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Title	Bifidobacterial ß-galactosidase-mediated production of galacto-oligosaccharides: structural and preliminary functional assessments
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Supplementary material

Supplementary Table S1. GOS sample quantification from GOS assay related to BgaC experiment

Enzyme	galactose (%)	glucose (%)	allo-lactose (%)	lactose (%)	lactulose (%)	GOS (%)
BgaC 12Lu	9.83	14.48	0.44	65.07	1.51	8.7
BgaC 16Lu	11.61	16.68	0.57	57.02	1.00	13.1

Supplementary Table S2a GOS sample quantification from GOS assay related to BgaD experiment 40% lactose, 55°C, 12, 16, 20, 30 Lu/g

Enzyme	galactose (%)	glucose (%)	allo-lactose (%)	lactose (%)	lactulose (%)	GOS (%)
BgaD 12Lu 08h	12.68	20.05	1.01	51.52	1.24	13.5
BgaD 12Lu 10h	14.67	22.62	1.28	47.42	1.05	13.0
BgaD 12Lu 12h	15.46	23.41	1.54	43.76	1.16	14.7
BgaD 16Lu 08h	14.76	22.68	1.30	46.41	1.15	13.7
BgaD 16Lu 10h	16.85	25.23	1.72	41.65	1.04	13.5
BgaD 16Lu 12h	17.65	25.96	1.71	38.07	1.07	15.5
BgaD 20LU 5H	10.72	17.29	0.64	55.40	1.35	14.6
BgaD 20LU 6H	11.61	18.40	0.74	50.83	1.18	17.2
BgaD 20LU 7H	12.45	19.54	0.84	48.96	1.18	17.0
BgaD 20LU 8H	13.42	20.87	0.93	46.50	1.10	17.2
BgaD 30LU 4H	11.28	17.83	0.84	46.55	1.14	22.4
BgaD 30LU 5H	14.05	21.38	1.02	41.89	1.03	20.6
BgaD 30LU 6H	15.05	22.56	1.18	38.73	0.97	21.5
BgaD 30LU 7H	16.51	24.35	1.35	37.45	0.98	19.4
BgaD 30LU 8H	17.61	25.50	1.50	34.67	0.86	19.8

Supplementary Table S2b GOS sample quantification from the GOS producing assay corresponding to BgaD used at 4, 8, 16, 24 Lu/g lactose, with 30% lactose, and performed at 55°C.

