Supplemental Material

The biofilms that formed in TSBs, TSBg, and TSBr media were stained with the Live/Dead BacLight® Bacterial Viability Kit and were examined by CLSM (Fig. S1). A biofilm formation assay in BHI or Todd Hewitt Broth (THB) supplemented with 0.25% (w/v) sucrose, glucose, or raffinose (Fig. S2). Comparison of the band patterns produced by the RAPD method products between genomic DNA and eDNA (Fig. S3). The effect of commercial inulin on the biofilm formation in TSBr (Fig. S4). Safranin staining of the biofilm formed on the sHA surface (Fig. S5). Effect of trisaccharides other than raffinose on biofilm formation of *S. mutans* (Fig. S6).

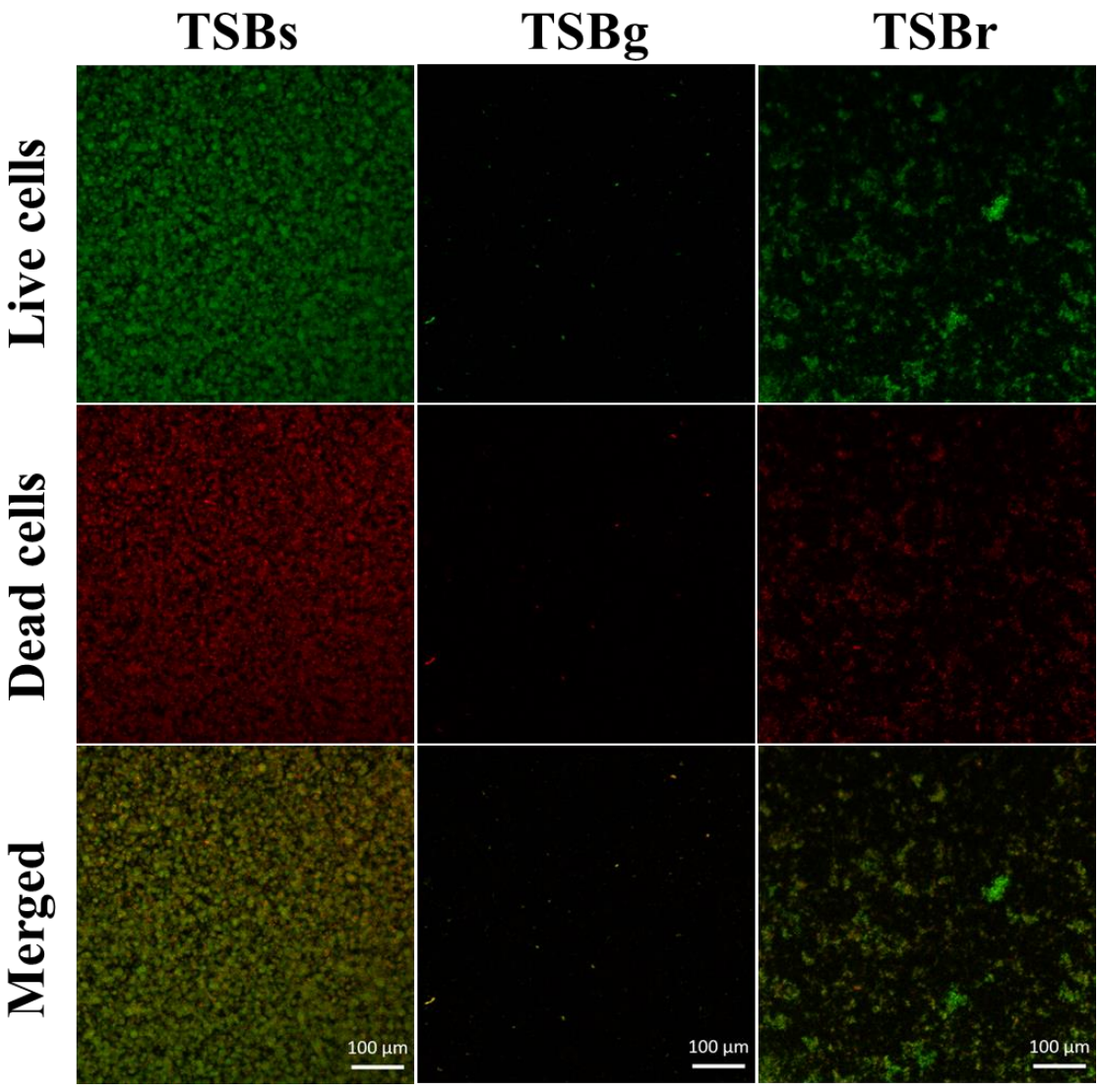

Fig. S1 CLSM analysis of the biofilm formed in TSB. *S. mutans* UA159 was cultivated in TSB with sucrose (TSBs), glucose (TSBg), or raffinose (TSBr) in a 96-well microtiter plate. The biofilms formed on the bottom of the wells were stained with the Live/Dead BacLight® Bacterial Viability Kit and were examined by CLSM. A representative image from three independent experiments is presented.

