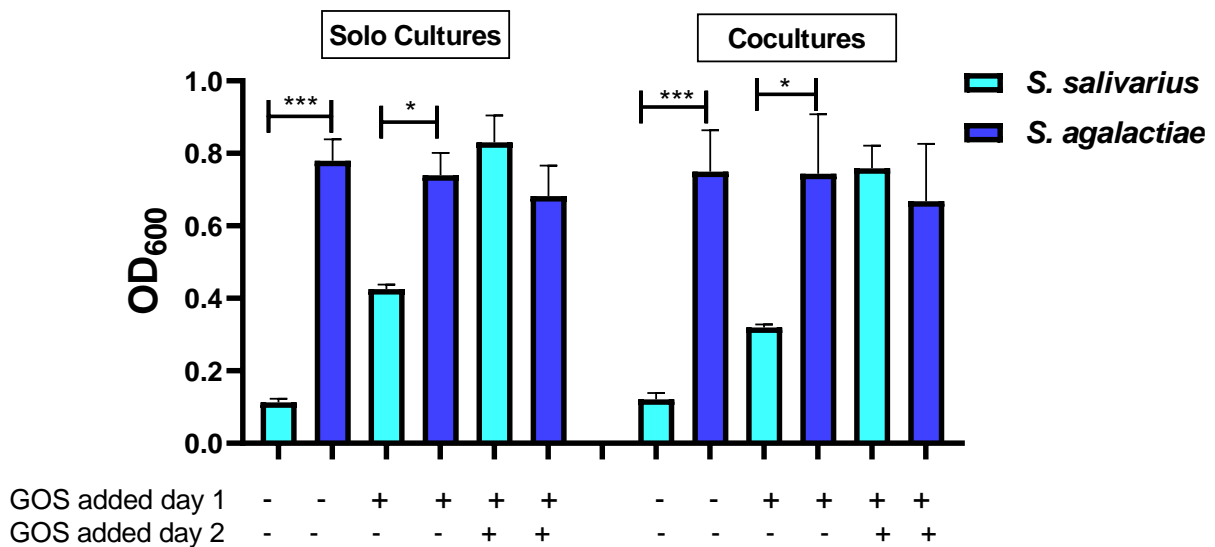
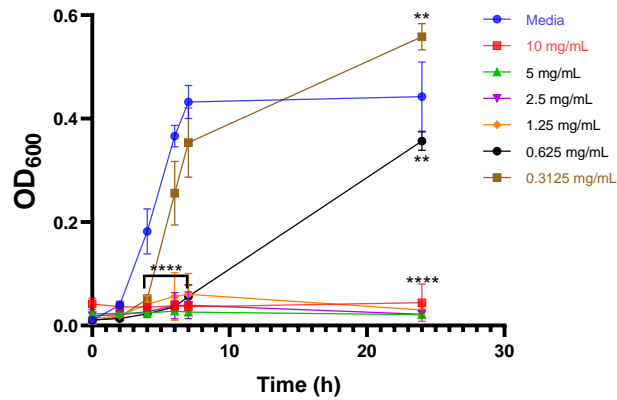


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|----|--|----|
| 1. | Overnight cultures treated with cell-free supernatants                       | S1 |
| 2. | Effects of the HMO cocktail at increasing concentrations on bacterial growth | S2 |
| 3. | Recorded pH of data collected in Figure 3 under ambient air                  | S2 |
| 4. | Recorded pH of data collected in Figure S4 under CO <sub>2</sub> conditions  | S2 |
| 5. | Lactic acid production standard curve  | S3 |
| 6. | Coculture assay under CO <sub>2</sub> conditions                             | S3 |

