Electronic Supplementary Material (ESI) for RSC Advances.

Modulating the release of *Escherichia coli* in double $W_1/O/W_2$ emulsion globules under hypo-osmotic pressure

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Supplementary Methods

S1. Measurement of oil globule size of double $W_1/O/W_2$ emulsions. The particle size distribution of oil globules in the $W_1/O/W_2$ emulsion was measured immediately after preparation and as a function of storage time using a laser diffraction particle size analyser (Malvern Mastersizer 2000, Malvern Instrument Ltd, Worcestershire, UK), equipped with a He-Ne laser (λ = 633 nm). The dispersion unit stirring speed was kept at 2000 rpm and the measurement range was 0.02–2000 µm. The optical parameters selected were: dispersed phase refractive index of n_D^{22} 1.39; oil globule absorbance of 0.01; and a dispersant liquid (distilled water) refractive index n_D^{22} 1.33; obscuration between 10% and 20%. Particle size calculations were based on the Mie Scattering theory and the volume mean diameter values (D [4, 3]), and the percentage of volume corresponding to each observed population were calculated using the Mastersizer 2000 software.

S2. Fluorescent and optical imaging of the $W_1/O/W_2$ emulsions and *E. coli*-GFP. The double $W_1/O/W_2$ emulsions with or without *E. coli*-GFP were observed using optical and fluorescent microscopy (Zeiss Axioplan) at room temperature. For optical microscopy imaging the sample was placed on a microscope slide and the image was acquired under objective lens 10x or 100x magnification with a digital colour camera system Motic Moticam 10 using a 10 megapixel CMOC camera via Motic Images Plus video acquisition software. For fluorescent microscopy imaging the sample was placed on a microscope slide and gently covered with a cover slip. For the osmotic balance alteration study the images were acquired under objective lens 40x magnification with a digital colour camera system Motic Moticam 10 using a 10 megapixel CMOC camera via Motic Images Plus video acquisition software. The light source used to excite the GFP was a light-emitting diode (LED) and the emission was observed at 509 nm. For the stability during storage study the images were acquired under objective lens 100x magnification (oil immersion) with a digital camera system Axiocam ICm1 using a 1.4 megapixel monochrome CCD camera via AxioVision Software (Zeiss). The light source used to excite the GFP was a mercury arc lamp and the emission was observed at 509 nm (GFP) and 461 nm (DAPI). Micrographs were overlaid using analysis software (ImageJ).

S3. Determining the total cell count and health of *E. coli* (MG1655) after encapsulation and release using epifluorescence microscopy. Due to the differences in density between the W_2 phase and the oil globules creaming or phase separation occurs when $W_1/O/W_2$ emulsion is un-mixed and it partitions into a serum phase (W_2 phase) and a cream layer (containing oil globules).[1] *E. coli* MG1655 in serum phase or de-ionised water (control) was stained by adding DAPI (4 µl/ml) and [$DiBAC_4$ (3)] (50 ng/ml) and incubated in the dark for 30 minutes. The serum phase was injected through a nucleopore black polycarbonate membrane filter with 0.2 µm pore size and 13mm diameter placed inside a Swinnex filter holder (Millipore) and the cells were counted using a fluorescent microscope (Zeiss Axiolab). The procedure is described in detail in Supplementary Methods section. A nucleopore black polycarbonate membrane filter with 0.2 µm pore size and 13mm diameter was mounted shiny side uppermost on a membrane Swinnex filter holder (Millipore) and the stained solution was passed through the membrane filter by injecting slowly with a syringe. The membrane filter was placed shiny side uppermost on a glass slide and a drop of immersion oil added to its surface and gently covered with a cover slip. The cells were then counted under objective lens of 100x magnification with a fluorescent microscope (Zeiss Axiolab) equipped with a mercury arc lamp and the emission was observed at 461 nm (DAPI) and 516 nm [$DiBAC_4$ (3)]. For each filter, 10 microscope (0.1 mm x 0.1 mm) fields were randomly selected and all cells within each field were counted and the average number of cells per field was calculated.[2] The serum phase volume was adjusted to yield a minimum of 400 total cell counts in 10 counting fields. The total number of *E. coli* cells from each sample of serum phase was calculated by multiplying the total number of fields with the average number of cells per field.

Supplementary Results

S1. Microscopic observation of a single $W_1/O/W_2$ emulsion oil globule in hypo-osmotic solution. To confirm that the increase in D (4, 3) of oil globules in hypo-osmotic solution was due to water movement from W_2 to W_1 and not solely as a result of coalescence between the oil globules we followed the evolution of a single oil globule under hypo-osmotic conditions. Fig. S8 shows snapshots of an oil globule taken after diluting the $W_1/O/W_2$ emulsion made with 40% W_1 containing NaCl and stabilised with 8% PGPR and 1% Tween80 in a hypo-osmotic solution. The oil globule showed a progressive increase in diameter over time with no observed coalescence confirming that swelling of the oil globule is occurring as a result of water movement from W_2 to W_1. The swelling phenomenon was a reproducible observation and occurred for all the oil globules under hypo-osmotic pressure.

S2. Video microscopy observation of *E. coli*-GFP release from $W_1/O/W_2$ emulsion oil globules. So far our results showed that the release of *E. coli*-GFP is significantly increased during osmotic balance alteration by increasing the amount of W_1 and decreasing the concentration of Tween80 or PGPR. Therefore we decided to further investigate the mechanism of bacterial release using fluorescence video microscopy. Using video-microscopy we were able to observe the bursting mechanism responsible for the release of *E. coli*-GFP from the oil globules after diluting the $W_1/O/W_2$ emulsion in a hypo-osmotic solution. Fig. S9 shows snapshots taken after diluting the $W_1/O/W_2$ emulsion made with 40% W_1 containing NaCl and stabilised with 2% PGPR and 1% Tween80 in a hypo-osmotic solution (see ESI, Video S1). The interfacial film of the oil globule ruptures and W_1 droplets with *E. coli*-GFP cells are released into the W_2 phase.

S3. Rheological characterisation of the primary W_1/O emulsion and phases. To understand how the concentration of W_1 and the concentration of surfactants affect the rheological properties of the $W_1/O/W_2$ emulsion, we measured the viscosity of the oil phase, W_2 phase and the primary W_1/O emulsion (Table S5). The viscosity of the oil phase was significantly ($P<0.05$) higher with increasing concentrations of PGPR. There was no significant difference in the viscosity between the W_2 with varying concentrations of Tween80. The viscosity of the W_1/O emulsion was significantly ($P<0.05$) higher at 40% W_1 compared to 20% W_1. These results suggest that the surfactant concentration affects the viscosity of the oil phase but not the W_2 phase while the viscosity of the primary W_1/O emulsion is affected by the amount of W_1.

