

# Polysaccharides of a fermented food, natto, suppress sucrose-induced hyperglycemia in an *in vivo* evaluation system and inhibit glucose uptake by human intestinal cells

Yasuhiko Matsumoto<sup>1,2</sup>, Miki Takahashi<sup>2,3</sup>, Kazuhisa Sekimizu<sup>2,3,\*</sup>

<sup>1</sup> Department of Microbiology, Meiji Pharmaceutical University, Tokyo, Japan;

<sup>2</sup> Teikyo University Institute of Medical Mycology, Tokyo, Japan;

<sup>3</sup> Genome Pharmaceuticals Institute Co., Ltd., Tokyo, Japan.

**SUMMARY** Natto is a well-known traditional Japanese food produced by fermenting soybeans with *Bacillus subtilis* var *natto*. Here we found that the water-soluble viscous fraction of natto inhibits sucrose- or glucose-induced hyperglycemia in silkworms. The water-soluble viscous fraction treated with DNase I, RNase A, and proteinase K, followed by phenol extraction also suppressed sucrose-induced hyperglycemia in silkworms. The enzyme-treated polysaccharide fraction of natto inhibits glucose uptake by Caco-2 cells, human intestinal epithelial cells. These findings suggest that the polysaccharide components of natto selected on the basis of their suppressive effects on sucrose-induced hyperglycemia in silkworms inhibit glucose uptake by human intestinal cells.

**Keywords** Glucose uptake, natto, polysaccharide, silkworm, sucrose-induced hyperglycemia

## 1. Introduction

Sucrose intake causes a rapid increase in blood glucose levels, a main factor in the development of diabetes. Suppression of the rapid postprandial increase in blood glucose is considered to be useful toward preventing the onset of diabetes, and the development of a food with such an effect is highly desired. For this purpose, mammalian experimental systems have been applied to evaluate increases in blood glucose levels and assessment of substances that may suppress these increases. These types of experiments, however, require a large number of mammals, which is problematic not only in terms of cost, but also from the view of animal welfare. To overcome these problems, we propose the use of silkworms as a model animal (1-3). We previously reported that silkworms ingesting a sucrose-containing diet exhibit an increase in blood glucose levels and that acarbose and voglibose, which are  $\alpha$ -glucosidase inhibitors, inhibit the blood glucose-increasing effect of sucrose ingestion (4). Furthermore, we identified a lactic acid bacterial strain, *Enterococcus faecalis* YM0831, that suppresses sucrose-induced hyperglycemia in silkworms as well as sucrose-induced hyperglycemia in humans (5). On the basis of these findings, we consider that the silkworm evaluation system could be useful for screening substances that suppress sucrose-induced hyperglycemia in humans.

Caco-2 cells are cultured cells derived from human colon adenocarcinoma used for evaluating the glucose absorption process in human intestinal epithelial cells (6,7). Thus, we used Caco-2 cells to evaluate the effect of substances that suppress sucrose-induced hyperglycemia in silkworms on a human cell type.

Polysaccharides derived from bacteria have diverse structures and physiologic activities (8-10). Polysaccharides produced by soil bacteria, such as *Rhizobium altiplani*, *Cupriavidus* sp., *Paenibacillus polymyxa*, *Pantoea eucalypti*, *Variovorax boronicumulans*, and *Xanthomonas cynarae*, inhibit sucrose-induced hyperglycemia in silkworms (11). Therefore, we hypothesized that polysaccharides secreted by other types of bacteria could suppress postprandial hyperglycemia.