	galactose (%)	glucose (%)	allo-lactose (%)	lactose (%)	lactulose (%)	GOS (%)
BgaD 4LU-0.5h	0.47	1.23	n.a.	92.71	1.65	3.9
BgaD 4LU-1h	0.78	1.96	n.a.	88.46	1.78	7.0
BgaD 4LU-2h	1.62	3.58	n.a.	84.35	1.46	9.0
BgaD 4LU-3h	2.53	5.19	n.a.	82.61	1.73	7.9
BgaD 4LU-4h	3.01	6.00	n.a.	78.97	1.42	10.6
BgaD 4LU-5h	3.54	6.89	n.a.	78.56	1.61	9.4
BgaD 4LU-6h	4.28	7.95	n.a.	76.01	1.36	10.4
BgaD 4LU-8h	4.81	8.75	0.07	73.89	1.27	11.2
BgaD 8LU-0.5h	1.20	2.77	n.a.	87.36	1.46	7.2
BgaD 8LU-1h	2.10	4.41	n.a.	81.55	1.32	10.6
BgaD 8LU-2h	4.25	7.89	n.a.	75.17	1.22	11.5
BgaD 8LU-3h	6.21	10.73	0.15	68.59	1.29	13.0
BgaD 8LU-4h	7.43	12.24	0.22	65.48	1.04	13.6
BgaD 8LU-5h	8.59	13.84	0.27	62.47	1.37	13.5
BgaD 8LU-6h	9.99	15.53	0.55	59.73	1.31	12.9
BbgIIV 8LU-8h	11.33	17.21	0.44	57.65	0.87	12.5
BgaD 16LU-0.5h	2.50	5.14	n.a.	81.64	0.95	9.8
BgaD 16LU-1h	4.31	8.05	0.08	75.33	0.98	11.2
BgaD 16LU-2h	11.06	16.90	0.46	55.42	1.14	15.0
BgaD 16LU-3h	8.08	13.29	0.27	64.45	1.09	12.8
BgaD 16LU-4h	13.05	19.36	0.60	52.67	0.79	13.5
BgaD 16LU-5h	14.73	21.27	0.73	48.55	0.93	13.8
BgaD 16LU-6h	16.78	23.46	1.03	45.05	0.94	12.7
BgaD 16LU-8h	18.37	25.25	1.20	41.26	0.82	13.1
BgaD 24LU-0.5h	3.81	7.37	n.a.	78.00	1.17	9.6
BgaD 24LU-1h	6.31	10.99	0.15	69.18	0.99	12.4
BgaD 24LU-2h	11.17	17.07	0.48	55.67	0.80	14.8
BgaD 24LU-3h	15.10	21.55	0.83	47.19	0.88	14.5
BgaD 24LU-4h	17.10	23.76	0.97	42.14	0.85	15.2
BgaD 24LU-5h	19.46	26.44	1.17	39.29	0.70	12.9
BgaD 24LU-6h	21.56	28.50	1.36	35.21	0.75	12.6
BgaD 24LU-8h	23.77	30.90	1.55	32.06	0.64	11.1

