Modulating effects of Symbiotic Lacto bacterium- and Yeast-fermented soy extract on life-style related Diseases.

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Recently, all over the world face to serious health problem. 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
* Lacto Bacterium: 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 redactase: 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

Melanoma Injection

Metastasis and Proliferation

Tumor colonies

Normal diet + Water or 1%, 2% LYS water or 1%, 2% lipoprotein water
Effect of LYS on Tumor Metastasis and Proliferation

Control

1% LYS

2% LYS

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

N=6, Mean ± S.E.
**p<0.01

Tumor Colonies

Control 1% 2% 1% 2%
Lipoprotein Ext. Positive control drug LYS

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Effect of LYS on Macrophage Activation

Male ddY mouse (30 g)

Mouse peritoneal macrophage

Wash peritoneal with PBS

Selection (Preculture)

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

37 ℃, 20 h

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

LPS; lipopolysaccharide from S. aureus

Collected from mouse peritoneal macrophage

Primary culture LYS or LPS

Measurement of NO production by Griess reagent

Activation Marker
Effect of LYS on Macrophage Activation

N=6

**p<0.01

LYS (µg/ml)
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)

Collected from mouse peritoneal macrophage

Primary culture LYS or LPS Bio-Beads (Fluorescence)

Measurement of fluorescence density
Effect of LYS on Macrophage Activation

N=6
**p<0.01

LYS (µg/ml)

**
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 (%)
Effects of LYS on Diabetes

Diabetic model mouse
KKay mouse ( KK )

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

KKay mouse
High Triglyceride
High Glucose

Blood Glucose Level
Glucose-Oxidase method

4 weeks
Effects of LYS on Diabetes

The diagram shows the effects of LYS treatment on diabetes parameters. The vertical bars indicate changes in various parameters following LYS treatment. The graph highlights a significant reduction in the blood glucose level (70 mg/dl) after LYS treatment.
Effects of LYS on Hyperlipemia

Normal level

High Cholesterol Diets
Conclusion

- By in vivo, hyperglycemia, hyperlipemia, and melanoma metastasis in lung was significantly suppressed by LYS according to its dose dependence.
- And also passive cutaneous anaphylaxis was significantly suppressed by LYS for inhibiting mast cell degranulation and decreased 1/4-fold.
- These data indicates that LYS inhibits hyperglycemia, hyperlipemia, tumor metastasis and IgE-mediated type I allergy by modulating the digestive and immune systems.
- The LYS, Lacto bacterium- and Yeast-fermented soy extract, is considered to be one of the preventive agents of life-style related diseases.
Preventive Agents of Life-style related Diseases

*Symbiotic Lacto Bacterium- Yeast-fermented Soy Extract*

**LYS**
Symbiotic Lacto Bacterium- Yeast-fermented Soy Extract

Digestive and Immune System
(Intestinal Immune System)

- Modulation

  - Immunocytes (Macrophage, leukocyte....) Modulation
  - Digestive Modulation

- Modulation of blood glucose and lipids

- Inhibition of Tumor Metastasis and Proliferation

- Inhibition of Allergic Reaction