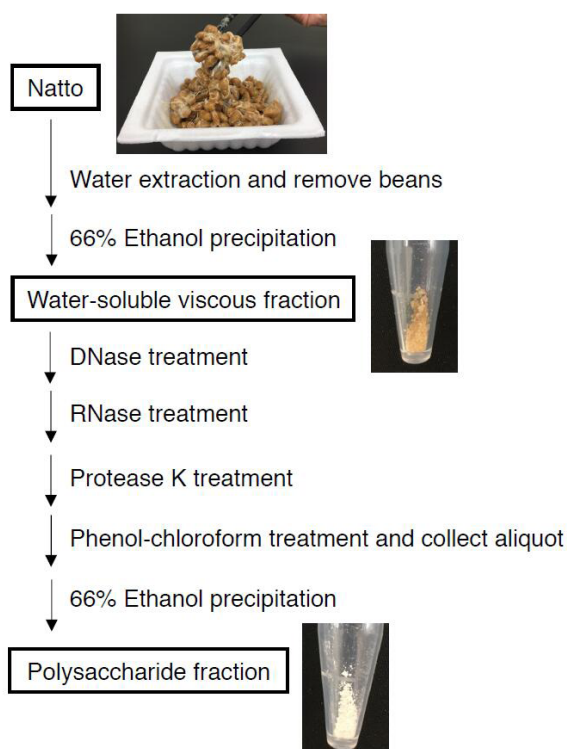
Natto is a traditional Japanese food produced by fermenting soybean with *Bacillus subtilis* var *natto*. *Bacillus subtilis* var *natto* grows on the surface of soybeans and produces various viscous polysaccharides. Natto has preventive effects on lifestyle-related diseases, and suppresses postprandial hyperglycemia (12,13). The active compounds in natto, however, have remained unknown. In this study, we examined whether the polysaccharides extracted from natto suppress sucrose-induced hyperglycemia in silkworms. Furthermore, we investigated whether the natto-derived polysaccharides identified in a

silkworm evaluation system to inhibit sucrose-induced hyperglycemia also inhibit glucose uptake by human intestinal Caco-2 cells.

## 2. Materials and Methods

### 2.1. Preparation of the water-soluble viscous fraction and enzyme-treated polysaccharide fraction of natto

The preparation scheme for the water-soluble viscous fraction and enzyme-treated polysaccharide fraction of natto is shown in Figure 1. Water was added to commercially available natto, and the mixture was thoroughly stirred. The soybeans were removed from the mixture and a water extract fraction was obtained. Two volumes of ethanol were added to the water extract fraction, and a filamentous precipitate was collected by centrifugation to obtain a water-soluble viscous fraction. Enzymatic treatment of the water-soluble viscous fraction was performed as described previously (11). The water-soluble viscous fraction was treated with DNase I (1,000 U/mL; Promega) and RNase A (10 µg/mL; NIPPON GENE CO., LTD.) for 24 h at 37°C, and then incubated overnight at 37°C with proteinase K (100 µg/mL). An equal volume of phenol:chloroform:isoamyl alcohol (50:49:1) was added to the fraction, and the samples were vigorously shaken. The upper layer fraction was collected after centrifugation, followed by the addition of two volumes of ethanol to the upper layer fraction. The precipitate was collected by centrifugation and dried to



**Figure 1.** Preparation scheme of the water-soluble viscous fraction and the enzyme-treated polysaccharide fraction of natto.

obtain an enzyme-treated polysaccharide fraction.

### 2.2. Measurement of sugar, DNA, RNA, and protein

The amounts of sugars, DNA, RNA, and proteins in the enzyme-treated polysaccharide fraction were determined using the phenol-sulfuric acid method, the fluorescent-based Qubit assay for DNA and RNA, and the Bradford assay, respectively, according to a previous report (11).

### 2.3. Sucrose or glucose tolerance tests of silkworms

Silkworms were reared as described previously (14,15). Silkworm sucrose or glucose tolerance tests were conducted according to the previously reported method (4). A diet containing 10% (w/w) sucrose or glucose was prepared by mixing an artificial diet (Silkmate 2S: Nihon Nosan Co., Ltd., Kanagawa, Japan) and D-sucrose or D-glucose. Test samples were mixed with the diet containing 10% (w/w) sucrose or glucose. A sucrose diet with or without polysaccharide samples was fed to 5<sup>th</sup>-instar silkworm larvae for 1 h, and then the silkworm hemolymph was collected by cutting the first proleg and glucose concentrations were measured with a glucometer (Accu-Chek, Roche).