Supplementary Table S3. GOS sample quantification from the GOS production assay corresponding to BgaA used at 4, 8 and 11 Lu/g lactose, with 50 % lactose as starting substrate level, and performed at 50°C.

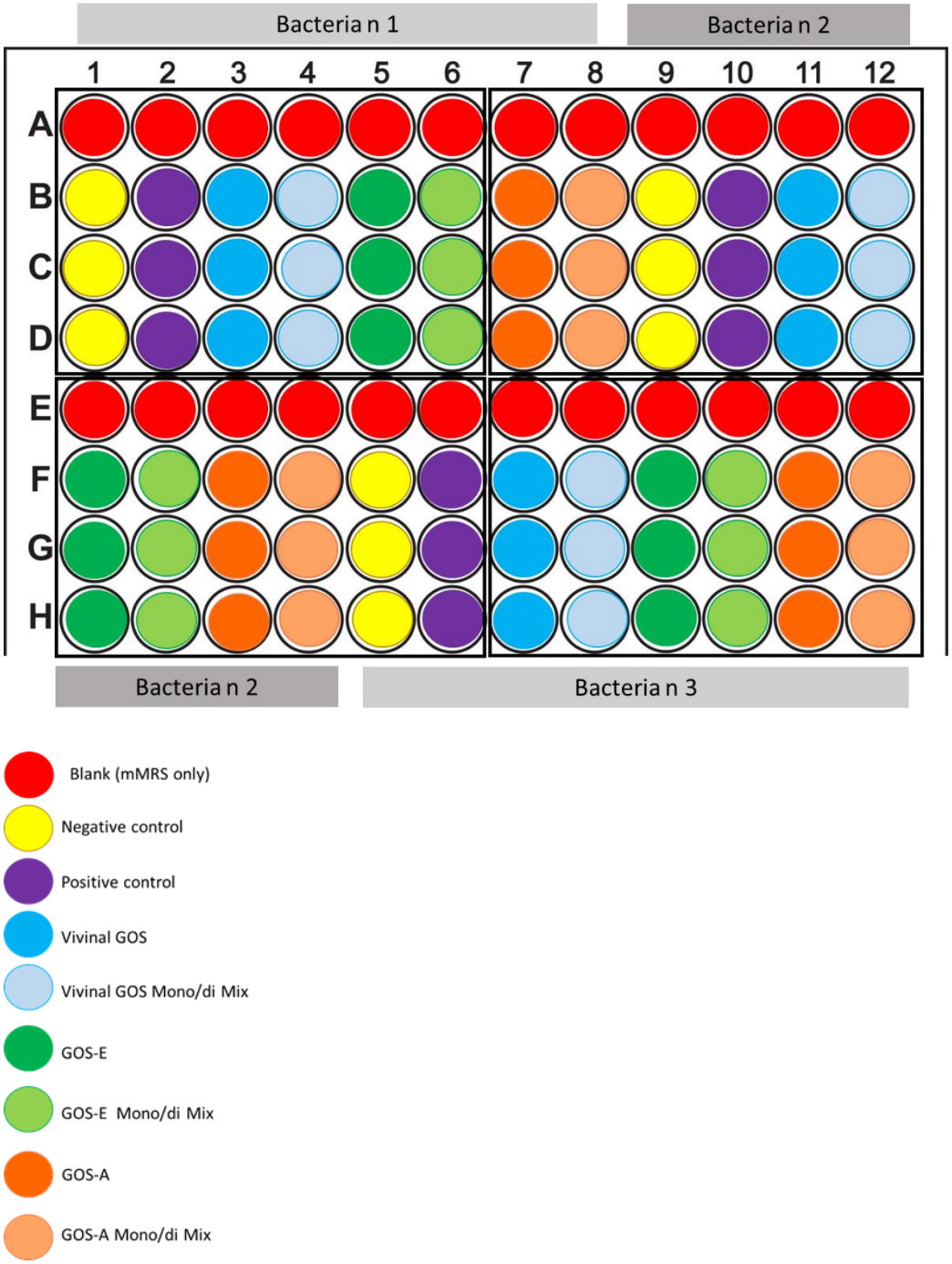
Enzyme	galactose (%)	glucose (%)	allo-lactose (%)	lactose (%)	lactulose (%)	GOS (%)
BgaA 4Lu 6h	2.64	5.81	2.43	75	1.22	12.9
BgaA 8Lu 1.5h	13.29	21.49	10.12	40.11	0.75	14.2
BgaA 8Lu 3h	19.66	29.08	11.43	20.39	0.48	19
BgaA 8Lu 5h	26.78	36.5	10.41	9.22	0.29	16.8
BgaA 8Lu 6h	29.04	38.69	9.48	6.57	0.18	16
BgaA 11Lu 1.5h	17.46	26.47	11.14	25.37	0.56	19
BgaA 11Lu 3h	26.9	36.87	10.36	9.42	n.a.	16.5
BgaA 11Lu 5h	34.43	43.74	6.91	2.47	n.a.	12.5
BgaA 11Lu 6h	35.83	44.72	5.84	1.55	n.a.	12.1

Supplementary Table S4. GOS sample quantification from the GOS production assay corresponding to BgaE used at 2, 4 and 6 Li.g lactose, with 50% lactose, and performed at 50 °C.

