Prebiotic effect of fructans from *Agave salmiana* in probiotic lactic acid bacteria and in children as supplement for malnutrition

Supplementary Information

Martinez-Gamiño Daniel[1], Garcia-Soto Mariano J.[2], Gonzalez-Acevedo Olivia[3], Godinez-Hernandez Cesar[4], Juarez-Flores Bertha[4], Ortiz-Basurto Rosa Isela[5], Rodriguez-Aguilar Maribel[6], Flores-Ramirez Rogelio[6], Martinez-Martinez Marco[2], Ratering Stefan[7], Schnell Sylvia[7], Bach Horacio[8], Martinez-Gutierrez Fidel[2, 9] *

[1] Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, México.

[2] Facultad de Ciencias Químicas, Universidad Autónoma de San Luis Potosí, México.

[3] Facultad de Enfermería y Nutrición, Universidad Autónoma de San Luis Potosí, México.

[4] Instituto de Investigación de Zonas Desérticas, Universidad Autónoma de San Luis Potosí, México.

[5] Laboratorio Integral de Investigación en Alimentos, Instituto Tecnológico de Tepic, México.

[6] Coordinación para la Innovación y Aplicación de la Ciencia y la Tecnología, Universidad Autónoma de San Luis Potosí, México.

[7] Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität Giessen, Germany.

[8] Division of Infectious Diseases, Department of Medicine, University of British Columbia, Canada.

[9] Centro de Investigación en Ciencias de la Salud y Biomedicina, Universidad Autónoma de San Luis Potosí, México.

*Correspondence:
Fidel Martinez-Gutierrez
Facultad de Ciencias Químicas, Universidad Autónoma de San Luis Potosí, Manuel Nava 6, Zona Universitaria, San Luis Potosí, SLP 78210, México.
ORCID: 0000-0002-2760-8273
E-mail: fidel@uaslp.mx Phone: +52 (444) 8262300 ext. 8566

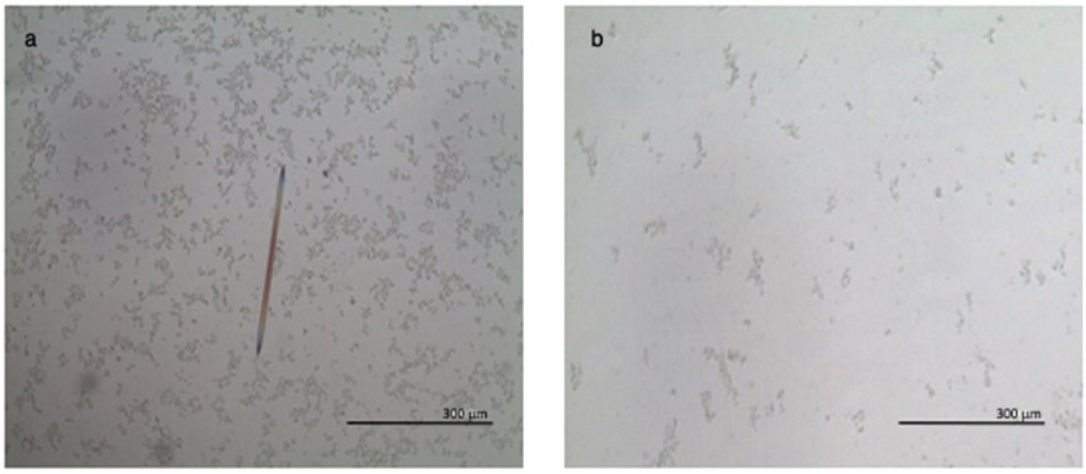

Figure S1. Juice from *Agave salmiana* with acicular calcium oxalate crystals observed with a microscope at 10× before (a) and after filtering (b).

Table S1. Averaged growth parameters of *L. lactis*, *L. acidophilus*, and *B. longum* in rMRS broth with 0 to 4 % fructans from *A. salmiana*, or a mix (*) of 3.6 % *A. salmiana* plus 0.4 % inulin Orafti® GR.