S4. Encapsulation efficiency (%) of *E. coli*-GFP cells in $W_1/O/W_2$ emulsions. Encapsulation efficiency, expressed as an extent of entrapment of *E. coli*-GFP cells in different $W_1/O/W_2$ emulsion formulations, was calculated by measuring the bacterial cell counts before and after the encapsulation process. Immediately after preparation all $W_1/O/W_2$ emulsion formulations showed high encapsulation efficiency (>99.8%) (Table S6). The encapsulation efficiency was significantly ($P<0.05$) reduced after 3 and 7 days (6.5% and 7.3% respectively) (Fig. S10) which probably occurred due to $W_1/O/W_2$ emulsion degradation over time.

S5. Viability of *E. coli* cells within $W_1/O/W_2$ emulsion during storage. In order to find out if the structure of the $W_1/O/W_2$ emulsion affects the viability of encapsulated *E. coli*-GFP within the W_1 of oil globules during storage we assessed the viability of the bacteria by culture in media. There was no significant difference of reduction in log CFU/ml (Fig. S11) of encapsulated *E .coli*-GFP cells compared to control after 3 and 7 days. These results suggest that the viability of bacterial cells is not affected by their presence in the W_1 of $W_1/O/W_2$ emulsion.

Supplementary Figures

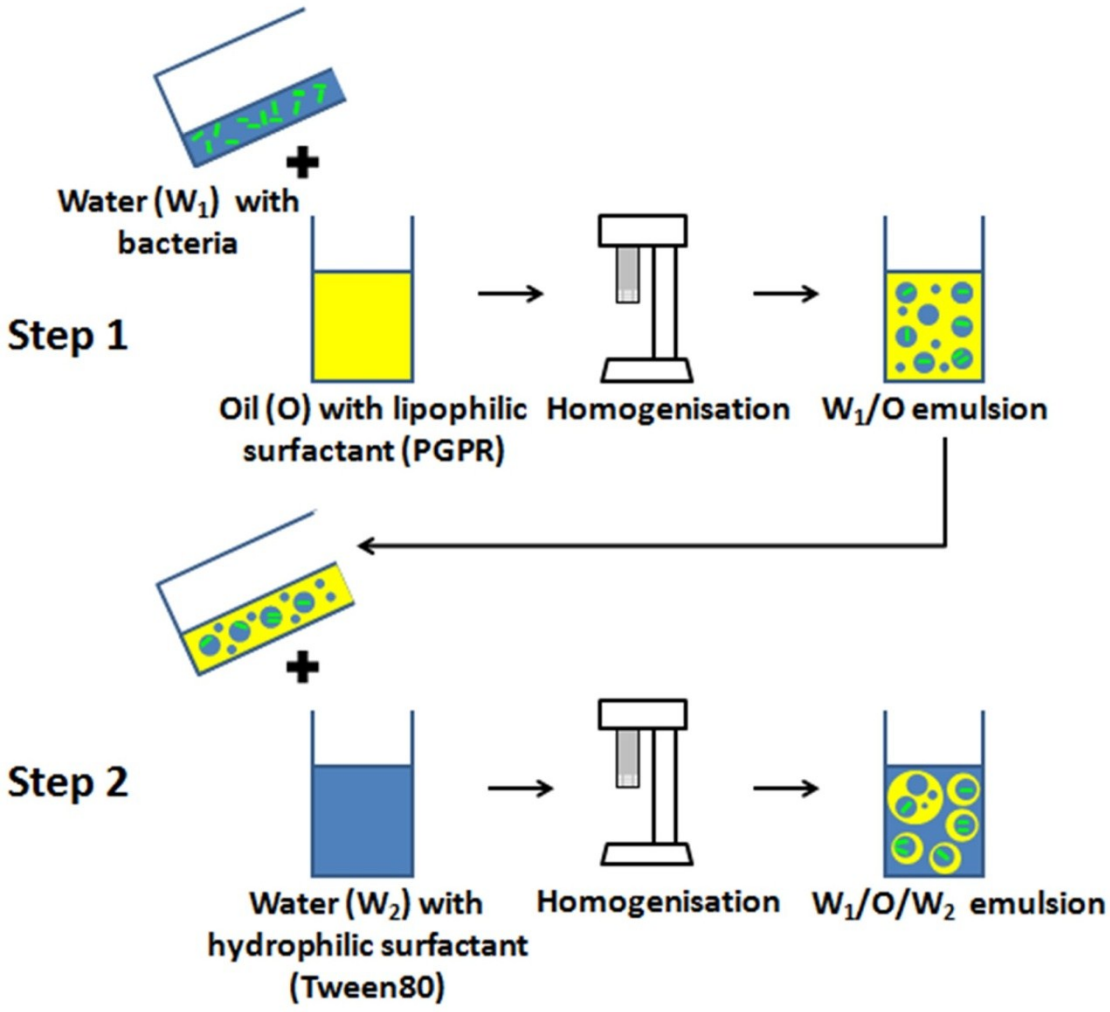

Figure S1. Schematic illustration of the two-step emulsification of $W_1/O/W_2$ emulsion and encapsulation of bacteria.