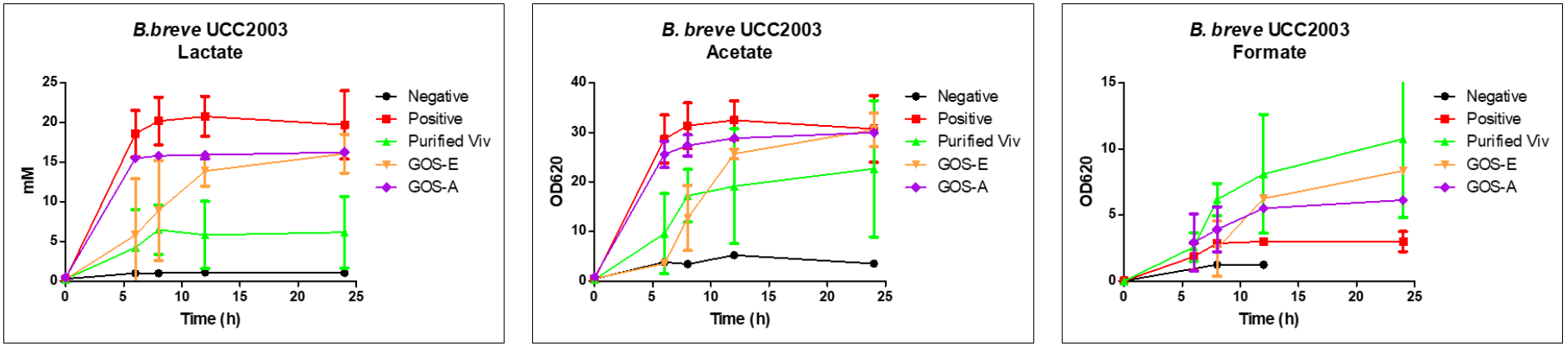
Enzyme	galactose (%)	glucose (%)	allo-lactose (%)	lactose (%)	lactulose (%)	GOS (%)
BgaE 2Lu 05h	3.6	12.11	9.93	50.24	1.04	23.1
BgaE 2Lu 10h	4.61	15.13	13.22	36.58	0.6	29.9
BgaE 2Lu 12h	4.99	16.19	14.32	33.16	0.58	30.8
BgaE 4LU 3h	6.07	18.98	17.01	24.94	0.44	32.6
BgaE 4LU 5h	7.88	22.65	19.17	13.5	0.33	36.5
BgaE 4LU 7h	9.76	25.63	19.64	7.93	0.22	36.8
BgaE 4LU 9h	10.31	25.58	18.09	5.63	0.13	40.3
BgaE 6LU 1.5h	5.21	16.87	15.21	32.75	0.67	29.3
BgaE 6LU 3h	7.66	22.23	19.16	13.97	0.33	36.6
BgaE 6LU 5h	10.06	25.94	19.17	6.62	0.19	38
BgaE 6LU 7h	12.6	29.28	18.16	4.14	0.08	35.7

Supplementary Table S5 Adhesion experiment conducted with *Salmonella typhimurium* 4/74 and PureViv.

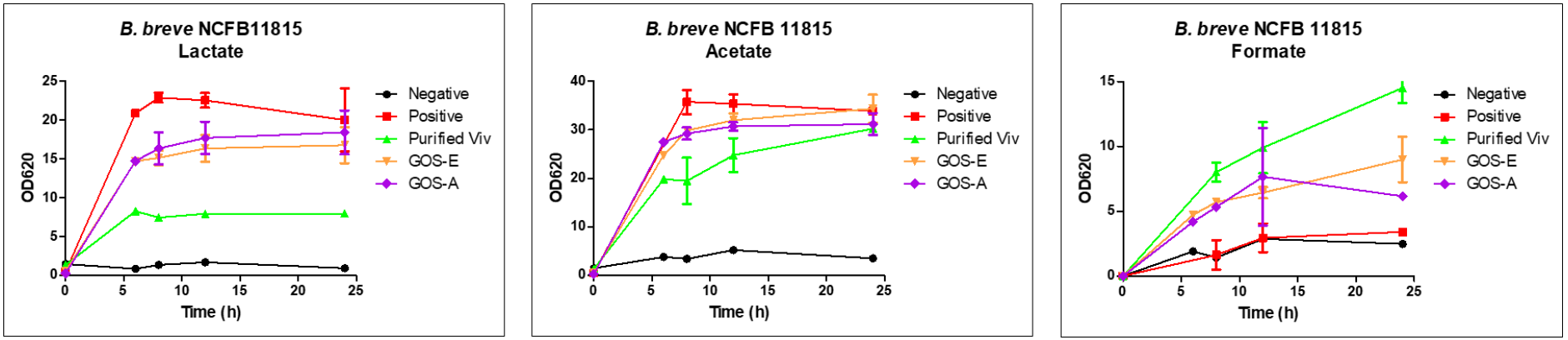
	Replicates (%)		
Substrate	**I**	**II**	**III**
Control	100.0	100.0	100.0
0.5 %	211.4	468.7	119.1
5 %	125.6	522.3	70.7
25 %	79.7	406.7	646.5
Mono Mix	0.0	72.9	80.3



