum</i> BB536 genome

Cf. Mouse, rabbit type
 CpG 1826
TCCATGACGTCCTGACGTT
 (Human: **TCGT**, Swine: **TCGT** or **TCGA**)

Kriege *et al.*, Nature, 1995
E. coli

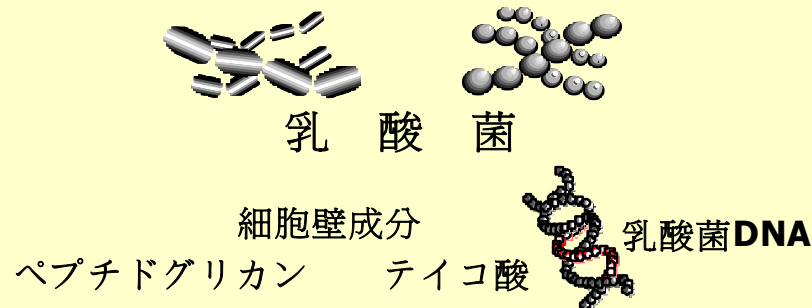
Establishment of a new evaluation system with transfectant of swine TLR9



Transfectant expressed swine TLR9 in CHO cell

Utilization of LAB selected by a new evaluation system with three assays

Components from LAB incorporated from intestinal M cell can reduce allergy symptoms !



Immunostimulating ODN motif

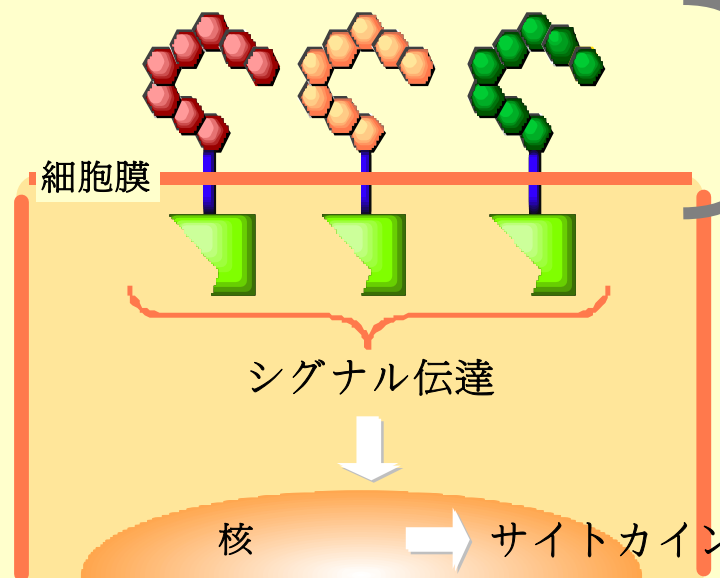
OLLB7 (*L.bulgaricus* NIAI B6)

5'-CGGCACGCTCACGATTCTTG-3'

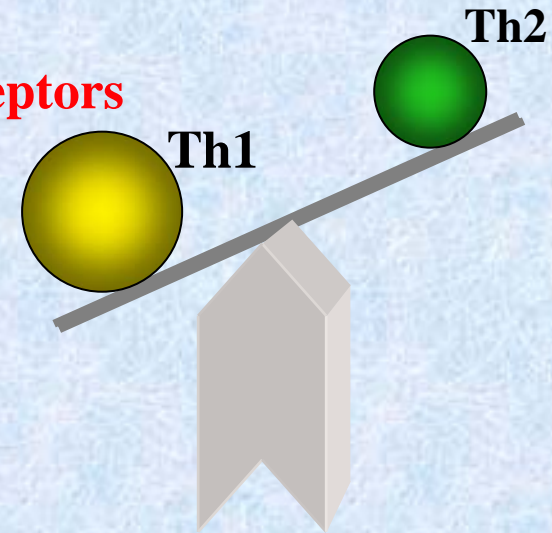
OLLG7L (*L.gasseri* JCM1131^T)

5'-ATTTATTATGACTTAGCT-3'

TLR2 TLR4 TLR9



Toll-like receptors
(TLR1-13)



