

1 Optimisation of bacterial release from a stable microfluidic-generated
2 water-in-oil-in-water emulsion

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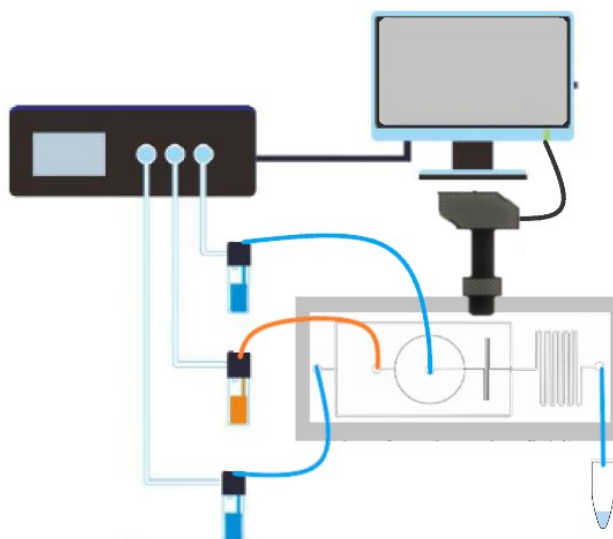
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14 **Supplementary information**

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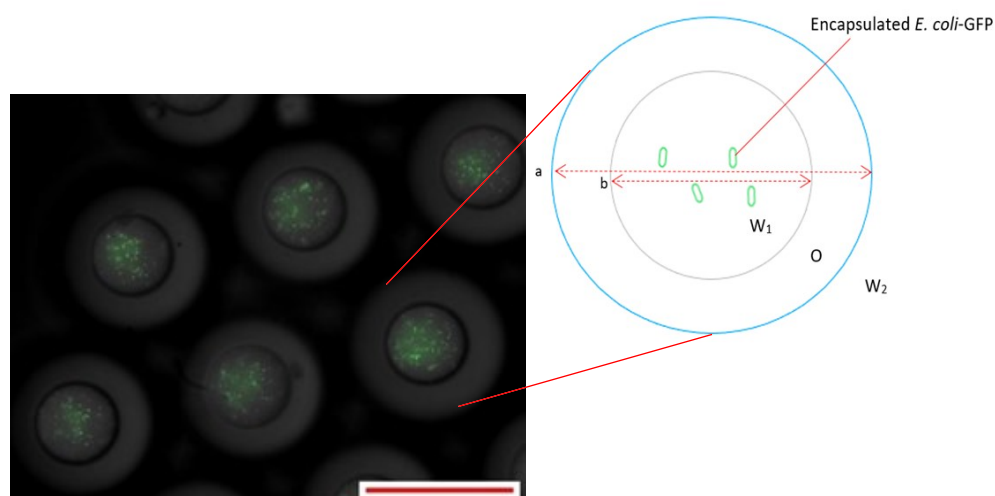


Figure S1. (A) Schematic representation of the microfluidic encapsulation whereby the different phases of W_1 , oil phase and W_2 were flushed through the inlet by using a pressure controller. The produced $W_1/O/W_2$ droplet were collected continuously at the outlet. (B) Image and schematic representation of the $W_1/O/W_2$ droplet containing *E. coli*-GFP. Droplet formation was monitored by using a microscope with FASTCAM. The diameter of the oil globule is given by a: 100 μm while the diameter of the inner W_1 phase is given by b: 50 μm . Scale bar represents 100 μm .

31 Table S1. The encapsulation efficiency of *E. coli*-GFP in W₁/O/W₂ emulsions. Data represent mean ±
 32 standard deviation taken from 3 independent experiments.