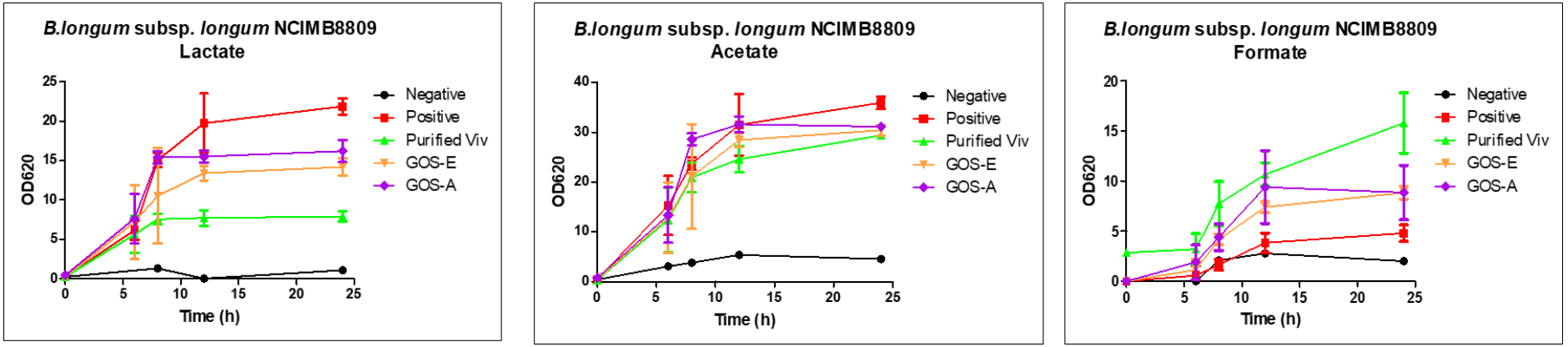
Supplementary Figure S1. 96 wells plate set up for bacterial growth assay.



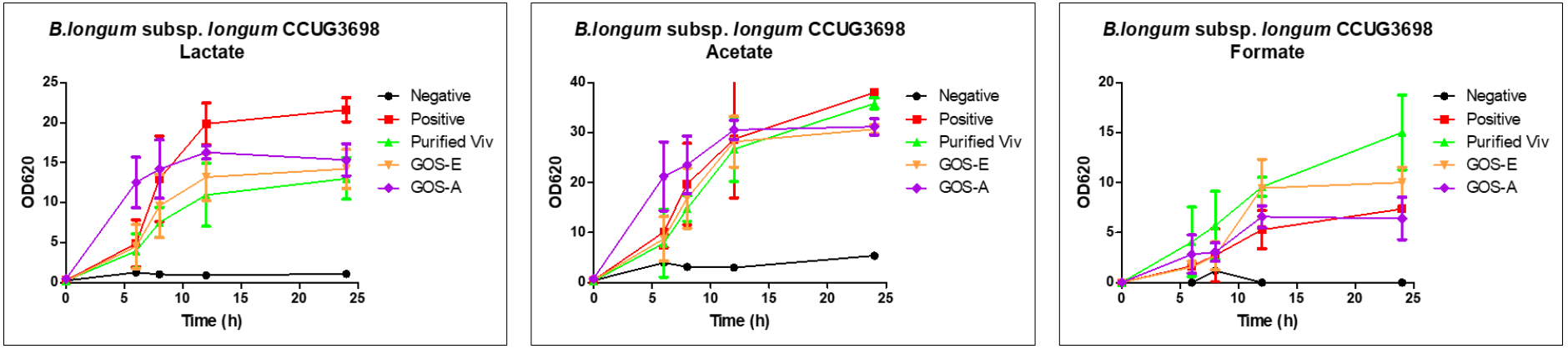
Supplementary Figure S2.a SCFA production resulting from *B. breve* UCC2003 growth on novel purified GOS preparations. Negative: no sugar, Positive: lactose, Purified Viv: Pure Viv, GOS-E: purified GOS obtained by enzymatic reaction employing BgaE, GOS-A: purified GOS obtain by enzymatic reaction employing BgaA.