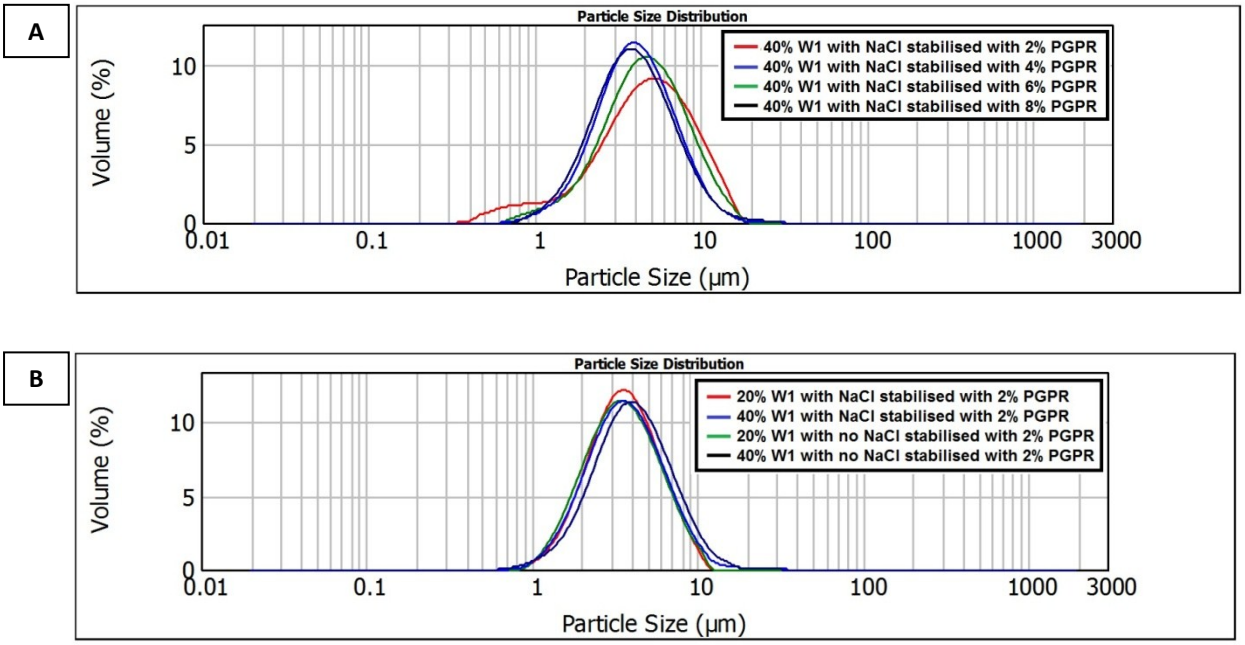

Figure S2. Particle size [D (4, 3)] analysis of the W_1 droplets from primary W_1/O emulsions. The formulations were as follows: A) W_1/O emulsion was prepared with 20% or 40% W_1 with or without bacteria stabilized with 2% PGPR. Measurements were taken at refractive index of 1.33; B) W_1/O emulsion was prepared with 20% or 40% W_1 with or without bacteria stabilized with 2% PGPR. Measurements were taken at refractive index of 1.33.

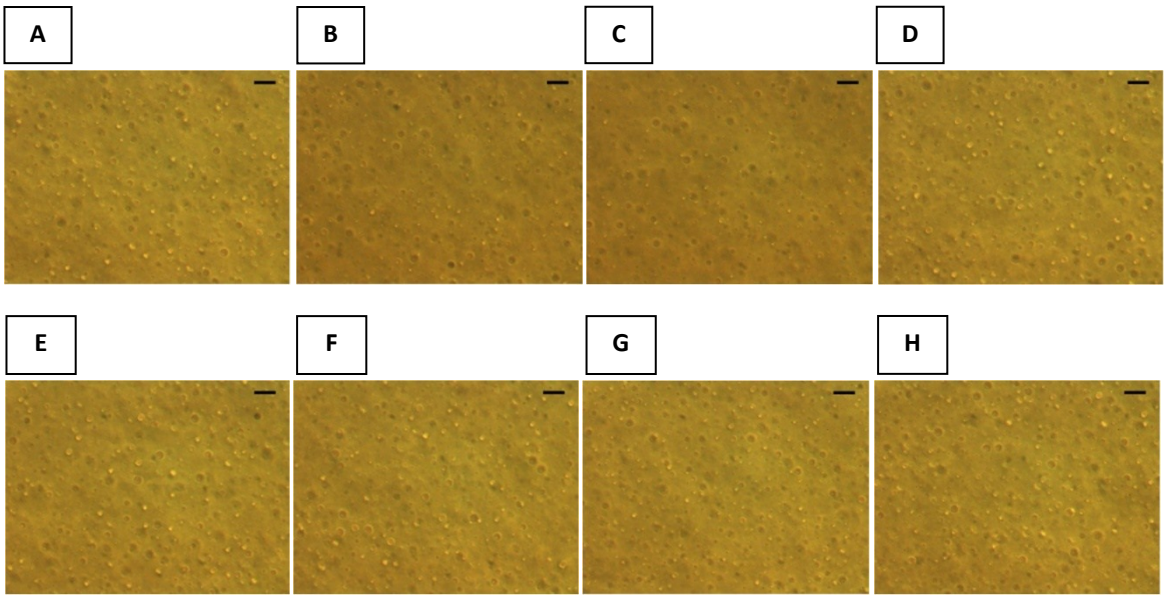

Figure S3. Optical images of primary W_1/O emulsions. Primary W_1/O emulsions were prepared with water (W_1) volume percentage of 20% or 40% with or without bacteria and stabilized with 2% PGPR. The formulations were as follows: A) 40% W_1 with 0.085 M NaCl stabilised with 2% PGPR; B) 40% W_1 with 0.085 M NaCl stabilised with 4% PGPR; C) 40% W_1 with 0.085 M NaCl stabilised with 6% PGPR; D) 40% W_1 with 0.085 M NaCl stabilised with 8% PGPR; E) 20% W_1 with 0.085 M NaCl stabilised with 2% PGPR; F) 20% W_1 with 0.085 M NaCl stabilised with 2% PGPR; G) 20% W_1 with no NaCl stabilised with 2% PGPR; F) 40% W_1 with no NaCl stabilised with 2% PGPR. Scale bar: 10µm.

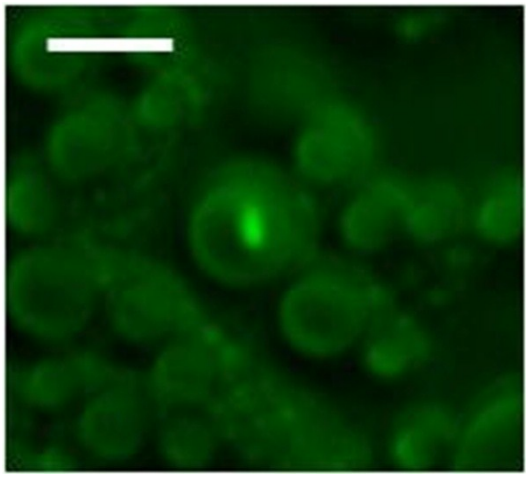

Figure S4. Fluorescent images of W_1 droplets encapsulating *E. coli*-GFP within the primary W_1/O emulsion. Scale bar: 5 μm.