### 2.4. Glucose uptake assay in Caco-2 cells

Glucose uptake by Caco-2 cells was determined by the previously described method (5). Caco-2 cells were obtained from American Type Cell Collection (ATCC, Manassas, VA, USA) and cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco, NY, USA) containing 10% fetal bovine serum (Gibco) and 1% penicillin/streptomycin (Gibco) at 37°C with 5% CO<sub>2</sub> in air. Caco-2 cells were cultured in a monolayer in a 96-well plate (Tissue culture test plate 96F: TPP, Switzerland). The cells were incubated for an additional 24 h in serum-free DMEM. Subsequently, the cells were washed with Na buffer [10 mM HEPES (pH 7.4), 140 mM NaCl, 20 mg/mL bovine serum albumin] and incubated in Na buffer for 15 min. After incubation, the cells were incubated in Na buffer with 50 µM 2-deoxy-2-[(7-nitro-2, 1, 3-benzoxadiazol-4-yl)amino]-D-glucose (2-NBDG: Cayman Chemical, MI, USA) for 10 min and then washed twice with ice-cold phosphate buffered saline (PBS) to remove the 2-NBDG. Fluorescence of the cells containing 2-NBDG was detected by fluorescence microscopy (IX73: Olympus, Tokyo, Japan) and calculated by Image J ver. 1.43u (National Institutes of Health, USA).

### 2.5. Chemicals

Acarbose was purchased from LKT Laboratories, Inc. (St. Paul, MN, USA). Voglibose was kindly provided by the Takeda Pharmaceutical Company (Osaka, Japan).

## 2.5. Statistical analysis

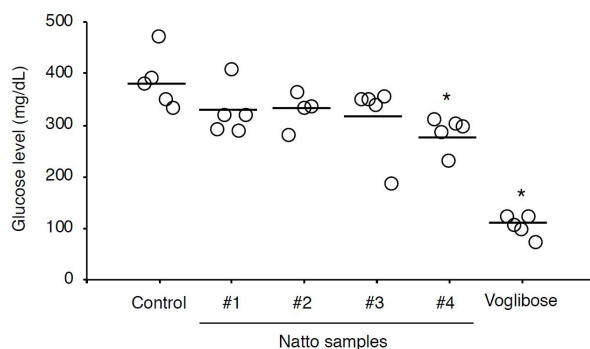
All experiments were performed at least twice. The significance of differences was calculated using a two-tailed Student's *t*-test at the significance level of  $\alpha = 0.05$ .

## 3. Results

### 3.1. Suppressive effect of a water-soluble viscous fraction obtained from natto on sucrose-induced hyperglycemia in silkworms

We prepared a water-soluble viscous fraction from four commercially available types of natto and examined the suppressive effects on sucrose-induced hyperglycemia in silkworms. The water-soluble viscous fraction obtained from natto (#4) significantly suppressed sucrose-induced hyperglycemia in silkworms (Figure 2). The water-soluble viscous fraction obtained from natto #4 suppressed sucrose-induced hyperglycemia in silkworms in a dose-dependent manner (Figure 3A). Indigestible dextrin is a typical water-soluble dietary fiber, the solution of which is viscous (16). Indigestible dextrin lowers postprandial hyperglycemia in rats (16). Indigestible dextrin (200 mg/g diet), however, did not suppress sucrose-induced hyperglycemia in silkworms (Figure 3B). These findings suggest that the viscous components of natto have suppressive activity on sucrose-induced hyperglycemia in silkworms and that the suppressive activity is greater than that of indigestible dextrin.