Formulation	Encapsulation efficiency (%)
<i>E. coli</i> /DI water in W ₁ (1% Tween 80)	99.99 ± 0.01
<i>E. coli</i> /DI water in W ₁ (5% Tween 80)	100
<i>E. coli</i> / LB broth in W ₁ (1% Tween 80)	99.98 ± 0.02
<i>E. coli</i> /LB broth in W ₁ (5% Tween 80)	99.98 ± 0.02
<i>E. coli</i> / 0.5% NaCl in W ₁ (1% Tween 80)	99.95 ± 0.02
<i>E. coli</i> / 0.5% NaCl in W ₁ (5% Tween 80)	99.97 ± 0.03

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46 Table S2. The effect of different LB broth formulations on the viability of *E. coli*-GFP (Log CFU/mL).
 47 Data represent mean standard deviation from 3 independent experiments.

Incubation time (hour)	Sodium chloride (0.5% w/v)	Tryptone	Yeast extract
0	6.27 ± 0.05	6.40 ± 0.04	7.03 ± 0.09
24	6.18 ± 0.03	7.98 ± 0.03	8.26 ± 0.03
Change in Log CFU/mL	-1.48	24.72	17.48

69 Table S3. Average density measurements for the aqueous and oil phase of W/O/W emulsions at ambient
70 temperature (25°C). The density for each sample was determined by measuring the mass per volume
71 for each sample. Data represent mean $\pm$ standard deviation taken from 3 independent experiments.

Solutions	Average density (g/mL)
DIW	0.997 ± 0.001
DIW with <i>E. coli</i> -GFP	0.973 ± 0.001
DIW with 1% w/v Tween 80	0.986 ± 0.002
DIW with 5% w/v Tween 80	0.994 ± 0.001
Mineral Oil	0.863 ± 0.001
Mineral Oil with 1.5% w/v PGPR	0.849 ± 0.003
0.5% w/v NaCl	1.004 ± 0.002
1.5% w/v NaCl	1.012 ± 0.003
2.0% w/v NaCl	1.011 ± 0.002
0.5% w/v NaCl with <i>E. coli</i> -GFP	1.004 ± 0.002
0.5% w/v NaCl w 1% w/v Tween 80	1.011 ± 0.003
0.5% w/v NaCl with 5% w/v Tween 80	1.023 ± 0.004

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Table S4. Change in mean W1 droplet and oil globule diameter (μm) at 30, 60 and 180 minutes with respect to 0 minutes of incubation. Samples were prepared in the presence or absence of *E. coli*-GFP in the W1 phase, with or without 1.5% w/v of NaCl in either W1 or W2 phase. Surfactant concentration was set at either 1% w/v or 5% w/v for Tween 80 in W2 phase. Data represent mean $\pm$ standard deviation from 3 independent experiments with N=900 droplet. The mean diameters were compared between samples within each incubation time (small letters) and between different incubation times within each sample (capital letters).