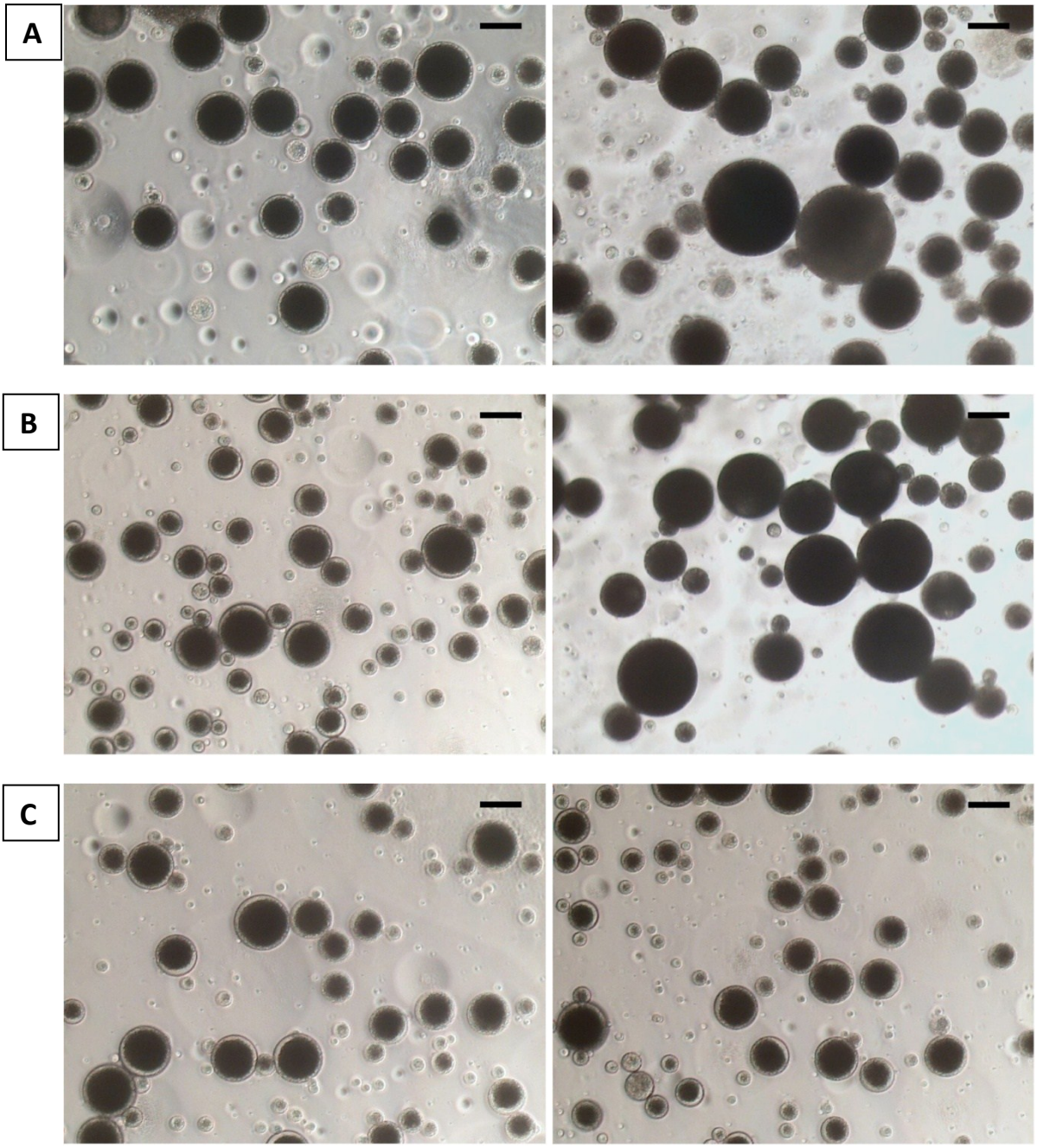

Figure S5. Optical microscopy images of $W_1/O/W_2$ emulsions at 0 (left) and 180 (right) minutes. The $W_1/O/W_2$ emulsions were prepared with inner-phase (W_1) volume percentage of 40% containing *E. coli*-GFP with or without 0.085 M NaCl in the W_1 phase and stabilized with 1% or 5% Tween80 in W_2 and 2% PGPR in the oil phase. The formulations were as follows: A) 40% W_1 and 1% Tween80 with 0.085 M NaCl in W_1; B) 20% W_1 and 1% Tween80 with 0.085 M NaCl in W_1; C) 40% W_1 and 1% Tween80 with no NaCl in W_2. Scale bar: 50µm.