Change the balance of helper T lymphocytes and reduce allergy symptoms

サイトカイン生産
IL-12など

Structural gene (ORF) of Gassericin A produced by *L. gasseri* LA39

430 440 450 460 470 480
AAGATACTATGGTTACTAAGTACGGACGTAATTTAGGTTTGAaCAAGGTAGAGTTGTTTG
M V T K Y G R N L G L N K V E L F A

490 500 510 520 530 540
CAATTTGGGCGGTTTTAGTAGTTGCTCTTTTATTGaCCACAGCGAACATTTATTGGATTG
I W A V L V V A L L L T T A N I Y W I A

cleavage site ↑

550 560 570 580 590 600
CTGATCAATTCGGGATTCATTTAGCGACTGGAACAGCCCGTAAGTTATTAGATGCAaTGG
D Q F G I H L A T G T A R K L L D A M A

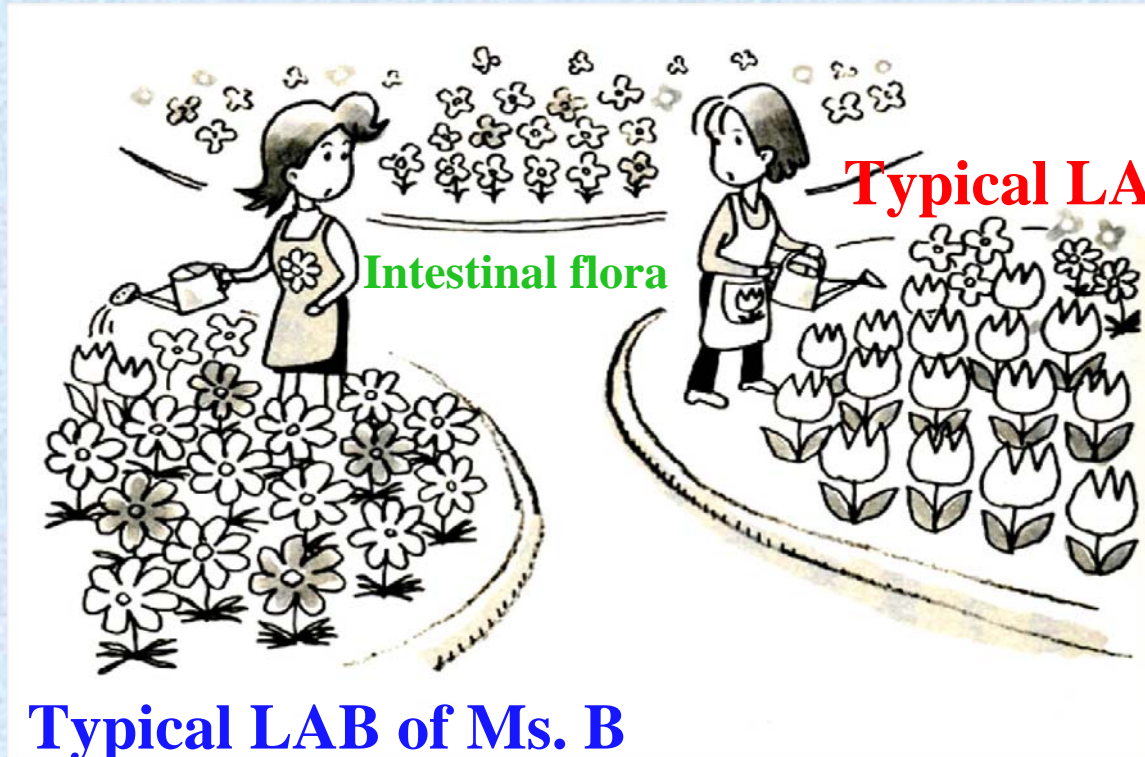
Mature type of Gasericin A

610 620 630 640 650 660
CTTCTGGTGCCTCATTGGGAAGTGCCTTTGCTGCTATTTTGGGCGTGACATTACCTGCAT
S G A S L G T A F A A I L G V T L P A W

670 680 690 700 710 720
GGGCTTTGGCAGCTGCAGGAGCATTGGGAGCGACTGCaCCcTAGTGATTATAAAGCTTTA
A L A A A G A L G A T A A *

(Kawai, Saito *et al.*, *Biosci. Biotechnol. Biochem.*,62, 887-892 (1998))

Each person can maintain health in whole body through keeping a good intestinal flora



What is future research subject ?

In personal level

- Search and utilization of high adhesive LAB to human intestine
- Competitive exclusion of pathogenic bacteria by probiotic LAB
- Addition of lacking LAB to personal intestinal flora