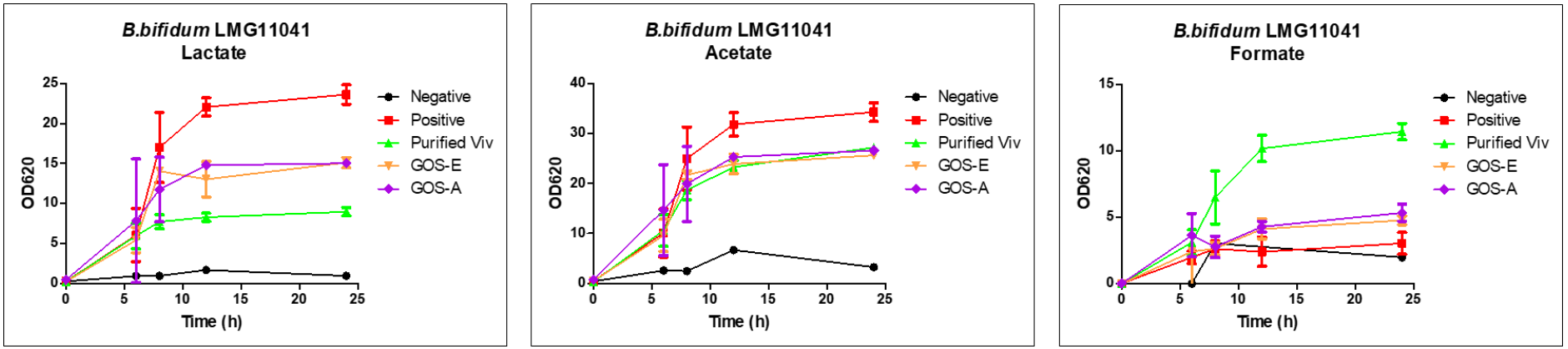
Supplementary Figure S2.b SCFA production resulting from *B. breve* NCFB11815 growth on novel purified GOS preparations. Negative: no sugar, Positive: lactose, Purified Viv: Pure Viv, GOS-E: purified GOS obtained by enzymatic reaction employing BgaE, GOS-A: purified GOS obtain by enzymatic reaction employing BgaA.



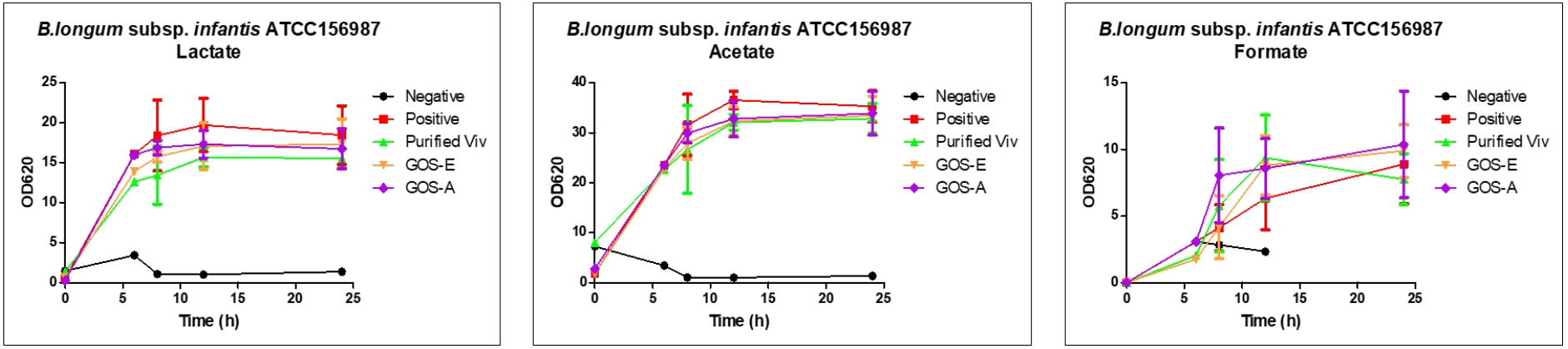
Supplementary Figure S2.c SCFA production resulting from *B. longum* subsp. *longum* NCIMB8809 growth on novel purified GOS preparations. Negative: no sugar, Positive: lactose, Purified Viv: Pure Viv, GOS-E: purified GOS obtained by enzymatic reaction employing BgaE, GOS-A: purified GOS obtain by enzymatic reaction employing BgaA.