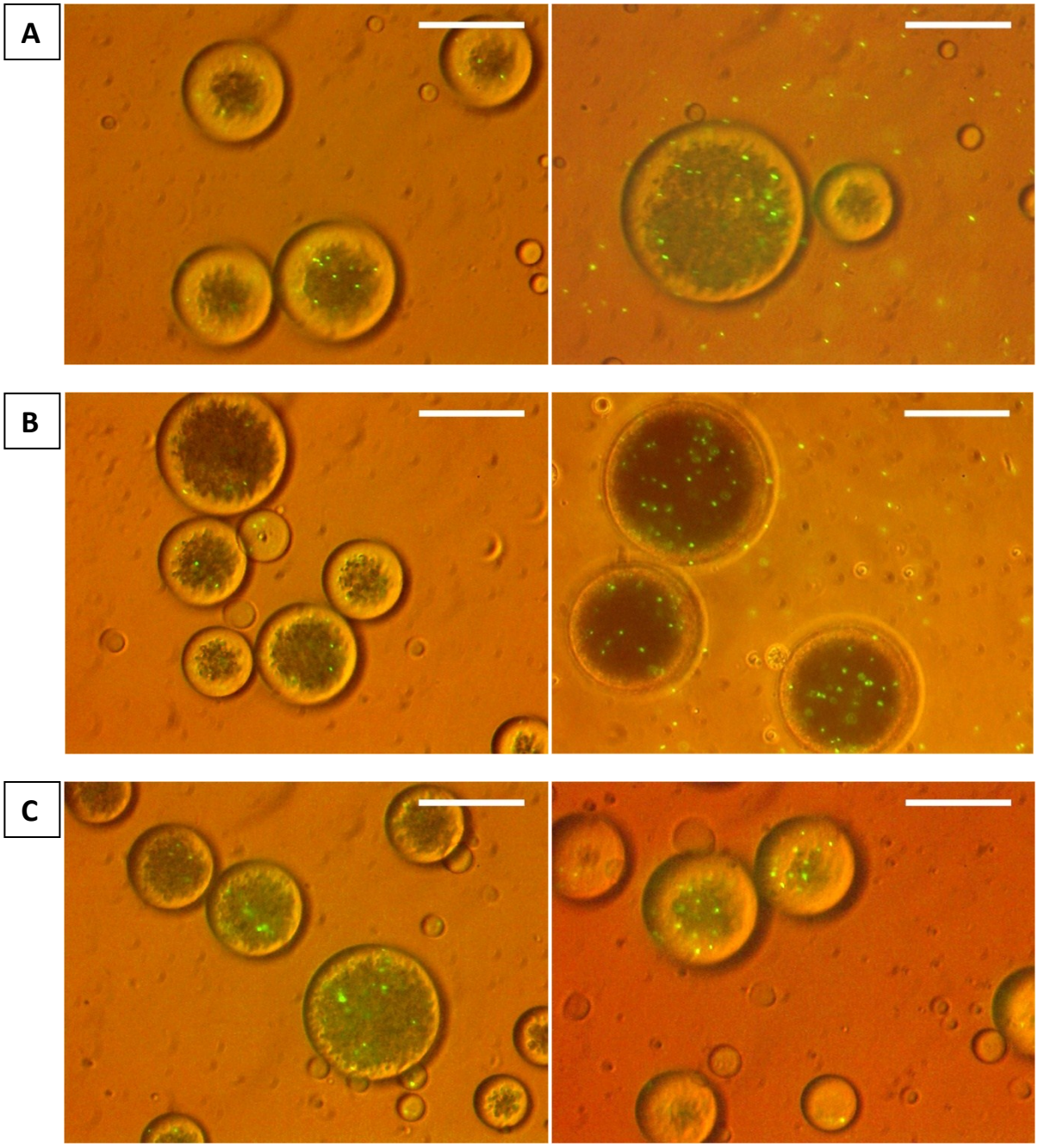

Figure S6. Photomicrographs composed from the optical and fluorescence images of *E.coli*-GFP within $W_1/O/W_2$ double emulsions at 0 (left) and 180 (right) minutes. The $W_1/O/W_2$ emulsions were prepared with inner-phase (W_1) volume percentage of 40% containing *E. coli*-GFP with or without 0.085 M NaCl in the W_1 phase and stabilized with 1% or 5% Tween80 in W_2 and 2% PGPR in the oil phase. The formulations were as follows: A) 40% W_1 and 1% Tween80 with 0.085 M NaCl in W_1; B) 20% W_1 and 1% Tween80 with 0.085 M NaCl in W_1; C) 40% W_1 and 1% Tween80 with no NaCl in W_1. Scale bar: 100µm.

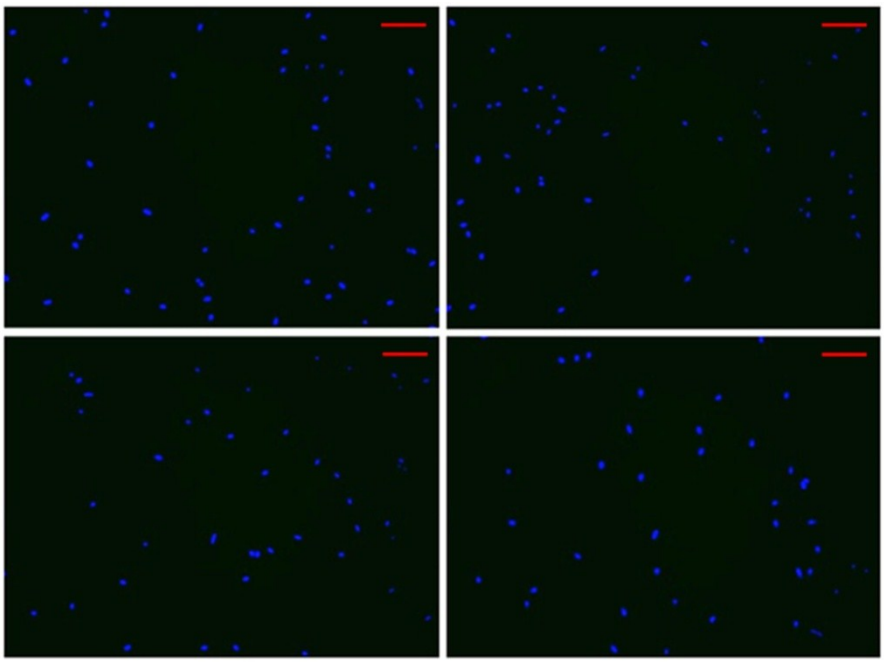

Figure S7. Photomicrographs composed from the optical and fluorescence images of released *E. coli* cells in serum phase (right) after 180 minutes stained with DAPI and $DiBAC_4$ (3). The $W_1/O/W_2$ emulsion was made with 40% W_1 with *E. coli* with 0.085 M NaCl in the W_1 phase stabilised with 1% Tween80 in W_2 and 2% PGPR in the oil phase. Scale bar: 10μm.

