

SUPPLEMENTARY MATERIAL

Isomelezitose overproduction by alginate-entrapped recombinant *E. coli* cells and in-vitro evaluation of its potential prebiotic effect

Martin Garcia-Gonzalez¹, Fadia V. Cervantes², Ricardo P. Ipiates³, Angeles de la Rubia³, Francisco J. Plou², and Maria Fernandez-Lobato^{1*}

¹Departamento de Biología Molecular, Instituto de Biología Molecular, Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Universidad Autónoma de Madrid. C/ Nicolás Cabrera, 1, 28049 Madrid, Spain

²Instituto de Catálisis y Petroleoquímica, CSIC, 28049 Madrid, Spain

³Departamento de Ingeniería Química, Universidad Autónoma de Madrid (UAM). Campus de Cantoblanco 28049 Madrid, Spain

* Corresponding author: Maria Fernandez-Lobato (mfernandez@cbm.csic.es)

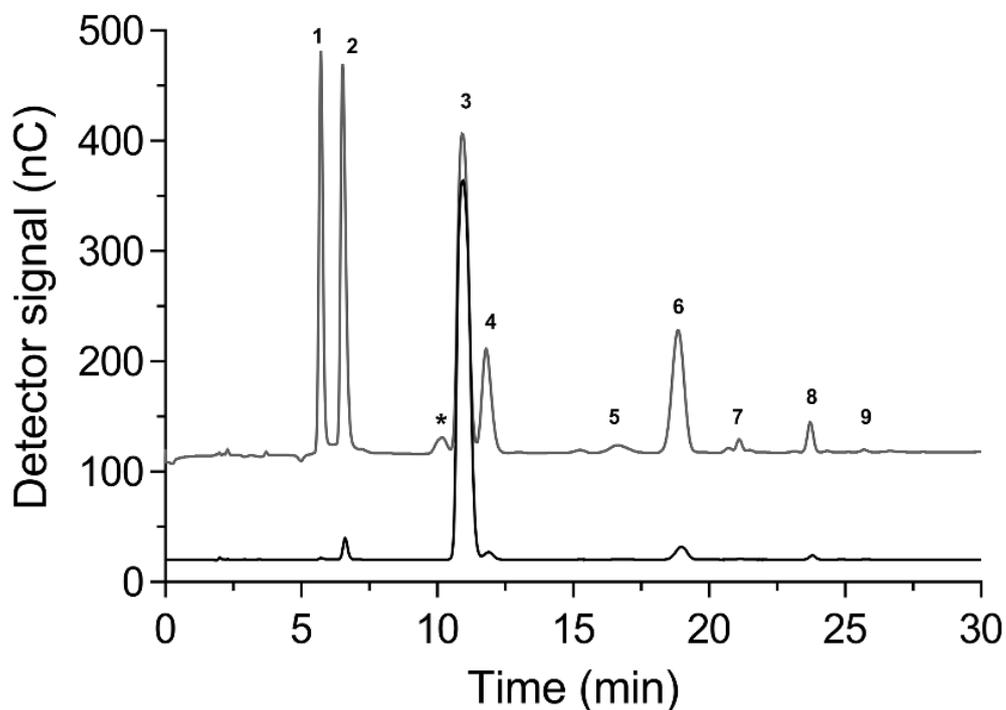


Figure S1. Analysis of hetero-GlcOS production catalysed by alginate-entrapped *E. coli* [MrαGlu-pET28b(+)] cells. HPAEC-PAD chromatograms of the reactions performed with not induced immobilized *E. coli* cells (black line) and with 1 mM IPTG induced cells (grey lines) are shown. Alginate-based biocatalyst with cells that were not induced with IPTG practically did not hydrolysed sucrose and so, the peaks corresponding to the transglucosylation products were negligible. Peak assignment: (1) D-glucose; (2) D-fructose; (3) sucrose; (4) trehalulose; (5) melezitose; (6) isomelezitose; (7) theanderose; (8) erlose; (9) esculosa; (*) unknown product.

