Bacterial polysaccharides inhibit sucrose-induced hyperglycemia in silkworms

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1. Introduction

Transient and rapid postprandial increases in blood glucose levels, referred to as a blood glucose spike, are a potential risk factor for diabetes. Suppression of the blood glucose spike is expected to be useful for preventing the onset of diabetes. Generally, blood glucose levels and their suppression by drugs are evaluated in mammalian animal models. We previously reported that an increase in blood glucose levels after glucose intake could also be evaluated in silkworms, an alternative model animal (1-3). Not only glucose, but also sucrose intake increases silkworm blood glucose levels (4). Glucose level increases in the silkworm hemolymph after sucrose intake are suppressed by acarbose and voglibose, α-glucosidase inhibitors that are used clinically for human diabetes patients (4). We propose that the silkworm evaluation system is useful for screening substances that suppress sucrose-induced hyperglycemia.

Bacterial polysaccharides have various structures and biologic activities (5-7). We recently reported a bacterial polysaccharide with high innate immunity-stimulating activity in a silkworm evaluation system (8). Bacterial polysaccharides can be obtained in large quantities at low cost. We therefore propose the use of bacterial polysaccharide libraries for screening seeds of medicines and supplements for human health. In this paper, we describe the collection of polysaccharide-producing bacteria and preliminary screening of polysaccharides that suppress sucrose-induced hyperglycemia in a silkworm model system.

2. Materials and Methods

2.1. Collection of polysaccharide-producing bacteria

Bacteria isolated from soil and plants that formed viscous colonies on agar plates were collected. The bacteria grown on the plates (10 cm) were recovered with a spreader and 15 ml of saline, and the cells were removed by centrifugation (8,000 rpm, 5 min). Ethanol (final concentration: 67%) was added to the centrifuge...
supernatant, and fibrous precipitates were collected by centrifugation. Bacterial 16S rRNA was sequenced and homology searches were performed to determine the bacterial species using the EZBiocloud database.

The crude polysaccharide fractions were treated enzymatically as follows. DNase I (1,000 U/mL; Promega) and RNase A (10 μg/mL; NIPPON GENE CO., LTD.) were added to the ethanol precipitate, dissolved in water, and incubated overnight at 37°C, and then further incubated overnight at 37°C with protease K (100 μg/mL). Phenol: chloroform: isoamyl alcohol (50:49:1) was added to the fraction, and the samples were vigorously shaken, followed by the addition of two volumes of ethanol to the upper layer fraction. The precipitates were then collected by centrifugation.

Table 1. Homology search of 16S rRNA sequences of bacteria producing extracellular polysaccharides

| Species                          | Strain   | Identities (%) |
|----------------------------------|----------|----------------|
| Bacillus megaterium              | 129-19   | 99             |
| Cupriavidus sp.                  | No.48    | 97             |
| Curtobacterium plantarum         | 126-8    | 99             |
| Enterobacter kobei               | 126-4b   | 97             |
| Enterobacter tabaci              | 118-13A  | 97             |
| Escherichia coli                 | 118-18   | 98             |
| Ewingella americana              | 221-5-2  | 99             |
| Gluconobacter cerinus            | 221-2-2  | 99             |
| Kosakonia sp.                    | 118-14   | 98             |
| Lelliottia ammonigena            | 112-13-1 | 99             |
| Novosphingobium panipatense      | 208-110  | 98             |
| Paenarthrobacter nicotinovorans  | 208-57   | 99             |
| Paenibacillus l tepi             | 110-24   | 99             |
| Paenibacillus polymyxa           | 126-6-1A | 99             |
| Pantoea eucalypti                | 118-5-1  | 98             |
| Pantoea sp.                      | 126-2    | 99             |
| Pantoea vagans                   | 126-1    | 99             |
| Paraburkholderiainsula           | 118-3-2  | 99             |
| Paracoccus aestuariivisens       | 126-5b   | 98             |
| Pseudomonas nitroreductens       | No.24    | 98             |
| Pseudomonas pallorionana         | 118-25A  | 99             |
| Rhizobium alitiplani             | No.26    | 99             |
| Variorovax boroniculamans        | 110-14   | 99             |
| Xanthomonas cynarae              | No.4     | 100            |

Bacterial species of 24 strains isolated in this study are listed. Bacterial species exhibiting the highest sequence homology are presented. When the species of the highest homologous bacterial strain could not be specified, only the names of the genus are shown.

2.2. Sucrose tolerance test of silkworms

Silkworms (Hu Yo x Tsukuba Ne, Ehime Sericulture Incorporated Company, Ehime, Japan) were reared as described previously (9,10). The silkworm sucrose tolerance test was conducted according to the previously reported method (4). Briefly, sucrose (10%) and test samples were mixed with silkworm artificial diet. Sucrose diet with or without polysaccharide samples was fed to 5th-instar larva of silkworms for 1 h, the silkworm hemolymph was collected, and glucose concentrations were measured with a glucometer (Accu-Chek, Roche).

3. Results

We collected bacteria that formed viscous colonies on YME agar plates. The isolated bacteria comprised 19 genera (Table 1). Ethanol precipitates of crude polysaccharides (see Materials and Methods) were mixed with silkworm diet containing 10% sucrose and fed to the silkworms. After 1 h, the blood glucose levels of silkworms were measured. Among the 24 polysaccharide fractions tested, 6 samples from Rhizobium alitiplani, Cupriavidus sp., Paenibacillus polymyxa, Pantoea eucalypti, Variorovax boroniculamans, and Xanthomonas cynarae exhibited suppressive effects on the increase in the blood sugar level of silkworms (Table 2). The differences in the glucose level between controls without bacterial samples and those with bacterial polysaccharides were statistically significant.

The crude polysaccharide fraction from R. alitiplani was treated with DNase I, RNase A, and proteinase K, and further extracted with phenol. The treatment had little effect on the sugar content, whereas the amounts of DNA and protein were greatly reduced to 1/20 and 1/28, respectively (Table 3). This phenol-extracted fraction also exhibited suppressive activity against sucrose-induced hyperglycemia (Figure 1).

4. Discussion

The findings of the present study demonstrated that bacterial polysaccharides from R. alitiplani...
polysaccharides that inhibit increases in blood glucose levels in humans. Bacteria secreting polysaccharides can be easily obtained as viscous colonies on agar plates. Polysaccharides secreted from bacteria have various structures depending on the bacterial species (6, 7). Furthermore, industrial mass production of bacterial polysaccharides is possible. Based on these properties, it is expected that the library of bacterial polysaccharides will be useful for screening compounds with physiologic activities, such as agents with blood sugar lowering effects.

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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. Rehm BH. Bacterial polymers: Biosynthesis, modifications and applications. Nat Rev Microbiol. 2010; 8:578-592.
6. Nwodo UU, Green E, Okoh AI. Bacterial exopolysaccharides: Functionality and prospects. Int J Mol Sci. 2012; 13:14002-14015.
7. Freitas F, Alves VD, Reis MA. Advances in bacterial exopolysaccharides: From production to biotechnological applications. Trends Biotechnol. 2011; 29:388-398.
8. Urai M, Aizawa T, Imamura K, Hamamoto H, Sekimizu K. Characterization of the chemical structure and innate immune-stimulating activity of an extracellular polysaccharide from Rhizobium sp. strain M2 screened using a silkworm muscle contraction assay. Drug Discov Ther. 2017; 11:238-245.
9. 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.
10. 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.

11. 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.

12. 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.

13. 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.

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