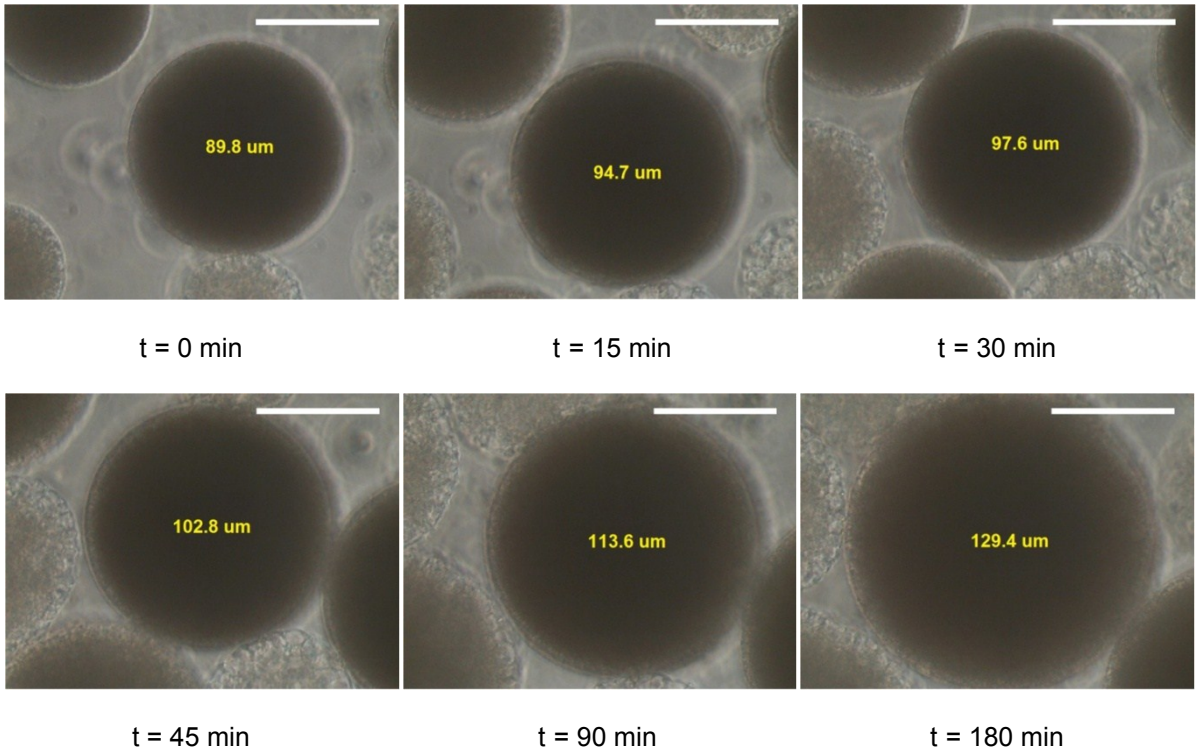

Figure S8. Optical snap shot images showing one $W_1/O/W_2$ emulsion oil globule swelling in hypo-osmotic solution over time (0, 15, 30, 45, 90 and 180 minutes). The $W_1/O/W_2$ emulsions were prepared with inner-phase (W_1) volume percentage of 40% containing *E. coli*-GFP with 0.085 M NaCl in the W_1 phase and stabilized with 1% Tween80 in W_2 and 8% PGPR in the oil phase. To alter the osmotic balance the $W_1/O/W_2$ globules were injected into a fresh W_2 phase with 1% Tween80 containing no salt. The diameter of the oil globule (µm) over time is shown in yellow. Scale bar: 50µm.

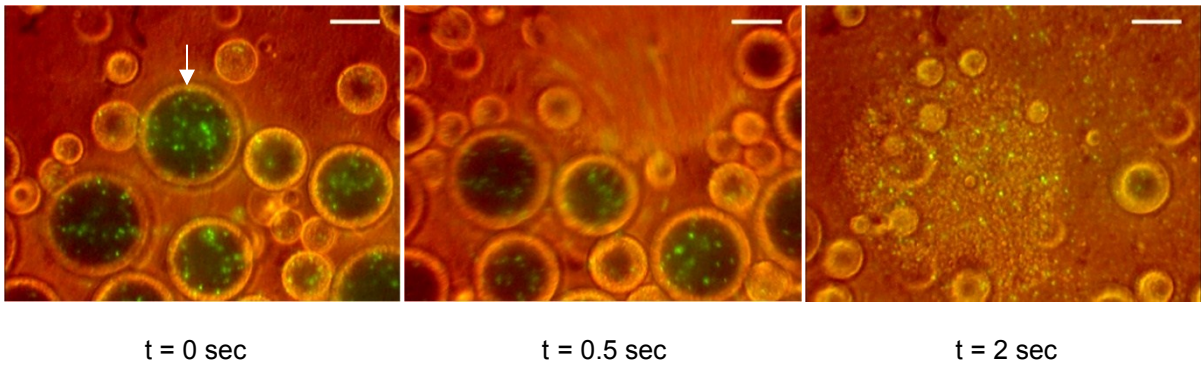

Figure S9. Fluorescence snap shot images obtained from video-microscopy showing oil globule bursting and release of W_1 droplets and *E. coli*-GFP after osmotic balance alteration. Fluorescent images of burst release of *E. coli*-GFP (green) from an oil globule (arrows) in a $W_1/O/W_2$ emulsion at different time points. The $W_1/O/W_2$ emulsion was prepared with inner W_1 phase volume percentage of 40% containing 0.085 M NaCl with bacteria and stabilized with 1% Tween80 in W_2 and 2% PGPR in the oil phase. Scale bar: 50µm.

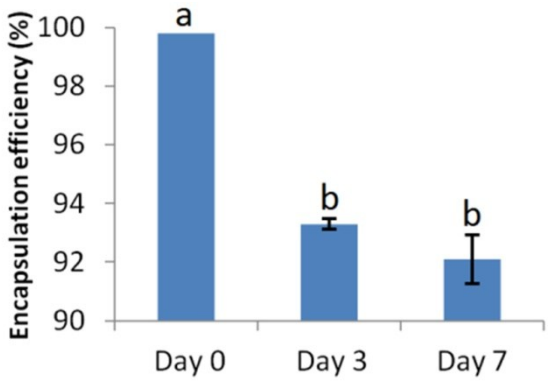

Figure S10. Encapsulation efficiency (%) of *E. coli*-GFP in $W_1/O/W_2$ emulsion at 0, 3 and 7 days. The $W_1/O/W_2$ emulsion was made with 20% W_1 and stabilized with 5% Tween80 in W_2 and 2% PGPR in the oil phase. Results are taken from a minimum of 3 independent experiments. Bars represent mean ± SEM taken from a minimum of 3 independent experiments. Mean values with different letters are significantly different ($P < 0.05$). The data was analysed with one-way ANOVA.

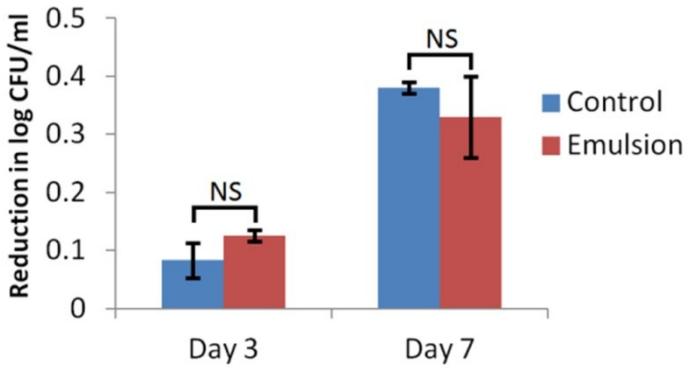

Figure S11. Viability of *E. coli*-GFP in $W_1/O/W_2$ emulsion after 3 and 7 days. The reduction in log number of *E. coli*-GFP cells in serum phase was quantified by plate counting after 3 and 7 days relative to day 0. Control was made with de-ionised water inoculated with *E. coli*-GFP. The $W_1/O/W_2$ emulsion was made with 20% W_1 and stabilized with 5% Tween80 in W_2 and 2% PGPR in the oil phase. Results are taken from a minimum of 3 independent experiments. Bars represent mean ± SEM taken from a minimum of 3 independent experiments. The data was analysed with Student's *t*-Test.