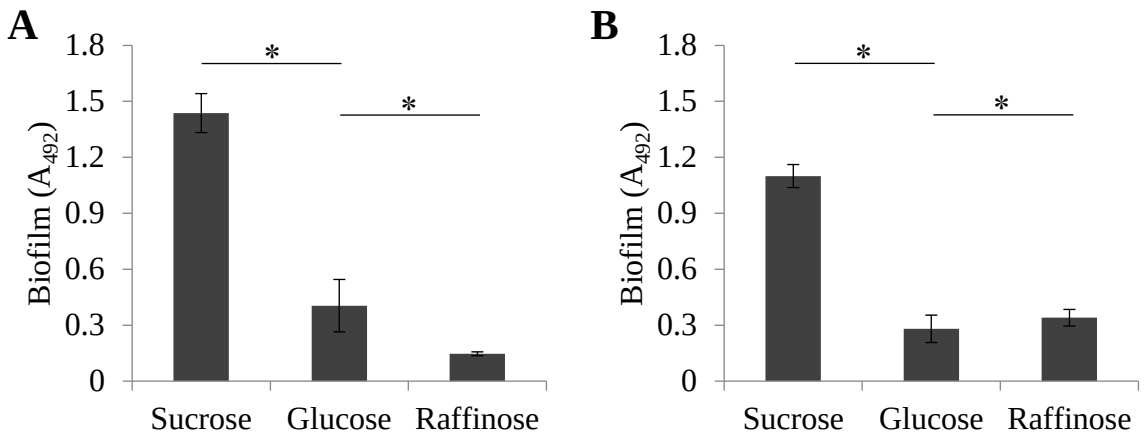

Fig. S2 Biofilm formation in BHI or THB supplemented with 0.25% (w/v) sucrose, glucose, or raffinose. A biofilm formation assay was performed by the same procedure as described in the Materials and Methods section. A) A biofilm formation assay in BHI supplemented with each sugar. B) A biofilm formation assay using Todd Hewitt Broth (THB, Difco Laboratories, Detroit, MI) supplemented with each sugar. These data are expressed as mean ± SD of three independent experiments. The asterisks indicate a significant difference between two strains (Student's *t*-test; $p < 0.05$).

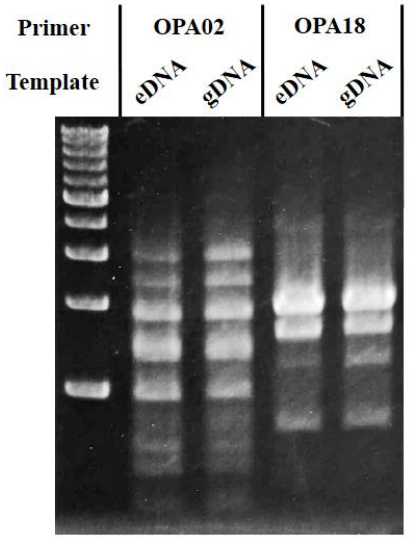

Fig. S3 Separation of the products of the random amplified polymorphic DNA (RAPD) method by agarose gel electrophoresis. RAPD was carried out using purified eDNA and gDNA as the PCR templates. We used two random primers OPA02 and OPA18, .