W ₁ /O/W ₂ samples		30 minutes (μm)		60 minutes (μm)		180 minutes (μm)	
		W ₁	Oil Globule	W ₁	Oil globule	W ₁	Oil Globule
With <i>E. coli</i>- GFP	No NaCl, 1% Tween 80	0.015 ^{aA} $\pm$ 0.001	-22.25 ^{aA} $\pm$ 1.10	0.036 ^{aA} $\pm$ 0.002	-22.23 ^{aA} $\pm$ 1.301	0.051 ^{aA} $\pm$ 0.002	-19.99 ^{aA} $\pm$ 1.241
	No NaCl, 5% Tween 80	0.017 ^{aA} $\pm$ 0.001	-22.26 ^{aA} $\pm$ 0.802	0.027 ^{aA} $\pm$ 0.001	-22.25 ^{aA} $\pm$ 0.913	0.032 ^{aA} $\pm$ 0.002	-22.23 ^{aA} $\pm$ 0.921
	1.5% NaCl in W ₁ , 1% Tween 80	0.947 ^{bA} $\pm$ 0.10	-22.24 ^{aA} $\pm$ 1.406	9.085 ^{bB} $\pm$ 0.62	-22.23 ^{aA} $\pm$ 0.923	15.304 ^{bC} $\pm$ 1.08	-18.04 ^{bB} $\pm$ 1.01
	1.5% NaCl in W ₁ , 5% Tween 80	0.317 ^{cA} $\pm$ 0.03	-22.256 ^{aA} $\pm$ 1.302	5.316 ^{cB} $\pm$ 0.51	-22.232 ^{aA} $\pm$ 1.311	9.865 ^{cC} $\pm$ 1.16	-22.22 ^{aA} $\pm$ 1.409
	1.5% NaCl in W ₂ , 1% Tween 80	-0.813 ^{dA} $\pm$ 0.06	-22.23 ^{aA} $\pm$ 1.105	-15.388 ^{dB} $\pm$ 1.02	-22.16 ^{aA} $\pm$ 0.805	-20.758 ^{dC} $\pm$ 1.04	-16.12 ^{cB} $\pm$ 0.903
	1.5% NaCl in W ₂ , 5% Tween 80	-0.381 ^{eA} $\pm$ 0.02	-22.23 ^{aA} $\pm$ 1.007	-6.732 ^{eB} $\pm$ 0.82	-22.23 ^{aA} $\pm$ 1.106	-10.534 ^{eC} $\pm$ 1.47	-22.2 ^{aA} $\pm$ 1.202
Without <i>E. coli</i>- GFP	No NaCl, 1% Tween 80	0.03 ^{aA} $\pm$ 0.002	-22.23 ^{aA} $\pm$ 0.801	0.038 ^{aA} $\pm$ 0.001	-22.23 ^{aA} $\pm$ 0.821	0.065 ^{aA} $\pm$ 0.002	-17.76 ^{aB} $\pm$ 0.701
	No NaCl, 5% Tween 80	0.025 ^{aA} $\pm$ 0.003	-22.24 ^{aA} $\pm$ 0.935	0.04 ^{aA} $\pm$ 0.004	-22.23 ^{aA} $\pm$ 0.905	0.048 ^{aA} $\pm$ 0.01	-22.22 ^{bA} $\pm$ 1.408
	1.5% NaCl in W ₁ , 1% Tween 80	1.021 ^{bA} $\pm$ 0.02	-22.22 ^{aA} $\pm$ 1.401	10.242 ^{bB} $\pm$ 1.62	-22.23 ^{aA} $\pm$ 1.207	16.527 ^{bC} $\pm$ 1.62	-16.98 ^{cB} $\pm$ 1.301
	1.5% NaCl in W ₁ , 5% Tween 80	0.439 ^{cA} $\pm$ 0.06	-22.23 ^{aA} $\pm$ 1.611	5.837 ^{cB} $\pm$ 1.13	-22.23 ^{aA} $\pm$ 1.503	10.104 ^{cC} $\pm$ 1.16	-22.22 ^{bA} $\pm$ 1.371
	1.5% NaCl in W ₂ , 1% Tween 80	-0.92 ^{dA} $\pm$ 0.12	-22.23 ^{aA} $\pm$ 0.781	-13.323 ^{dB} $\pm$ 0.91	-22.13 ^{aA} $\pm$ 0.838	-18.906 ^{dC} $\pm$ 1.02	-15.28 ^{dB} $\pm$ 0.911
	1.5% NaCl in W ₂ , 5% Tween 80	-0.368 ^{eA} $\pm$ 0.05	-22.22 ^{aA} $\pm$ 1.108	-9.048 ^{eB} $\pm$ 0.56	-22.22 ^{aA} $\pm$ 1.265	-13.015 ^{eC} $\pm$ 1.14	-22.2 ^{bA} $\pm$ 1.153

Data were analysed with one-way ANOVA.
^{abcde} means $\pm$ standard deviation and ^{ABC} means $\pm$ standard deviation with different letters are significantly different at $P < 0.05$.