### 3.2. Suppressive effect of a natto polysaccharide fraction on sucrose-induced hyperglycemia in silkworms

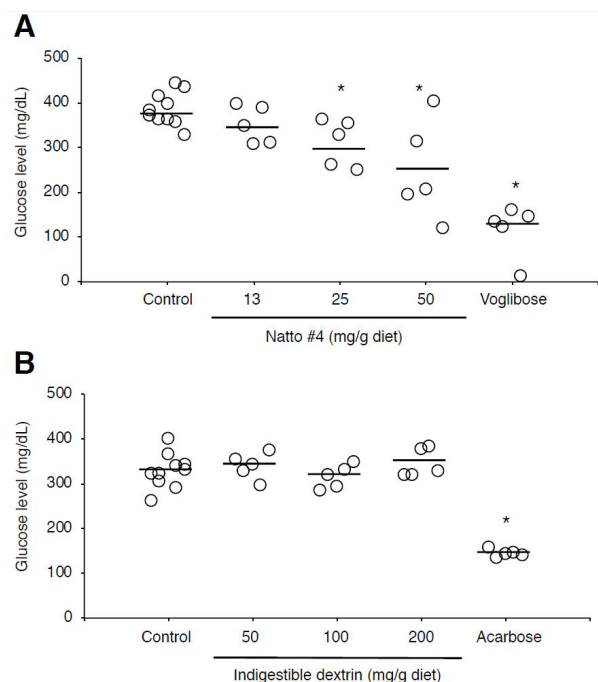


**Figure 2. Inhibitory effects of water-soluble viscous fractions of natto on sucrose-induced hyperglycemia in silkworms.** A water-soluble viscous fraction (50 mg/g diet) prepared from four commercially available types of natto, and voglibose (40 mg/g diet) were mixed with a 10% sucrose-containing diet. Silkworms were fed a diet containing 10% (w/w) sucrose with or without commercially available natto #1-4 (50 mg/g diet) and acarbose (40 mg/g diet) for 1 h. Glucose levels in the silkworm hemolymph were measured ( $n = 4-5$ /group). Bars indicate the mean value of each sample. Statistically significant differences between the control and experimental groups were evaluated using Student's *t*-test. \*:  $p < 0.05$ .

The water-soluble viscous fraction obtained from natto was treated with DNase, RNase, proteinase K, and phenol to obtain an enzyme-treated polysaccharide fraction. The enzyme-treated polysaccharide fraction contained (per gram dry weight) 32% sugar, and less than 1% DNA, RNA, and protein (Table 1). Addition of the enzyme-treated polysaccharide fraction to a sucrose-containing diet suppressed sucrose-induced hyperglycemia in silkworms, similarly to the water-soluble viscous fraction (Figure 4). This finding suggests that the natto-derived polysaccharides suppress sucrose-induced hyperglycemia.

### 3.3. Suppressive effect of a water-soluble viscous fraction obtained from natto on glucose-induced hyperglycemia in silkworms

Sucrose in the intestinal tract is cleaved into glucose and fructose by  $\alpha$ -glucosidase and is absorbed into the intestinal cells (4). Acarbose and voglibose,