Probiotic Parameters	Fructans (%)	λ (h)	ε (h)	μ (h^{-1})	*A*	DT (h)	TR (×)
L. Lactis $\mu_x = 0.605\ h^{-1}$ $K_s = 0.81$ g/L	0	2.2	5.9	0.477	3.94	1.5	52
	1	1.5	6.4	0.557	5.00	1.2	149
	2	1.4	6.4	0.588	5.31	1.2	202
	4	1.3	6.6	0.589	5.47	1.2	237
L. acidophilus $\mu_x = 0.859\ h^{-1}$ $K_s = 5.18$ g/L	0	25.1	5.5	0.316	2.45	2.2	12
	0.4	1.5	8.4	0.374	4.41	1.9	83
	3.6	2.0	5.2	0.751	5.49	0.9	243
	4*	2.2	4.7	0.835	5.49	0.8	243
B. longum $\mu_x = 0.732\ h^{-1}$ $K_s = 1.07$ g/L	0	1.9	6.0	0.499	4.21	1.4	67
	0.4	2.5	6.8	0.578	5.57	1.2	263
	3.6	2.7	6.5	0.711	6.55	1.0	702
	4*	2.7	6.4	0.723	6.55	1.0	696

Parameters: lag (λ) and exponential (ε) phases, specific growth rate (μ), asymptote (*A*) of $\ln(N/N_0)$, duplication time (DT), number of times replicated (TR), maximum growth rate (μ_x), and half-velocity constant (K_s) when $\mu/\mu_x = 0.5$.

Table S2. Age and characteristics at birth of malnourished and normal-weight children.

	Dx	*n*	AVG	SD	Difference	*p*	95 % CI	
							Inferior	Superior
Child's age (years)	1	6	5.17	0.82	0.53	0.235	−0.40	1.47
	2	6	4.64	0.62			−0.41	1.48
Weight at birth (kg)	1	6	2.498	1.260	−0.4	0.485	−1.643	0.836
	2	6	2.901	0.518			−1.733	0.926
Mother's age (years)	1	6	38.83	5.41	7.67	0.107	−1.99	17.32
	2	6	31.16	9.13			−2.30	17.63
Pregnancy term (weeks)	1	6	35.33	3.55	3.67	0.591	−11.07	18.40
	2	6	31.66	15.80			−12.87	20.21
Number of pregnancies	1	6	2.66	1.50	−0.17	0.859	−2.24	1.91
	2	6	2.83	1.72			−2.25	1.91
Number of children	1	6	2.66	1.36	0	1.0	−1.75	1.75
	2	6	2.66	1.36			−1.75	1.75

Dx: Diagnosis, 1 = normal-weight, 2 = malnourished.

Table S3. Dietary characteristics between normal-weight and malnourished children.

Consumption of:	Dx	*n*	AVG	SD	Difference	*p*	95 % CI	
							Inferior	Superior
Coffee per day	1	6	0.16	0.40	−0.17	0.535	−0.76	0.43
	2	6	0.33	0.51			−0.76	0.43
Soft drinks per day	1	6	0.50	0.54	0.17	0.587	−0.51	0.85
	2	6	0.33	0.51			−0.51	0.85
Salty snacks per day	1	6	0.00	0.00	−0.33	0.144	−0.80	0.13
	2	6	0.33	0.51			−0.87	0.20
Fruits per week	1	6	4.50	3.14	−0.33	0.828	−3.64	2.97
	2	6	4.83	1.83			−3.75	3.09
Vegetables per week	1	6	3.00	3.68	1.17	0.520	−2.75	5.08
	2	6	1.83	2.22			−2.87	5.20

Dx: Diagnosis, 1 = normal-weight, 2 = malnourished.

Table S4. Weight and height of malnourished and normal-weight children before and after the intervention, including z-scores for height-for-age (HAZ), weight-for-age (WAZ), weight-for-height (WHZ).

			Diet with fructans								Without fructans (control)							
			Malnourished				Normal-weight				Malnourished				Normal-weight			
Measure	Event	*n*	AVG	SD	Diff.	*p*	AVG	SD	Diff.	*p*	AVG	SD	Diff.	*p*	AVG	SD	Diff.	*p*
Weight (kg)	**B**efore	6	14.30	1.223	0.27	0.712	17.78	1.550	0.14	0.874	15.12	0.700	0.0	1.000	17.88	1.598	0.05	0.959
	After	6	14.57	1.244			17.92	1.439			15.12	0.725			17.93	1.681		
Height (cm)	B	6	99.70	2.881	0.02	0.991	108.38	3.271	0.09	0.963	102.62	2.842	0.05	0.976	108.65	4.666	0.05	0.986
	A	6	99.72	2.880			108.47	3.285			102.67	2.805			108.70	4.710		
HAZ*	B	6	−1.50	0.548			0.0	0.0			−1.50	0.548			0.0	0.0		
	A	6	−1.50	0.548			0.0	0.0			−1.50	0.548			0.0	0.0		
WAZ	B	6	−1.17	0.408			0.00	0.000	0.17	0.332	−1.33	0.516	0.17	0.592	0.0	0.0		
	A	6	−1.17	0.408			0.17	0.408			−1.50	0.548			0.0	0.0		
WHZ*	B	6	−0.50	0.548			0.33	0.516			−0.17	0.753			0.33	0.516		
	A	6	−0.50	0.548			0.33	0.516			−0.17	0.753			0.33	0.516		

**t*-test not calculated as the difference is zero.

Table S5. Lactic acid bacteria in MRS, Gram-negatives in MCA, and pH measured in fecal samples from malnourished and normal-weight children before and after consuming fructans from *A. salmiana*.