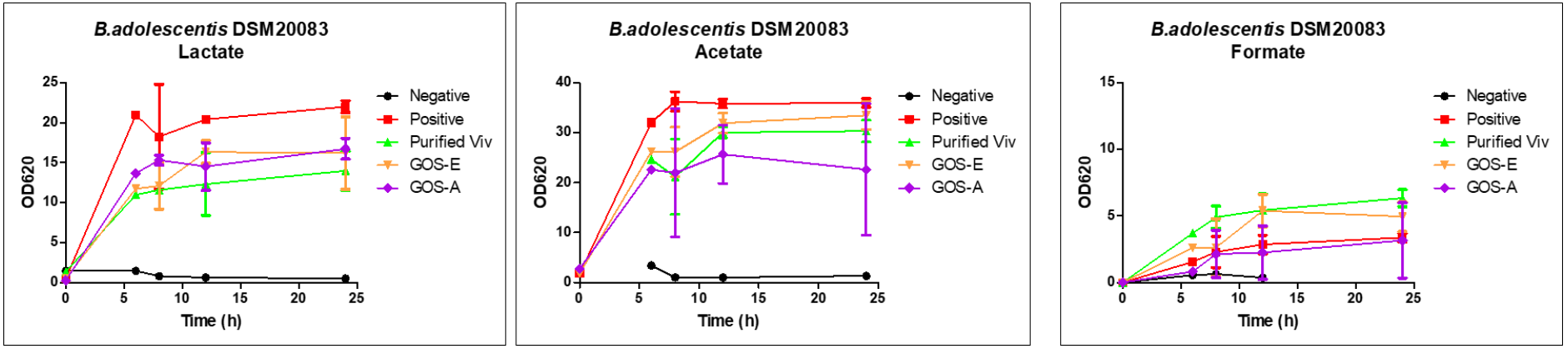
Supplementary Figure S2.d SCFA production resulting from *B. longum* subsp. *longum* CCUG3698 growth on novel purified GOS preparations. Negative: no sugar, Positive: lactose, Purified Viv: Pure Viv, GOS-E: purified GOS obtained by enzymatic reaction employing BgaE, GOS-A: purified GOS obtain by enzymatic reaction employing BgaA.



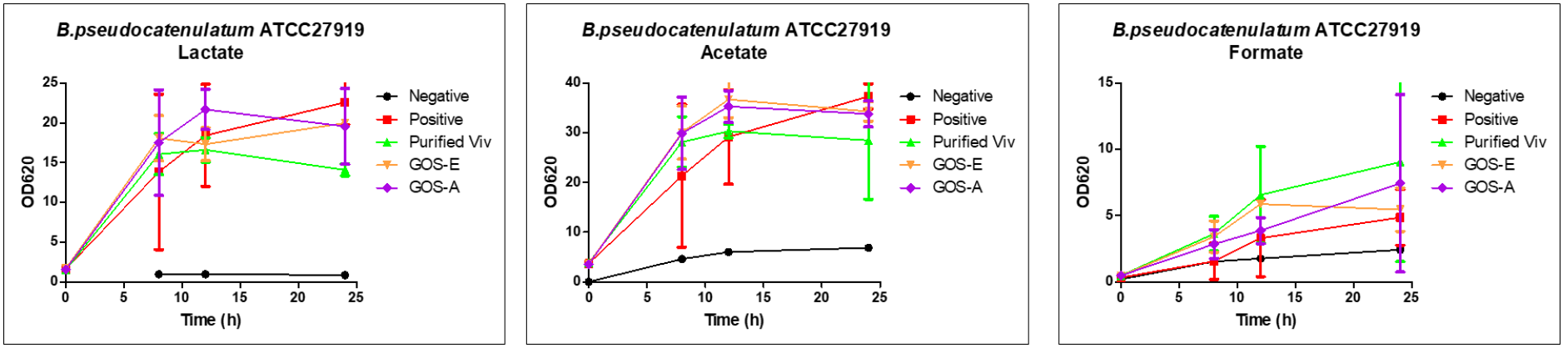
Supplementary Figure S2.e SCFA production resulting from *B.bifidum* LMG11041 growth on novel purified GOS preparations. Negative: no sugar, Positive: lactose, Purified Viv: Pure Viv, GOS-E: purified GOS obtained by enzymatic reaction employing BgaE, GOS-A: purified GOS obtain by enzymatic reaction employing BgaA.