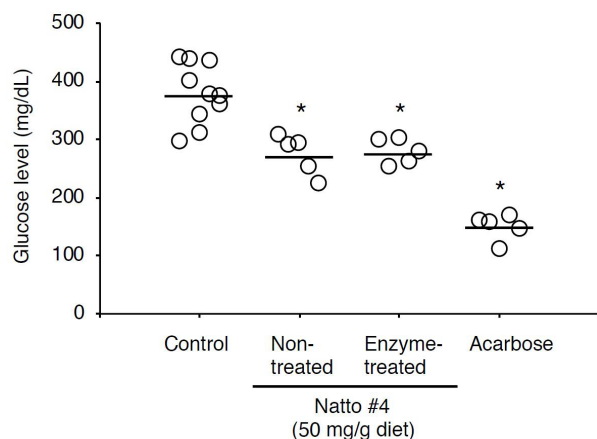


**Figure 3. Dose-dependent inhibitory effects of a water-soluble viscous fraction obtained from natto on sucrose-induced hyperglycemia in silkworms.** (A) Dose-dependent inhibitory effects of a water-soluble viscous fraction obtained from natto #4 on sucrose-induced hyperglycemia in silkworms. Silkworms were fed a diet containing 10% (w/w) sucrose with or without the water-soluble viscous fraction obtained from natto #4 (13, 25, 50 mg/g diet) and voglibose (40 mg/g diet) for 1 h. Glucose levels in the silkworm hemolymph were measured ( $n = 5-10$ /group). Bars indicate the mean value of each sample. Statistically significant differences between the control and experimental groups were evaluated using Student's *t*-test. \*:  $p < 0.05$ . (B) Dose-dependent inhibitory effects of indigestible dextrin on sucrose-induced hyperglycemia in silkworms. Silkworms were fed a diet containing 10% (w/w) sucrose with or without indigestible dextrin (50, 100, 200 mg/g diet) and acarbose (40 mg/g diet) for 1 h. Glucose levels in the silkworm hemolymph were measured ( $n = 5-10$ /group). Bars indicate the mean value of each sample. Statistically significant differences between the control and experimental groups were evaluated using Student's *t*-test. \*:  $p < 0.05$ .

**Table 1. Amounts of sugar, DNA, RNA, and protein in Natto #4 enzyme-treated fraction**

Fraction	Sugar (mg/g)	DNA (mg/g)	RNA (mg/g)	Protein (mg/g)
Enzyme-treated polysaccharide sample	320	0.04	0.3	3.4

The amounts of sugars, DNA, RNA, and proteins in the enzyme-treated polysaccharide fraction were determined by the phenol-sulfuric acid method, the fluorescent-based Qubit assay for DNA and RNA, and Bradford assay, respectively. The amounts of sugars, DNA, RNA, and proteins in 1 g of dry weight of the enzyme-treated fraction are shown.

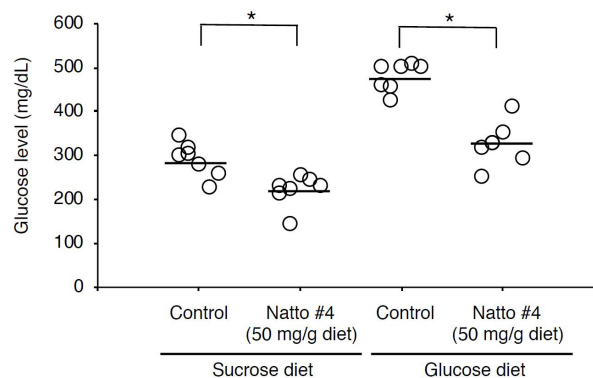


**Figure 4. Inhibitory effects of the polysaccharide fraction of natto on sucrose-induced hyperglycemia in silkworms.** A water-soluble viscous fraction obtained from natto #4 was treated with DNase, RNase, proteinase K, and phenol to obtain an enzyme-treated polysaccharide fraction (Enzyme-treated natto #4). The water-soluble viscous fraction (Non-treated natto #4, 50 mg/g diet) prepared from natto #4, an enzyme-treated polysaccharide fraction (Enzyme-treated natto #4, 50 mg/g diet), and acarbose (40 mg/g diet) were mixed with a 10% sucrose containing diet. Silkworms were fed a diet containing 10% (w/w) sucrose with or without the water-soluble viscous fraction (Non-treated natto #4, 50 mg/g diet), the enzyme-treated polysaccharide fraction (Enzyme-treated natto #4, 50 mg/g diet), or acarbose (40 mg/g diet) for 1 h. Glucose levels in the silkworm hemolymph were measured ( $n = 5-10/\text{group}$ ). Bars indicate the mean value of each sample. Statistically significant differences between the control and experimental groups were evaluated using Student's  $t$ -test. \*:  $p < 0.05$ .

$\alpha$ -glucosidase inhibitors, inhibit sucrose-induced hyperglycemia, but do not suppress glucose-induced hyperglycemia (4). *E. faecalis* YM0831, which has inhibitory activity against glucose transport in the isolated silkworm intestine suppresses both sucrose- and glucose-induced hyperglycemia in silkworms (5). In the present study, addition of the water-soluble viscous fraction obtained from natto to the diet suppressed sucrose- and glucose-induced hyperglycemia in silkworms (Figure 5). These findings suggest that the water-soluble viscous fraction obtained from natto inhibits the transport of glucose in the intestine, similar to *E. faecalis* YM0831.