Malnourished							Normal-weight						
ID	pH		MRS log(CFU/mL)		MCA log(CFU/mL)		ID	pH		MRS log(CFU/mL)		MCA log(CFU/mL)	
	Before	**A**fter	B	A	B	A		**B**efore	**A**fter	B	A	B	A
6	6.61	4.73	8.2	8.3	7.28	6.8	18	6.57	7.46	8.38	8.32	8.82	8.1
22	7.51	7.31	6.52	7.75	6	7.52	12	5.69	5.6	9.43	9.45	8.42	6.85
23*	5.96	7.12	8.87	8.39	8.08	8.44	24	6.63	5.26	8.88	9.11	8.21	6.8
16	6.53	6.13	9.05	9.07	8.52	9.37	11	7.15	7.16	8.99	10.52	8.57	9.22
10*	5.77	6.95	8.54	8.03	7.97	7.22	15	6.35	5.88	8.94	10.6	7.75	9.12
4	7.25	6.66	8.06	7.99	10.21	7.43	20*	6.23	5.91	8.33	7.90	8.29	7.52
AVG	6.61	6.48	8.21	8.26	8.01	7.79	AVG	6.44	6.21	8.83	9.31	8.34	7.94
SD	0.77	0.46	0.91	0.46	1.39	0.94	SD	0.54	0.89	0.41	1.11	0.36	1.07
Diff.		−0.13		0.05		−0.22	Diff.		−0.23		0.48		−0.40
p		0.729		0.901		0.754	*p*		0.6		0.344		0.406

MRS: deMan-Rogosa-Sharpe agar, MCA: MacConkey agar.

Table S6. Volatile organic compounds (VOCs) found in fecal samples from malnourished and normal-weight children before and after consuming fructans from *A. salmiana*.

Compound	Malnourished						Normal-weight					
	Before		After				Before		After			
	AVG	SD	AVG	SD	Diff.	*p*	AVG	SD	AVG	SD	Diff.	*p*
2-Methylbutanoic acid	256.5	69.2	232.7	269.2	−23.8	0.844	222.4	64.6	249.9	147.4	27.5	0.563
n-Octanal	268.1	25.3	267.5	74.3	−0.6	0.844	231.0	115.4	255.6	70.2	24.6	0.063
2-Octenal	356.6	103.6	238.6	95.3	−118.0	0.688	293.6	135.7	248.1	78.5	−45.5	0.688
Acetylpyrazine	512.0	145.6	442.7	251.0	−69.3	0.844	365.6	282.9	423.0	211.7	57.4	0.156
Butanone	183.3	31.9	164.5	65.0	−18.8	0.438	198.3	71.3	196.3	47.1	−2.0	0.999
Isobunatol	137.7	71.9	107.7	61.0	−30.0	0.031	125.8	19.1	122.9	115.2	−2.9	0.688
2-Methylpropanal	107.0	36.6	90.2	17.8	−16.8	0.031	107.1	27.3	94.2	14.5	−12.9	0.313
Ethylpirazyne	226.7	135.1	151.2	48.4	−75.5	0.156	168.0	120.7	132.3	69.3	−35.7	0.219
Butane-2,3-dione	199.0	82.9	203.5	33.9	4.5	0.688	239.8	73.5	214.7	56.8	−25.1	0.563
Heptane	1328.3	129.2	1307.7	49.6	−20.6	0.844	1290.8	172.0	1296.5	105.5	5.7	0.438
Propyl acetate	1222.0	63.9	1253.9	59.7	31.9	0.999	1295.8	185.3	1191.7	175.8	−104.1	0.438
Hexadecafluoroheptane	891.2	590.8	513.3	118.4	−377.9	0.219	640.5	257.9	541.5	263.8	−99.0	0.688
Trimethylamine (TMA)	4641.2	2897.0	4046.8	785.3	−594.4	0.156	5471.5	822.8	3999.5	724.4	−1472.	0.031
2-Ethyl-methylpyrazine	113.1	47.3	100.6	57.7	−12.5	0.999	82.0	76.2	145.6	82.7	63.6	0.063
1-Methoxy-2-propanol	62.0	156.6	56.6	172.5	−5.4	0.423	84.7	98.5	76.7	146.0	−8.0	0.999
cis-3-Hexenol	96.8	107.0	0.0	140.2	−96.8	0.201	181.7	177.9	0.0	72.9	−181.7	0.156