Inhibitory Effects of Symbiotic Lactobacterium- and Yeast-Fermented Soy Extract on Tumor Metastasis and Allergic Reaction

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Recently, all over the world face serious health problems. Patients of neoplasm, allergy, and virus infection involved in immune systems are increasing continuously, since our immune systems are getting worse with daily life, food and endocrine disrupting chemicals. In the course of our studies for developing immune-response modifiers from functional foods including fermented materials and traditional medicine, we found that symbiotic Lacto bacterium- and Yeast-fermented soy extract (LYS) inhibited tumor metastasis and allergic reaction by the modulation of immune systems.
Symbiotic Lacto bacterium- and Yeast-fermented Soy Extract (LYS)

Soy (Not genetically modified)

1) Water, r.t., 24 h
2) Homogenize
3) 100 °C, 1h
4) Filtration

Soy Extract

Symbiotic Fermentation

1) Enzyme Reaction (Cellulase, Amylase, Protease)
2) Fermentation
   Lactobacterium; E. faecalis, L. helveticus,
   L. casei, L. sp
   Yeast; Saccharomyces cerevisiae

Symbiotic Lacto bacterium- and Yeast-fermented Soy Extract (LYS)

1) 30 °C, 4 d
2) 100 °C, 1h
3) Freeze dry

Apply for various Assays
Activities of LYS

- Gastro protection (Inhibition of gastric lesions, ~1g/kg rat or mouse)
- Antiflatuents (Regulation of intestinal function)
- Anti-diabetes (Suppression of high blood glucose level, Inhibition of aldose reductase: improvements of complication)
- Control of immune systems (anti-allergy, immune-response activator)
Effect of LYS on Tumor Metastasis and Proliferation

Female C57/BL6 (4 weeks)

1 week

Injection (i.v.)
B16F10 melanoma, 10^5 cells/mouse

2 weeks

Same condition as above

Count tumor colonies in lung

Lung

Normal diet + Water or 1%, 2% LYS water or 1%, 2% lipoprotein water

Metastasis and Proliferation

Tumor colonies
Effect of LYS on Tumor Metastasis and Proliferation

Control

1% LYS

2% LYS

**p<0.01
Effect of LYS on Tumor Metastasis and Proliferation

- Control
- Lipoprotein Ext.
- Positive control drug
- LYS

Tumor Colonies

N=6, Mean ± S.E.

**p<0.01
Effect of LYS on Macrophage Activation

Male ddY mouse (30 g)

Wash peritoneal with PBS

Selection (Preculture)

Mouse peritoneal macrophage

LYS (~300 µg/ml) or LPS (10 µg/ml, positive control)

37 ºC, 20 h

Measurement of Nitric oxide (NO) in the culture medium by Griess reagent

LPS; lipopolysaccharide from S. aureus
Effect of LYS on Macrophage Activation

$N=6$

$**p<0.01$
Effect of LYS on Macrophage Activation

Mouse peritoneal macrophage

- LYS (~300 μg/ml)
  or LPS (10 μg/ml)
- 37 °C, 1 h

Fluorescent Beads for phagocytic activity
- 37 °C, 1 h

Trypan Blue (quenching)

Measurement of fluorescence density of activated macrophage (Ex.532 nm, Em.526 nm)
Effect of LYS on Macrophage Activation

N=6
**p<0.01
Effect of LYS on Allergic Reaction

Male SD rat (120 g)

Anti-DNP IgE

Injection

LYS, 1000mg/kg/day, 3times, p.o.

1% Evans blue-saline containing 0.75 mg DNP-BSA, i.v.

30 min

Measurement of the pigmentation area.

Dorsal skin sites Injected intradermally with DNP-IgE

LYS, p.o.

DNP-BSA saline, i.v.

Measurement of the pigmentation area.
Effect LYS on Allergic Reaction

Control

LYS
1000mg/kg/3times

N=6
**p<0.01
Effect of LYS on Allergic Reaction

Mast Cell (RBL Cell) Degranulation by DNP-BSA

LYS Conc. (%) vs. Degranulation (%)
Conclusion

• By in vivo administration of LYS, melanoma metastasis in lung was significantly suppressed depending on its dose dependence. At dose level of 2% LYS, colonies of melanoma were decreased 1/3-fold. And anti-metastasis activity of LYS was 20% stronger than positive control drug of lipoprotein.
• Passive cutaneous anaphylaxis was significantly suppressed by LYS for inhibiting mast cell degranulation and decreased 1/4-fold.
• These data indicates that LYS inhibits tumor metastasis and IgE-mediated type I allergy by modulating the immunocompetent cells activation.
• The LYS, symbiotic Lacto bacterium- and Yeast-fermented soy extract, is considered to be one of the immunomodulator.
Anti-Tumor Metastasis and Anti-Allergy of LYS

LYS
Symbiotic Lacto Bacterium- Yeast-fermented Soy Extract

Immunocytes (Macrophage, leukocyte....) Modulation

Activation Inhibition

Inhibition of Tumor Metastasis and Proliferation

Inhibition of Allergic Reaction

Immunomodulator, Immune-response modifiers