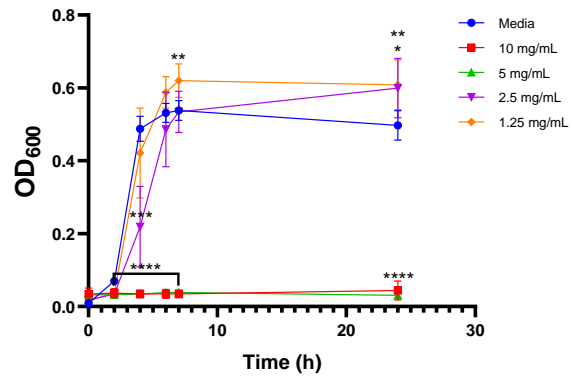


**Figure S1.** Overnight cultures treated with cell-free supernatants. Suppression of *S. salivarius* by *S. agalactiae* is still observed when the supernatants from overnight cultures are cocultured with whole cells. Cultures with GOS supplementation were added at ca. 5 mg/ml. Growth was quantified via OD<sub>600</sub> readings at 24 h. Data displayed represent the relative mean growth ratios  $\pm$  SEM of three independent experiments, each with three technical replicates. \*\*\* represents  $p = 0.0002$  or  $p = 0.0004$  and \* represents  $p = 0.0127$  by one-way ANOVA with post hoc Dunnett's multiple comparison test comparing the mean growth of each condition with the mean of every other condition.

### A. *S. salivarius*



### B. *S. agalactiae*



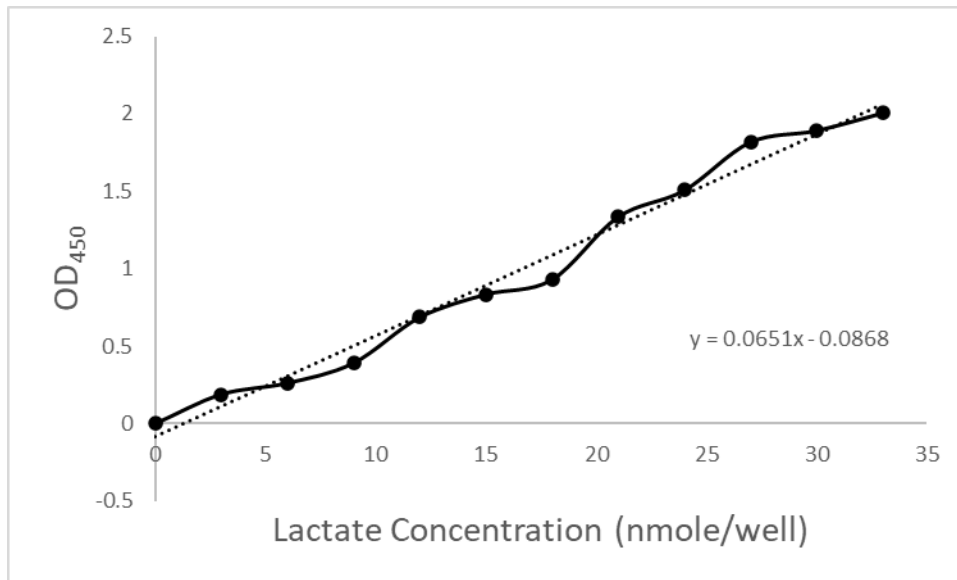
**Figure S2.** Effects of the HMO cocktail at increasing concentrations on (A) *S. salivarius* (ATCC 19258) and (B) *S. agalactiae* (GB00002). Growth was quantified via OD<sub>600</sub> readings at 0, 2, 4, 6, 7, and 24 h. Mean OD<sub>600</sub> for each time point is indicated by the corresponding symbols. Data displayed represent the relative mean growth ratios  $\pm$  SEM of three independent experiments, each with three technical replicates. In (A) \*\*\*\* represents  $p < 0.0001$ , and \*\* represents  $p = 0.033$  and  $p = 0.0031$  by two-way ANOVA with *post hoc* Dunnett's multiple comparison test comparing the growth of *S. salivarius* in each HMO supplementation concentration to the growth of *S. salivarius* in medium alone. In (B) \*\*\*\* represents  $p < 0.0001$ , \*\*\* represents  $p = 0.0007$ , \*\* represents  $p = 0.0032$  and  $p = 0.0016$ , and \* represents  $p = 0.0179$  by two-way ANOVA with *post hoc* Dunnett's multiple comparison test comparing the growth of *S. agalactiae* in each HMO supplementation condition to the growth of *S. agalactiae* in medium alone.

