

Condition	Osmolality ¹ (Osm)	Δ Osmolality ² (Osm)	D_{MEDIAN} ($\mu\text{m}^2/\text{s}$)	IQR ³ ($\mu\text{m}^2/\text{s}$)	Range ⁴ ($\mu\text{m}^2/\text{s}$)	n ⁵
CDM	0.53	0	7	5.1 - 10	1.4 - 18	84
CDM + NaCl	0.99	0.46	3	1.9 - 4.8	1.2 - 8.2	24
CDM + NaCl	1.44	0.91	0.838	0.48 - 0.92	0.25 - 2.7	34
CDM + NaCl	1.93	1.4	0.23	0.14 - 0.44	0.034 - 3.9	40
CDM + NaCl	2.25	1.72	0.087	0.041 - 0.72	0.015 - 4.7	46
Pi buffers	0.54	0.01	4.3	2.9 - 6.8	0.29 - 15	80
Pi buffers + Salt ⁶	1.01	0.48	0.41	0.28 - 0.68	0.061 - 1.9	44
Pi buffers + Salt ⁶	1.36	0.83	0.11	0.068 - 0.21	0.025 - 0.3	41
Pi buffers + NaCl	2.36	1.83	0.091	0.037 - 0.29	0.016 - 2.3	41

Table S1. Diffusion coefficients of GFP in the cytoplasm of *Lactococcus lactis* at standard osmotic conditions of cell growth and upon osmotic upshift in CDM^{RP} or phosphate-based media.

- 1) Osmolality of the medium determined as described in the Experimental Procedures section
- 2) Osmotic upshift: difference in osmolality between the measurement condition and the osmolality of the growth medium
- 3) The interquartile range (IQR), giving values containing the middle half of the data
- 4) Minimal and maximal D values measured
- 5) Number of individual cells measured
- 6) Salt is either KCl or NaCl, since there was no difference between data sets, they were combined