

1 **Table S1.** Composition of mouse diet (Diet 86)

Ingredient	Concentration (% w/w)
Wheat	40.00
Broll	21.50
Barley	20.00
Meat & bone meal	6.00
Fish meal	5.00
Mollases	2.50
Prelac	2.00
Dried blood meal	1.70
Sodium chloride	0.50
Tallow	0.45
Limestone	0.2
Premix	0.15

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### 3 **Supplementary Materials and Methods**

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#### 5 *Chemical analysis of exopolysaccharide*

6 The total carbohydrate and fructose contents of the exopolysaccharide were  
7 determined colorimetrically by the phenol-sulphuric acid method (Dubois *et al.*,  
8 1956) and the ketose-specific modification of the anthrone method (Pollock and  
9 Jones, 1979), respectively, using fructose as a standard.

10 For fructanase treatment, exopolysaccharide (70 mg) was dissolved in 0.1 M  
11 sodium acetate (pH4.5) at 2 mg per ml and incubated with fructanase (~3000 units;  
12 Megazyme) at 40°C for 20 h. The reaction was stopped by boiling for 5 minutes, the  
13 sample centrifuged (3,000 x *g*, 15 minutes, room temperature), and the soluble  
14 material was dialysed against distilled water (molecular weight cut-off 12,000), then  
15 lyophilised.

16 For molecular weight analysis, exopolysaccharide and fructanase-treated  
17 exopolysaccharide was separated by size-exclusion HPLC (SEC) on three columns  
18 (TSK-Gel G5000PWXL, G4000PWXL and G3000PWXL, 300 x 7.8 mm, Tosoh Corp.,  
19 Tokyo, Japan) connected in series. Samples (500µg) dissolved in distilled water (10  
20 mg per ml) were eluted with water (0.5 ml per minute) and detected with a Waters  
21 490E UV detector set at 280 nm, a DAWN-EOS multi-angle laser light scattering  
22 (MALLS) detector with a laser at 690 nm (Wyatt Technology Corp., Santa Barbara,  
23 CA, USA) and a Waters 2410 refractive index monitor. The molecular weight of the  
24 eluted material was estimated by comparison of peak elution positions with

25 pullulan standards (5.8 – 850 kDa) and by MALLS using ASTRA software (Version  
26 4.73.04, Wyatt Technology Corp.) using a  $dn/dc$  of 0.143 ml per gram .

27 Exopolysaccharide (100  $\mu$ g) was separated by high performance anion-  
28 exchange chromatography (HPAEC) on a CarboPac PA-100 (4 x 250 mm) column  
29 using Dionex ICS 3000 (Dionex Corp., Sunnyvale, CA, USA) with 100 mM sodium  
30 hydroxide as eluant using a gradient of sodium acetate (Cairns *et al.*, 1999).

31 To determine the linkages between the glycosyl residues, exopolysaccharide  
32 (~200  $\mu$ g) was derivatised as described by Bonnett *et al.* (1994). Samples dissolved  
33 in dimethylsulfoxide were methylated using NaOH and CH<sub>3</sub>I. After extraction into  
34 chloroform, the methylated polysaccharides were hydrolysed with TFA, reduced  
35 and acetylated. The resulting partially methylated alditol acetates were separated  
36 by GC (HP5-MS, 30 m x 0.25 mm i.d., 0.25  $\mu$ m film thickness, Agilent) with the GC  
37 oven programmed from 50°C (1 min) to 130°C at a rate of 25°C per minute, and then  
38 to 230°C at a rate of 3°C per minute and analysed by MS using a Hewlett Packard  
39 5973 MSD. Identifications were based on peak retention times and by comparison  
40 of electron impact mass spectra with published spectra.

41 For NMR spectroscopy, <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Bruker  
42 500 MHz spectrometer at 30°C. Samples were dissolved in deuterium oxide (99.6  
43 atom%), and transferred to 5 mm o.d. NMR tubes. Assignments were made from  
44 double quantum filtered (DQF) COSY, heteronuclear single quantum coherence  
45 (HSQC) COSY and HSQC TOCSY experiments and by comparing the spectra with data  
46 in public databases.

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48 *Analysis of mouse stomach contents*

49 Sucrose concentrations in stomach contents were measured as follows. Four  
50 batches of pooled stomach contents from *Lactobacillus*-free mice (13 mice) and four  
51 batches of pooled stomach contents from mice colonised with strain 100-23  
52 (13mice) were homogenised in sterile water, centrifuged at low speed (106 x *g*) to  
53 remove particulate material, then lyophilized. Soluble sugars were extracted from  
54 the lyophilized material after extraction in methanol (62.5 % v/v) at 55°C for 1 h  
55 with vortexing. Extracts were centrifuged to remove particulates, and the  
56 supernatants were analysed by HPLC (Dionex Ultimate 3000). Aliquots (20 µl) were  
57 separated by HPLC on a Prevail carbohydrate ES 5µ column (250 x 4.6 mm; Grade  
58 Davidson Discovery Science), in a solvent mix of 25% water and 75% acetonitrile at  
59 a flow rate of 1 ml per min. The column temperature was kept at 30°C. Peaks were  
60 detected using an ELSD (Polymer Laboratories, Alphatech Systems Ltd, gas flow 1.0,  
61 nebulising temperature 40°C, evaporating temperature 90°C), and identified by  
62 their retention times against standards (fructose (BDH) 7.8 min, glucose (BDH) 10.4  
63 min, sucrose (Merck) 14.7 min, maltose (BDH) 18.0 min). The area under the peaks  
64 was integrated using Millennium (software version 3.2, Waters Corporation) and  
65 quantified against standard curves produced during the analysis.

66 To detect exopolysaccharide in stomach contents, the pooled stomach contents  
67 (prepared as above) of 19 mice colonised with strain 100-23 and pooled stomach  
68 contents from 20 *Lactobacillus*-free mice were dissolved in water and dialysed  
69 exhaustively against distilled water (molecular weight cut-off 1000 Da) and  
70 lyophilised to yield 16 mg and 7 mg dried material from colonised and control mice,

71 respectively. These were dissolved at 1 mg per ml and analysed by HPAEC as  
72 described above.

### 73 **References**

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93 **Figure S1. A**, molecular weight analysis of *L. reuteri* exopolysaccharide by size-  
94 exclusion chromatography on three columns (TSK-Gel 5000<sub>PWXL</sub>, 4000<sub>PWXL</sub>,  
95 3000<sub>PWXL</sub>) connected in series. Arrows indicate the elution volumes of pullulan  
96 molecular weight standards. **B-D**, high-performance anion-exchange  
97 chromatography of chicory inulin (B), *L. reuteri* exopolysaccharide (C), and stomach  
98 contents of mice colonised with *L. reuteri* 100-23 (D). The oligosaccharides were  
99 separated on a CarboPac PA-100 column, eluted with a gradient of NaOAc and  
100 monitored by pulsed amperometric detection. **E and F**, proton (E) and <sup>13</sup>C (F) NMR  
101 of *L. reuteri* exopolysaccharide in D<sub>2</sub>O at 500 MHz.  
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