**Table S1.** Recorded pH of data collected in Figure 3 under ambient air.

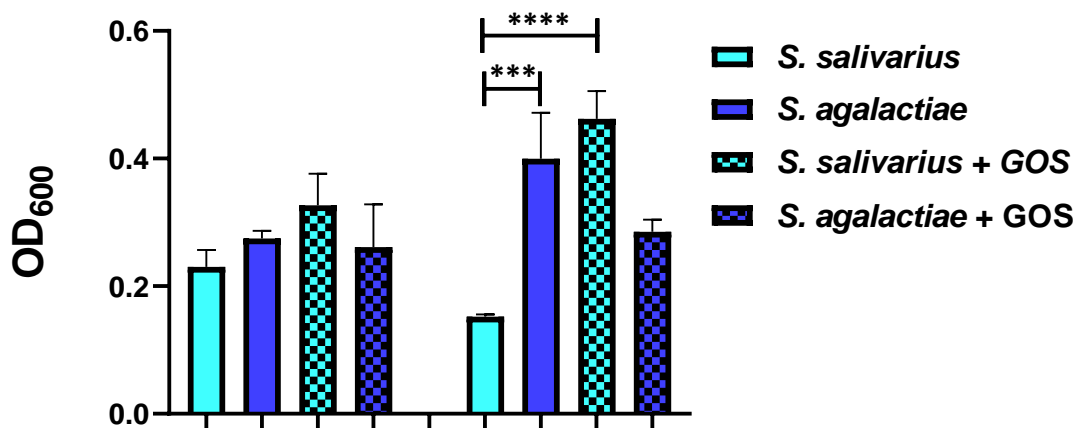
Condition	Time (h)					
	0	2	4	6	8	24
<i>S. agalactiae</i>	7	7	6	6	6	5
<i>S. salivarius</i>	7	7	7	7	7	6
<i>S. agalactiae</i> + GOS	7	7	6	6	6	6
<i>S. salivarius</i> + GOS	7	7	7	6	6	6
Coculture - GOS	7	7	6	6	6	5
Coculture + GOS	7	7	7	6	6	6

**Table S2.** Recorded pH of data collected in Figure S4 under CO<sub>2</sub> conditions.

Condition	Time (h)					
	0	2	4	6	8	24
<i>S. agalactiae</i>	7	7	7	6	6	5
<i>S. salivarius</i>	7	7	7	7	7	6
<i>S. agalactiae</i> + GOS	7	7	7	6	6	6
<i>S. salivarius</i> + GOS	7	7	7	7	7	6
Coculture - GOS	7	7	7	6	6	5
Coculture + GOS	7	7	7	6	6	6



**Figure S3.** The amount of lactic acid present in each sample is calculated from the lactic acid production standard curve created fresh during each new assay run. The concentration (C) calculated using the equation  $S_a/S_v = C$  in which  $S_a = x$  (calculated from standard curve) and  $S_v =$  sample volume added to each well. The concentration is converted to  $\text{ng}/\mu\text{L}$  using the lactic acid molar mass ( $89.07 \text{ ng}/\text{nmole}$ ).



**Figure S4.** *S. agalactiae* suppresses growth of *S. salivarius* in coculture; GOS supplementation reverses this suppression. This assay was performed in a  $\text{CO}_2$  incubator. The first four bars represent controls in which *S. salivarius* (ATCC 19258) and *S. agalactiae* (GB00002) were grown separately, either with or without GOS supplementation at ca.  $5 \text{ mg}/\text{ml}$ . The last four bars represent the two strains grown in coculture either with or without GOS supplementation at ca.  $5 \text{ mg}/\text{ml}$ . Growth was quantified via  $\text{OD}_{600}$  readings at 24 h. Data displayed represent the relative mean growth ratios  $\pm$  SEM of three independent experiments, each with three technical replicates. \*\*\*\* represents  $p < 0.0001$  by one-way ANOVA with *post hoc* Dunnett's multiple comparison test comparing the mean growth of each condition with the mean of every other condition.