#### 3.4. Inhibitory effect of a natto polysaccharide fraction on glucose uptake by Caco-2 cells

*E. faecalis* YM0831 inhibits glucose uptake by Caco-2 cells derived from human colon adenocarcinoma



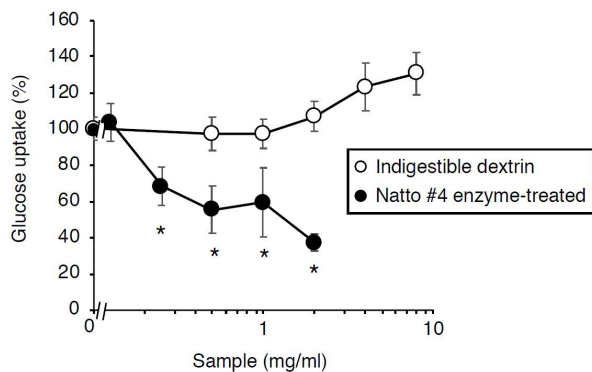
**Figure 5. Inhibitory effects of the water-soluble viscous fraction of natto on glucose-induced hyperglycemia in silkworms.** The water-soluble viscous fraction (50 mg/g diet) prepared from four commercially available types of natto was mixed with 10% sucrose- or glucose-containing diet, respectively. Silkworms were fed a diet containing 10% (w/w) sucrose or glucose with or without the water-soluble viscous fractions (50 mg/g diet) for 1 h. Glucose levels in the silkworm hemolymph were measured ( $n = 6-7/\text{group}$ ). Bars indicate the mean value of each sample. Statistically significant differences between control and experimental groups were evaluated using the Student's  $t$ -test. \*:  $p < 0.05$ .

(5). Here we found that addition of the enzyme-treated polysaccharide fraction of natto inhibited glucose uptake by Caco-2 cells (Figure 6). On the other hand, even when indigestible dextrin was added, glucose uptake by Caco-2 cells was not inhibited (Figure 6). These findings suggest that the enzyme-treated polysaccharide fraction of natto has high activity to inhibit glucose uptake by intestinal tract cells compared with indigestible dextrin.

#### 4. Discussion

In this study, we found that a natto-derived, water-soluble viscous fraction and its enzyme-treated polysaccharide fraction suppressed sucrose-induced hyperglycemia in silkworms and inhibited glucose uptake by human intestinal cells. The polysaccharides obtained from natto are expected to inhibit glucose uptake by intestinal cells in human individuals and suppress postprandial hyperglycemia.

Although ingestion of natto was previously reported to suppress hyperglycemia, its active substances were not identified (12, 13). Natto contains viscous substances such as poly- $\gamma$ -glutamic acid and levan, a polysaccharide (17). Poly- $\gamma$ -glutamic acid is used as a base material for an insulin drug delivery system (18). There are no reports that polyglutamic acid has



**Figure 6. Inhibitory effect of natto-derived polysaccharide fraction on glucose uptake by human intestinal cell, Caco-2 cells.** Various concentrations of enzyme-treated polysaccharide fraction (Enzyme-treated natto #4) and indigestible dextrin were added to the uptake system of 2-NBDG in Caco-2 cells and fluorescence uptake by the Caco-2 cells was measured.  $n = 3/\text{group}$ . Statistically significant differences between the control and experimental groups were evaluated using Student's  $t$ -test. \*:  $p < 0.05$ .

blood glucose-suppressing activity following sucrose intake. Levan has various functions, such as moisture retention, and is used for food additives, cosmetics, and drug development (19). Research suggests, however, that administration of levan derived from natto does not have a blood glucose-suppressive effect in normal rats or diabetic rats treated with streptozotocin, and does not improve symptoms of diabetes (20). Therefore, this report is the first to demonstrate that natto-derived viscous components suppress the increase in blood glucose after sucrose ingestion. Our results suggest that the viscous component of natto suppresses postprandial hyperglycemia by inhibiting glucose uptake in intestinal tract cells. The molecular structure and mechanism of action of the polysaccharides in natto that inhibit glucose uptake in human intestinal cells require further clarification.