Supplementary Tables

Table S1. The rotational speeds of the rotor used to homogenize the different formulations of primary W_1/O and double $W_1/O/W_2$ emulsion.

W_1/O percentage	Tween80 percentage	PGPR percentage	Rotor speed (rpm) for W_1/O	Rotor speed (rpm) for $W_1/O/W_2$
20	1	2	3000	2000
20	5	2	3000	1800
40	1	2	5000	2700
40	5	2	5000	2300
40	1	4	2000	2700
40	1	6	1500	2700
40	1	8	1200	2700
40	0.5	2	5000	2900
40	10	2	5000	2000

Table S2. Change in mean oil globule diameter size (µm) of $W_1/O/W_2$ emulsions without osmotic balance alteration measured by light scattering [D (4, 3)] after 45, 90 and 180 minutes relative to time 0 minutes incubated at 25°C. The $W_1/O/W_2$ emulsions were prepared with different inner-phase (W_1) volume percentage of 20% or 40% in the presence or absence of *E. coli*-GFP stabilised with or without 0.085 M NaCl stabilised with 1% or 5% Tween80 in W_2 and 2% PGPR in the oil phase. Results are taken from a minimum of 3 independent experiments.

	$W_1/O/W_2$ emulsion formulations	45 minutes	90 minutes	180 minutes
No *E. coli*-GFP	20% W_1, 1% Tw80, 0.085 M NaCl in W_1 and W_2	1.14 ± 1^{a}	-2.19 ± 0.9^{a}	-1.13 ± 1.4^{a}
	20% W_1, 1% Tw80, no NaCl in W_1 and W_2	-0.97 ± 0.4^{a}	0.44 ± 2.4^{a}	-0.73 ± 1.3^{a}
	20% W_1, 5% Tw80, 0.085 M NaCl in W_1 and W_2	2.2 ± 2.2^{a}	-1.83 ± 1.6^{a}	-2.7 ± 1.6^{a}
	20% W_1, 5% Tw80, no NaCl in W_1 and W_2	-2 ± 0.4^{a}	$-0.95.\pm0.6^{a}$	-0.28 ± 0.2^{a}
	40% W_1, 1% Tw80, 0.085 M NaCl in W_1 and W_2	-0.6 ± 0.3^{a}	-0.67 ± 0.3^{a}	-0.65 ± 0.8^{a}
	40% W_1, 1% Tw80, no NaCl in W_1 and W_2	1.15 ± 0.5^{a}	0.86 ± 0.6^{a}	0.61 ± 0.5^{a}
	40% W_1, 5% Tw80, 0.085 M NaCl in W_1 and W_2	0.08 ± 0.6^{a}	-0.01 ± 0.6^{a}	0.26 ± 0.6^{a}
	40% W_1, 5% Tw80, no NaCl in W_1 and W_2	0.49 ± 0.3^{a}	0.14 ± 0.1^{a}	0.37 ± 0.1^{a}
With *E. coli*-GFP	20% W_1, 1% Tw80, 0.085 M NaCl in W_1 and W_2	0.88 ± 0.5^{ab}	0.49 ± 0.5^{ab}	0.97 ± 0.3^{ab}
	20% W_1, 1% Tw80, no NaCl in W_1 and W_2	0.93 ± 0.9^{ab}	-0.57 ± 0.8^{ab}	2 ± 0.4^{ab}
	20% W_1, 5% Tw80, 0.085 M NaCl in W_1 and W_2	-0.36 ± 1^{ab}	-0.11 ± 0.9^{ab}	-0.11 ± 0.7^{a}
	20% W_1, 5% Tw80, no NaCl in W_1 and W_2	1.95 ± 0.7^{ab}	0.69 ± 0.5^{ab}	1.11 ± 0.9^{ab}
	40% W_1, 1% Tw80, 0.085 M NaCl in W_1 and W_2	-0.614 ± 0.5^{ab}	-1.5 ± 0.7^{ab}	-1.2 ± 1.7^{a}
	40% W_1, 1% Tw80, no NaCl in W_1 and W_2	2.3 ± 1^{ab}	2.1 ± 1.3^{ab}	2.2 ± 0.8^{a}
	40% W_1, 5% Tw80, 0.085 M NaCl in W_1 and W_2	0.7 ± 0.4^{ab}	-4.1 ± 0.3^{a}	0.32 ± 0.7^{a}
	40% W_1, 5% Tw80, no NaCl in W_1 and W_2	1.93 ± 0.4^{ab}	4.3 ± 0.9^{b}	0.29 ± 1.1^{a}

The data was analysed with one-way ANOVA.

$^{a, b}$ means ± standard deviation with different letters are significantly different

Table S3. Change in mean oil globule diameter size (μm) of $W_1/O/W_2$ emulsions by light scattering [D (4, 3)] and creaming thickness after 3 and 7 days relative to day 0 incubated at 25°C. The $W_1/O/W_2$ emulsions were prepared with 20% W_1 and stabilised with 5% Tween80 in W_2 and 2% PGPR in the oil phase with no bacteria (control) or *E. coli*-GFP in W_1. Results are taken from a minimum of 3 independent experiments.

$W_1/O/W_2$ formulation	Day	Change in D (4, 3)	Change in creaming thickness
Control (no bacteria)	3	-0.29±0.19[a]	NC
	7	-0.17±0.32[a]	NC
E. coli in W_1	3	-0.07±1.34[a]	NC
	7	-0.03±0.06[a]	NC

The data was analysed with one-way ANOVA.

[a] means ± standard deviation with different letters are significantly different

NC = no change

Table S4. Change in percentage of cream layer thickness of $W_1/O/W_2$ emulsions without osmotic balance alteration after 45, 90 and 180 minutes relative to time 0 minutes incubated at 25°C. The $W_1/O/W_2$ emulsions were prepared with different inner-phase (W_1) volume percentage of 20% or 40% with (A) or without (B) *E. coli*-GFP with 0.085 M NaCl in the W_1 phase stabilised with 1% or 5% Tween80 in W_2 and 2% PGPR in the oil phase. Results are taken from a minimum of 3 independent experiments.