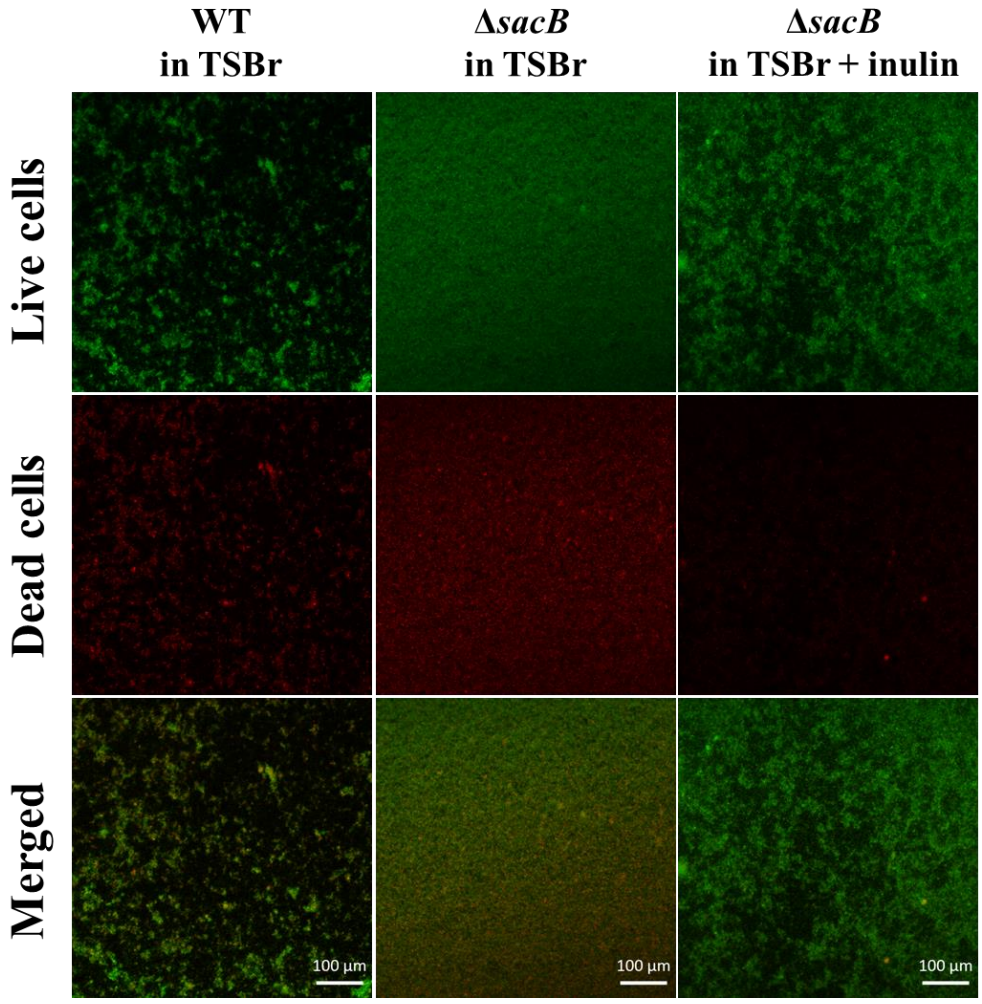

Fig. S4 Complementation of the morphology by the addition of commercial inulin. To clear whether the defective morphology was restored by exogenous fructan, Δ*sacB* was cultivated in TSBr supplemented with 200 µg/ml of commercial inulin. The biofilms formed on the bottom of the 96-well microtiter plate were stained with the Live/Dead BacLight® Bacterial Viability Kit and were examined by CLSM. A representative image from three independent experiments is presented.

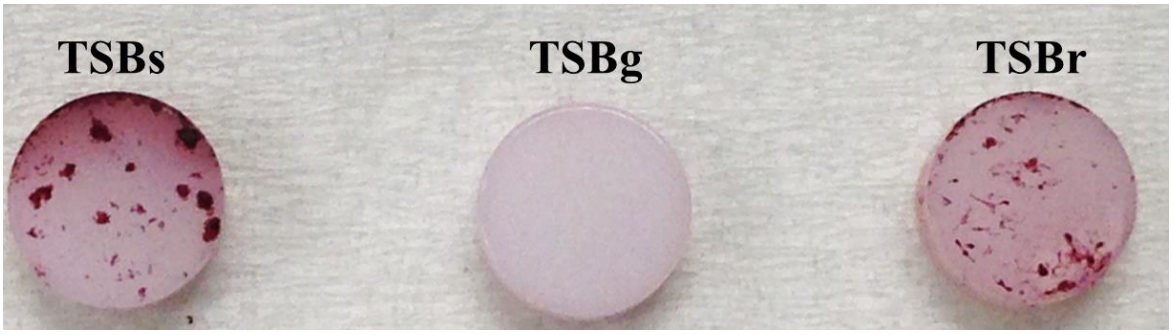

Fig. S5 Biofilm formation on the sHA surface. Biofilm formation was performed by the same procedure as described in the Materials and Methods section. The biofilm was stained with a safranin solution for 15 min. After that, these disks were washed two times with DW. A representative result of three independent experiments is presented. The disk's diameter is 5 mm.