Experiments using the silkworm evaluation system revealed that the viscous component of natto has a suppressive effect against sucrose- and glucose-induced hyperglycemia. Silkworms are useful for large-scale *in vivo* evaluation because they are inexpensive and can be reared in large groups (21-27). We reported the discovery of several active substances that suppress postprandial hyperglycemia using the silkworm evaluation system (4,11,28). The inhibitory effects of each of the four natto products we examined on sucrose-induced hyperglycemia in silkworms differed. Therefore, we will next search for a highly active natto using the silkworm evaluation system. We propose that the silkworm evaluation system is useful for developing functional fermented foods.

In the present study, we found that natto-derived polysaccharides identified by an *in vivo* evaluation system using silkworms inhibited glucose uptake by human intestinal cells. The development of more functional natto using the silkworm evaluation system

and verification of the effectiveness of natto for humans are future challenges.

### Acknowledgements

We thank Kana Hashimoto and Mari Maeda (Genome Pharmaceuticals Institute Co., Ltd., Tokyo, Japan) for their technical assistance in rearing the silkworms. The project was supported by JSPS KAKENHI grant number JP15H05783 (Scientific Research (S) to K.S.) and JSPS KAKENHI grant number JP17K08288 (Scientific Research (C) to Y.M.). The project was also supported by the Supporting Industry Program of the Ministry of Economy, Trade and Industry and Genome Pharmaceuticals Institute Co., Ltd. (Tokyo, Japan). The authors declare no conflicts of interest.

### References

1. Matsumoto Y, Sumiya E, Sugita T, Sekimizu K. An invertebrate hyperglycemic model for the identification of anti-diabetic drugs. *PloS one*. 2011; 6:e18292.
2. Matsumoto Y, Sekimizu K. Evaluation of anti-diabetic drugs by using silkworm, *Bombyx mori*. *Drug Discov Ther*. 2016; 10:19-23.
3. Matsumoto Y, Ishii M, Hayashi Y, Miyazaki S, Sugita T, Sumiya E, Sekimizu K. Diabetic silkworms for evaluation of therapeutically effective drugs against type II diabetes. *Sci Rep*. 2015; 5:10722.
4. Matsumoto Y, Ishii M, Sekimizu K. An *in vivo* invertebrate evaluation system for identifying substances that suppress sucrose-induced postprandial hyperglycemia. *Sci Rep*. 2016; 6:26354.
5. Matsumoto Y, Ishii M, Hasegawa S, Sekimizu K. *Enterococcus faecalis* YM0831 suppresses sucrose-induced hyperglycemia in a silkworm model and in humans. *Commun Biol*. 2019; 2:157.
6. Andrade N, Silva C, Martel F. The effect of oxidative stress upon intestinal sugar transport: an *in vitro* study using human intestinal epithelial (Caco-2) cells. *Toxicol Res (Camb)*. 2018; 7:1236-1246.
7. Schreck K, Melzig MF. Intestinal saturated long-chain fatty acid, glucose and fructose transporters and their inhibition by natural plant extracts in Caco-2 cells. *Molecules*. 2018; 23.
8. Rehm BH. Bacterial polymers: biosynthesis, modifications and applications. *Nat Rev Microbiol*. 2010; 8:578-592.
9. Nwodo UU, Green E, Okoh AI. Bacterial exopolysaccharides: functionality and prospects. *Int J Mol Sci*. 2012; 13:14002-14015.
10. Freitas F, Alves VD, Reis MA. Advances in bacterial exopolysaccharides: from production to biotechnological applications. *Trends Biotechnol*. 2011; 29:388-398.
11. Ishii M, Matsumoto Y, Sekimizu K. Bacterial polysaccharides inhibit sucrose-induced hyperglycemia in silkworms. *Drug Discov Ther*. 2018; 12:185-188.
12. Weng Y, Yao J, Sparks S, Wang KY. Nattokinase: an oral antithrombotic agent for the prevention of cardiovascular disease. *Int J Mol Sci*. 2017; 18. doi: 10.3390/ijms18030523.
13. Schallmeyer M, Singh A, Ward OP. Developments in the use of *Bacillus* species for industrial production. *Can J*