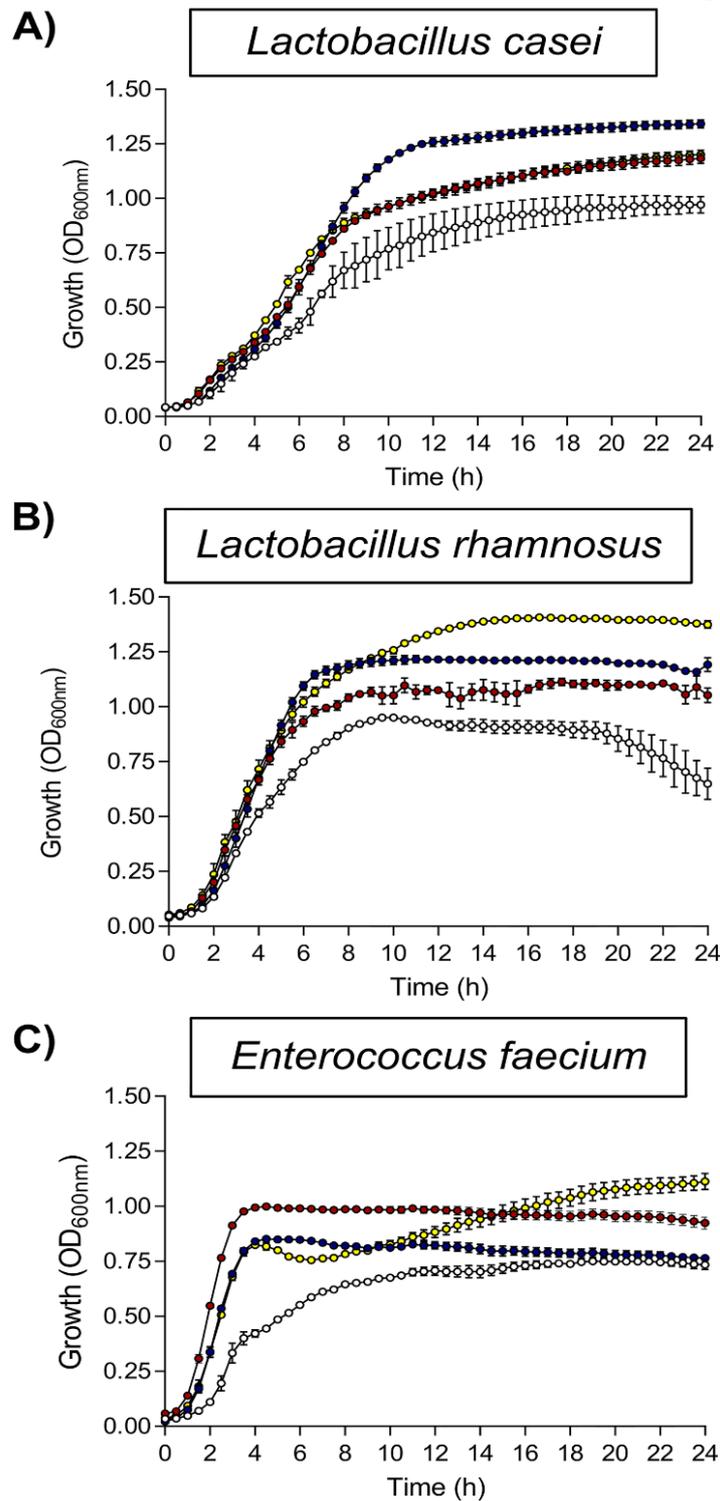


Figure S2. Growth curves of the indicated probiotic bacteria. Bacteria were cultured at 37 °C and under aerobic conditions in MRS medium with no carbon source (white circles), or supplemented with glucose, fructose and sucrose at the same concentration in the hetero-GlcOS mixture (yellow circles), 2.0% (w/v) “Actilight” FOS mixture (blue circles) or 2.0% (w/v) hetero-GlcOS mixture (green circles). Error bars represent the standard deviations from three independent analysis.

Table S1. Sugar composition of the reaction mixture treated with alginate-entrapped *Komagataella phaffii* cells.

Carbohydrate (% w/w)	Glucose	Fructose	Sucrose	Trehalulose	Isomelezitose	Rest of hetero- GlcOS
Before	18	25.5	13	19	15	9.5
After	1.2	0.6	22	33	26	17.2

Reaction conditions: 30 h incubation with orbital shaking (200-250 rpm) at 30 °C.

Table S2. Composition of the commercial fructo-oligosaccharides mixture “Actilight” determined by HPAEC-PAD.

Carbohydrate	Concentration (% w/w)
Glucose	0.4
Fructose	0.7
Sucrose	11.1
1-Kestose	33.0
Neokestose	2.3
Nystose	35.6
Neonystose	4.2
1-Fructosylnystose	7.3
1-Fructosylfructosylnystose	3.3
Other FOS	2.2

The HPAEC-PAD method is described in Section 3.10 of *Materials and Methods*.