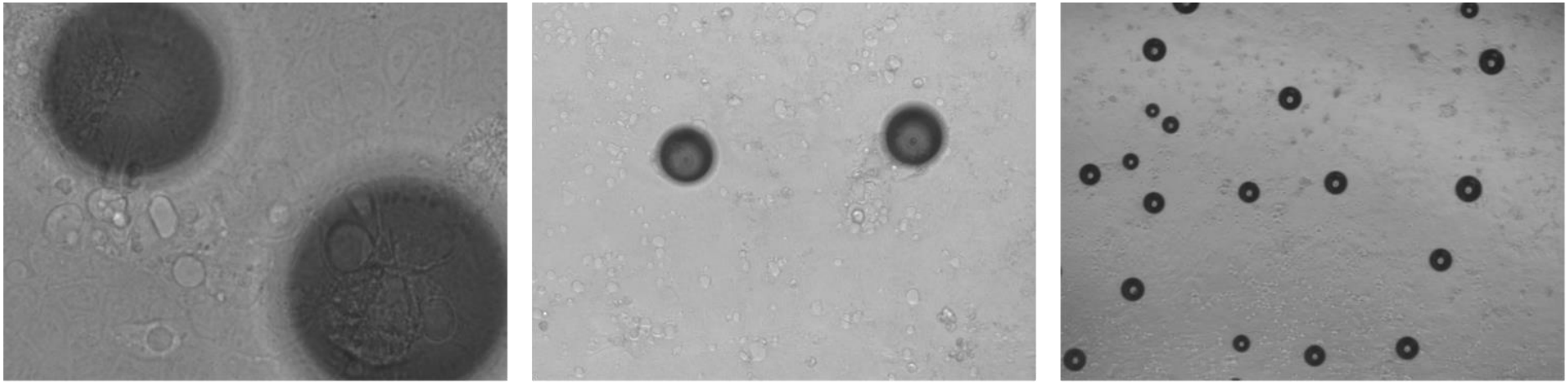
Supplementary Figure S2.f SCFA production resulting from *B.longum* subsp. *infantis* ATCC156987 growth on novel purified GOS preparations. Negative: no sugar, Positive: lactose, Purified Viv: Pure Viv, GOS-E: purified GOS obtained by enzymatic reaction employing BgaE, GOS-A: purified GOS obtain by enzymatic reaction employing BgaA.



Supplementary Figure S2.g SCFA production resulting from *B.adolescentis* DSM20083 growth on novel purified GOS preparations. Negative: no sugar, Positive: lactose, Purified Viv: Pure Viv, GOS-E: purified GOS obtained by enzymatic reaction employing BgaE, GOS-A: purified GOS obtain by enzymatic reaction employing BgaA.



Supplementary Figure S2.h SCFA production resulting from *B.pseudocatenulatum* ATCC27919 growth on novel purified GOS preparations. Negative: no sugar, Positive: lactose, Purified Viv: Pure Viv, GOS-E: purified GOS obtained by enzymatic reaction employing BgaE, GOS-A: purified GOS obtain by enzymatic reaction employing BgaA.



Supplementary Figure S3. C2BBe1 cell line infected with *Salmonella typhimurium* 4/74, micro bubble formation.