- Microbiol. 2004; 50:1-17.
14. Kaito C, Akimitsu N, Watanabe H, Sekimizu K. Silkworm larvae as an animal model of bacterial infection pathogenic to humans. *Microb Pathog.* 2002; 32:183-190.
  15. Kurokawa K, Kaito C, Sekimizu K. Two-component signaling in the virulence of *Staphylococcus aureus*: a silkworm larvae-pathogenic agent infection model of virulence. *Methods Enzymol.* 2007; 422:233-244.
  16. Wakabayashi S, Kishimoto Y, Matsuoka A. Effects of indigestible dextrin on glucose tolerance in rats. *J Endocrinol.* 1995; 144:533-538.
  17. Oner ET, Hernandez L, Combie J. Review of Levan polysaccharide: From a century of past experiences to future prospects. *Biotechnol Adv.* 2016; 34:827-844.
  18. Khalil IR, Burns AT, Radecka I, Kowalczyk M, Khalaf T, Adamus G, Johnston B, Khechara MP. Bacterial-derived polymer poly-γ-glutamic acid (γ-PGA)-based micro/nanoparticles as a delivery system for antimicrobials and other biomedical applications. *Int J Mol Sci.* 2017; 18. doi: 10.3390/ijms18020313.
  19. Srikanth R, Reddy CH, Siddartha G, Ramaiah MJ, Uppuluri KB. Review on production, characterization and applications of microbial levan. *Carbohydr Polym.* 2015; 120:102-114.
  20. de Melo FC, Zaia CT, Celligoi MA. Levan from *Bacillus subtilis* Natto: its effects in normal and in streptozotocin-diabetic rats. *Braz J Microbiol.* 2012; 43:1613-1619.
  21. Nwibo DD, Hamamoto H, Matsumoto Y, Kaito C, Sekimizu K. Current use of silkworm larvae (*Bombyx mori*) as an animal model in pharmaco-medical research. *Drug Discov Ther.* 2015; 9:133-135.
  22. Panthee S, Paudel A, Hamamoto H, Sekimizu K. Advantages of the silkworm as an animal model for developing novel antimicrobial agents. *Front Microbiol.* 2017; 8:373.
  23. Paudel A, Panthee S, Urai M, Hamamoto H, Ohwada T, Sekimizu K. Pharmacokinetic parameters explain the therapeutic activity of antimicrobial agents in a silkworm infection model. *Sci Rep.* 2018; 8:1578.
  24. Matsumoto Y, Ishii M, Shimizu K, Kawamoto S, Sekimizu K. A silkworm infection model to evaluate antifungal drugs for cryptococcosis. *Med Mycol J.* 2017; 58:E131-E137.
  25. Ishii M, Matsumoto Y, Nakamura I, Sekimizu K. Silkworm fungal infection model for identification of virulence genes in pathogenic fungus and screening of novel antifungal drugs. *Drug Discov Ther.* 2017; 11:1-5.
  26. Meng X, Zhu F, Chen K. Silkworm: a promising model organism in life science. *J Insect Sci.* 2017; 17. doi: 10.1093/jisesa/iex064.
  27. Matsumoto Y, Sekimizu K. Silkworm as an experimental animal for research on fungal infections. *Microbiol Immunol.* 2019; 63:41-50.
  28. Ishii M, Matsumoto Y, Sekimizu K. Inhibitory effects of alpha-cyclodextrin and its derivative against sucrose-induced hyperglycemia in an *in vivo* evaluation system. *Drug Discov Ther.* 2018; 12:122-125.

Received January 9, 2020; Accepted February 15, 2020

\*Address correspondence to:

Dr. Kazuhisa Sekimizu, Teikyo University Institute of Medical Mycology, 359 Otsuka, Hachioji, Tokyo, 192-0395, Japan.

E-mail: sekimizu@main.teikyo-u.ac.jp