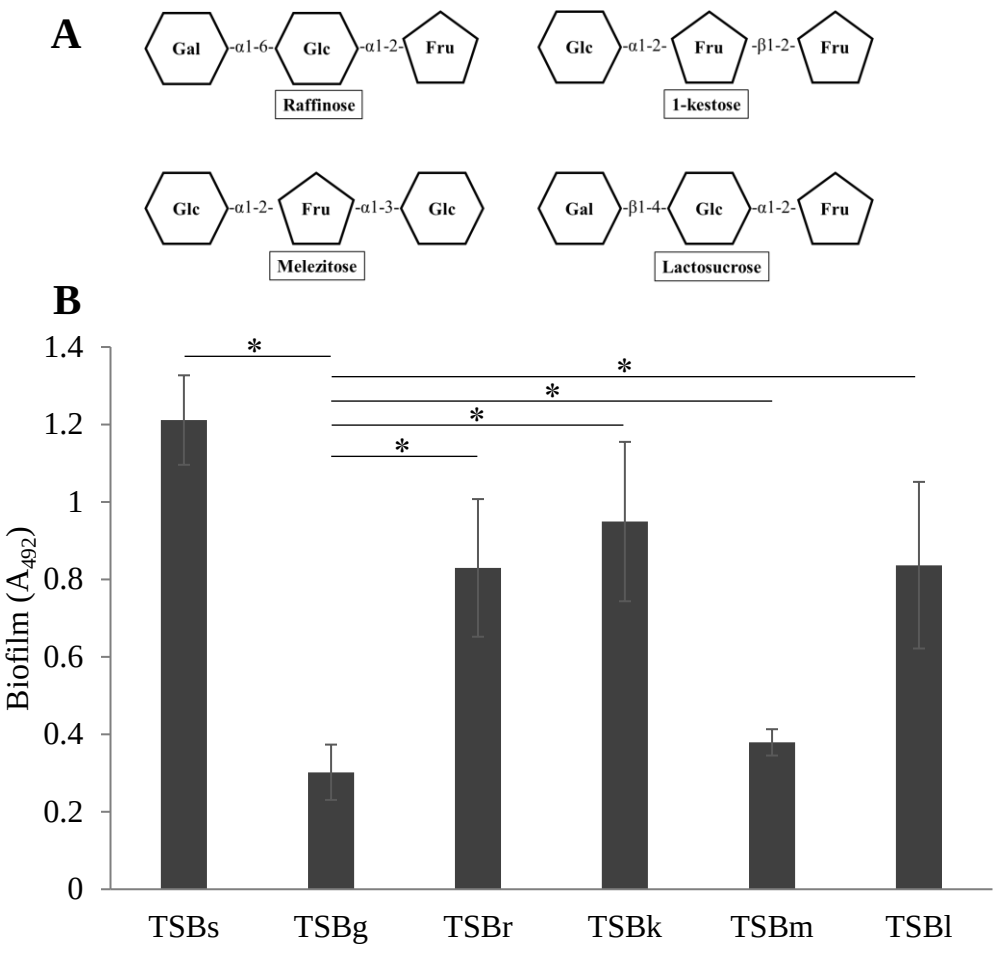

Fig. S6 Effect of trisaccharides other than raffinose on biofilm formation of *S. mutans*. A) The structure of trisaccharides used in this study. B) A biofilm formation assay in TSB supplemented with 0.25% (w/v) of sucrose (TSBs), glucose (TSBg), raffinose (TSBr), 1-kestose (TSBk), melezitose (TSBm), or lactosucrose (TSBl). *S. mutans* could not grow in TSBm. However, biofilm was formed in TSBk and TSBl, and the biofilm levels were comparable to TSBr. TSBs and TSBg is a positive control and negative control for biofilm formation, respectively. These data are presented as mean ± SD of three independent

65 experiments. The asterisks indicate a significant difference between two strains (Student's *t*-

66 test; $p < 0.05$).