	$W_1/O/W_2$ emulsion formulations	45 minutes	90 minutes	180 minutes
No *E. coli*-GFP	20% W_1, 1% Tw80, 0.085 M NaCl in W_1 and W_2	1.39±2[a]	6.5E-15±6.8[a]	7E-15±6.8[a]
	20% W_1, 1% Tw80, no NaCl in W_1 and W_2	1.39±2[a]	7E-15±6.8[a]	7E-15±6.8[a]
	20% W_1, 5% Tw80, 0.085 M NaCl in W_1 and W_2	2.77±3.8[a]	1.39±2[a]	2.77±3.8[a]
	20% W_1, 5% Tw80, no NaCl in W_1 and W_2	7E-15±6.8[a]	2.77±3.8[a]	7E-15±6.8[a]
	40% W_1, 1% Tw80, 0.085 M NaCl in W_1 and W_2	7E-15±6.8[a]	-1.39±2[a]	-8.33±6.7[a]
	40% W_1, 1% Tw80, no NaCl in W_1 and W_2	7E-15±6.8[a]	-5.33±6.7[a]	-8.33±6.7[a]
	40% W_1, 5% Tw80, 0.085 M NaCl in W_1 and W_2	7E-15±6.8[a]	-1.39±2[a]	-8.33±0[a]
	40% W_1, 5% Tw80, no NaCl in W_1 and W_2	7E-15±6.8[a]	-1.39±2[a]	-11.11±6.8[a]
With *E. coli*-GFP	20% W_1, 1% Tw80, 0.085 M NaCl in W_1 and W_2	-2.77±4.8[a]	7E-15±8.3[a]	5.55±4.8[a]
	20% W_1, 1% Tw80, no NaCl in W_1 and W_2	-2.77±4.8[a]	7E-15±8.3[a]	2.77±4.8[a]
	20% W_1, 5% Tw80, 0.085 M NaCl in W_1 and W_2	6.5E-15±8.3[a]	-2.77±4.8[a]	-2.77±2.4[a]
	20% W_1, 5% Tw80, no NaCl in W_1 and W_2	6.5E-15±8.3[a]	-2.77±4.8[a]	-4.16±4.2[a]
	40% W_1, 1% Tw80, 0.085 M NaCl in W_1 and W_2	6.5E-15±8.3[a]	-2.77±4.8[a]	-2.77±4.8[a]
	40% W_1, 1% Tw80, no NaCl in W_1 and W_2	6.5E-15±8.3[a]	-2.77±4.8[a]	-4.16±11[a]
	40% W_1, 5% Tw80, 0.085 M NaCl in W_1 and W_2	7E-15±8.3[a]	7E-15±8.3[a]	-2.77±4.8[a]
	40% W_1, 5% Tw80, no NaCl in W_1 and W_2	6.5E-15±8.3[a]	-4.16±4.1[a]	6.5E-15±8.3[a]

The data was analysed with one-way ANOVA.

[a] means ± standard deviation with different letters are significantly different

Table S5. Viscosity (mPas.s) measurements of the oil phase, W_2 phase and primary W_1/O emulsion at 25°C. The oil phase was prepared with sunflower oil without or with PGPR (2%, 4%, 6%, or 8%). The W_2 phase was prepared without or with Tween80 (0.5%, 1%, 5% or 10%). Primary W_1/O emulsions were prepared with W_1 volume percentage of 20% or 40% in the presence of *E. coli*-GFP with 0.085 M NaCl in the W_1 phase and stabilised and 2% PGPR in the oil phase. Results are taken from a minimum of 3 independent experiments.

Component	Viscosity (mPa.s)
Oil phase	
Sunflower	14.21±0.89[a]
Sunflower oil + 2% PGPR	22.73±4.05[a]
Sunflower oil + 4% PGPR	22.05±0.9[ab]
Sunflower oil + 6% PGPR	34.53±6.29[bc]
Sunflower oil + 8% PGPR	44.58±3.92[c]
W_2 phase	
Water	0.83±0.47[a]
Water + 0.5% Tween80	0.44±0.52[a]
Water + 1% Tween80	0.47±0.01[a]
Water + 5% Tween80	0.54±0.01[a]
Water + 10% Tween80	0.94±0.07[a]
Primary W_1/O emulsion with *E. coli*-GFP in W_1	
W_1/O (20:80)	20.96±1.66[a]
W_1/O (40:60)	55.26±8.59[b]

The data was analysed with one-way ANOVA and Student's *t*-Test.

[a, b, c] means ± standard deviation with different letters are significantly different

Table S6. The encapsulation efficiency (%) of *E. coli*-GFP and D (4, 3) of different $W_1/O/W_2$ emulsions. Results show means ± standard deviation taken from a minimum of 3 independent experiments.

Formulations	Encapsulation efficiency (%)	D (4,3)
40% W_1, 1% Tw80, 2% PGPR (NaCl)	99.9±0.03	70.6±5.4
40% W_1, 1% Tw80, 4% PGPR (NaCl)	99.8±0.06	71.6±3.6
40% W_1, 1% Tw80, 6% PGPR (NaCl)	99.9±0.03	71.9±4.5
40% W_1, 1% Tw80, 8% PGPR (NaCl)	99.8±0.03	72.6±4.4
40% W_1, 0.5% Tw80, 2% PGPR (NaCl)	99.8±0.01	66.6±0.9
40% W_1, 5% Tw80, 2% PGPR (NaCl)	99.9±0.05	69.6±3.7
40% W_1, 10% Tw80, 2% PGPR (NaCl)	99.8±0.05	73.6±1.3
20% W_1, 1% Tw80, 2% PGPR (NaCl)	99.9±0.01	67±0.2
20% W_1, 5% Tw80, 2% PGPR (NaCl)	99.8±0.16	66.9±0.1
20% W_1, 1% Tw80, 2% PGPR (water)	99.9±0.02	70.9±4
20% W_1, 5% Tw80, 2% PGPR (water)	99.8±0.03	70.5±5.2
40% W_1, 1% Tw80, 2% PGPR (water)	99.9±0.06	71.5±0.4
40% W_1, 5% Tw80, 2% PGPR (water)	99.8±0.02	70±2.7

Supplementary Videos

Video S1. Fluorescence video-microscopy showing bursting of oil globule and release of *E. coli*-GFP after altering the osmotic balance by injecting in the $W_1/O/W_2$ emulsion in a hypo-osmotic solution. The $W_1/O/W_2$ emulsion was prepared with inner W_1 phase volume percentage of 40% with 0.085 M NaCl containing *E. coli*-GFP and stabilized with 1% Tween80 in W_1 and 2% PGPR in the oil phase.

References

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2. J. E. Hobbie, R. J. Daley and S. Jasper, Use of nuclepore filters for counting bacteria by fluorescence microscopy, *Applied and Environmental Microbiology*. 1977, **33**(5), 1225.