Table S1. Final pH and aerobic plate counts (APC) of LB colonies on MRS after 20-h B-gal reaction in NDM with added sugars (mean ± SD, n=3).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Strain |  | β-gal Prep |  | Milk |  | pH |  | Final LB APC (log CFU/mL) |
| B548 |  | Lysate |  | NDM\* |  | 5.2 ± 0.3bc |  | n/a# |
|  |  |  |  | Sucrose |  | 5.1 ± 0.1bcd |  | n/a |
|  |  |  |  | CS\*\* |  | 5.4 ± 0.4bc |  | n/a |
|  |  |  |  | Sucrose + CS |  | 5.3 ± 0.2bc |  | n/a |
|  |  | Whole Cells |  | NDM |  | 4.2 ± 0.2f |  | ND## |
|  |  |  |  | Sucrose |  | 4.4 ± 0.1f |  | ND |
|  |  |  |  | CS |  | 4.4 ± 0.4ef |  | ND |
|  |  |  |  | Sucrose + CS |  | 4.5 ± 0.2def |  | 3.4 ± 0.5 |
| LB11 |  | Lysate |  | NDM |  | 5.0 ± 0.1cde |  | n/a |
|  |  |  |  | Sucrose |  | 5.2 ± 0.1bc |  | n/a |
|  |  |  |  | CS |  | 5.1 ± 0.1bc |  | n/a |
|  |  |  |  | Sucrose + CS |  | 5.3 ± 0.1bc |  | n/a |
|  |  | Whole Cells |  | NDM |  | 4.0 ± 0.0f |  | 5.7 ± 0.2 |
|  |  |  |  | Sucrose |  | 4.2 ± 0.1f |  | 7.0 ± 0.1 |
|  |  |  |  | CS |  | 4.4 ± 0.1ef |  | 6.3 ± 0.2 |
|  |  |  |  | Sucrose + CS |  | 4.3 ± 0.1f |  | 6.6 ± 0.1 |
| YB1 |  | Lysate |  | NDM |  | 5.2 ± 0.2bc |  | n/a |
|  |  |  |  | Sucrose |  | 5.2 ± 0.1bc |  | n/a |
|  |  |  |  | CS |  | 5.2 ± 0.0bc |  | n/a |
|  |  |  |  | Sucrose + CS |  | 5.4 ± 0.4bc |  | n/a |
|  |  | Whole Cells |  | NDM |  | 4.3 ± 0.0f |  | 5.3 ± 0.1 |
|  |  |  |  | Sucrose |  | 4.4 ± 0.2ef |  | 5.8 ± 0.8 |
|  |  |  |  | CS |  | 4.2 ± 0.1f |  | 6.2 ± 0.1 |
|  |  |  |  | Sucrose + CS |  | 4.3 ± 0.0f |  | 6.5 ± 0.3 |
| K. lactis |  | Enzyme Prep |  | NDM |  | 5.4 ± 0.3bc |  | n/a |
|  |  |  |  | Sucrose |  | 5.6 ± 0.1b |  | n/a |
|  |  |  |  | CS |  | 5.6 ± 0.0b |  | n/a |
|  |  |  |  | Sucrose + CS |  | 5.5 ± 0.0bc |  | n/a |
| Control |  | No β-gal |  | NDM |  | 6.7 ± 0.1a |  | n/a |
|  |  |  |  | Sucrose |  | 6.3 ± 0.2a |  | n/a |
|  |  |  |  | CS |  | 6.5 ± 0.1a |  | n/a |
|  |  |  |  | Sucrose + CS |  | 6.4 ± 0.3a |  | n/a |

\*NDM, nonfat dry milk

\*\*CS, corn syrup

#n/a, samples were not enumerated for APC

##ND, no colonies were detected above 2 log CFU/mL

a-fDifferent letters indicate significantly different pH values (p<0.050, 2-way ANOVA)

Table S2. Free glucose and fructose (mean ± SD, n = 3) released by 2 different bottles of Lactozyme in 799 mM sucrose in 50 mM sodium phosphate (pH 6.5) after 20 h at 50 °C.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Glucose (mM) |  | Fructose (mM) |
| Bottle 1 | 15.8 ± 0.8 |  | 14.5 ± 0.4 |
| Bottle 2 | 16.6 ± 1.2 |  | 14.4 ± 1.3 |

Table S3. The glucose (mean ± SD) released by the enzyme preps (50 °C, 20 h) in autoclaved 14.4% sucrose in reconstituted nonfat dried milk (NDM) or autoclaved NDM.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Medium |  | Enzyme Preparation |  | Glucose (mM) |
| NDM |  | KL B-gal |  | 97.3 ± 3.7 |
|  |  | B548 Lysate |  | 114.3 ± 11.3 |
|  |  | LB11 Lysate |  | 86.6 ± 21.3 |
|  |  | YB1 Lysate |  | 107.4 ± 3.7 |
|  |  | Negative Control |  | 1.0 ± 0.0 |
| 14.4% Sucrose in NDM |  | KL B-gal |  | 163.5 ± 8.5 |
|  |  | B548 Lysate |  | 103.1 ± 0.4 |
|  |  | LB11 Lysate |  | 104.8 ± 3.4 |
|  |  | YB1 Lysate |  | 110.8 ± 8.9 |
|  |  | Negative Control |  | 6.7 ± 0.0 |

Table S4. Free glucose (n=1) in the starting mMRS used to determine if the oligosaccharides resulting from B-gal reactions have the potential to support growth of BB 2141 and LR 1428.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Milk |  | Glucose (mM) |  | Protein (µg/mL) |
| Initial | NDM |  | 7.4 |  | 186.8 |
|  | Sucrose |  | 5.9 |  | 174.0 |
|  | Corn Syrup |  | 11.9 |  | 141.0 |
|  | Sucrose + CS |  | 12.2 |  | 133.9 |
| K. lactis | NDM |  | 6.2 |  | 217.3 |
|  | Sucrose |  | 4.5 |  | 201.6 |
|  | Corn Syrup |  | 12.0 |  | 168.7 |
|  | Sucrose + CS |  | 10.7 |  | 165.3 |
| YB1 | NDM |  | 6.1 |  | 259.4 |
|  | Sucrose |  | 5.8 |  | 171.0 |
|  | Corn Syrup |  | 13.6 |  | 148.3 |
|  | Sucrose + CS |  | 11.4 |  | 168.0 |



Figure S1. HPAEC-PAD of carbohydrates diluted 100-fold resulting from B-gal from L. bulgaricus strains (A) or from KL B-gal prep at 50 °C for 20 h. Numbers and letters indicate external standards (1a, galactose; 1b, glucose; 1c, fructose; R, raffinose; 2, lactose; V, Verbascose; 3, maltose; 4, malto-triose; 5, malto-tetraose; 6, malto-pentaose; 7, malto-hexaose).