

1 Supplementary materials

2 1. Supplementary methods

3 1.1. Metabolites extraction

4 Single colonies were inoculated in 10 mL M9 media in test tubes, incubate at 37 °C aerobically
5 until OD₆₀₀ reached ~1.0. The cells were centrifuged at 2,800× g for 10 minutes, supernatants were
6 removed and the pellets were resuspended in M9 at an OD₆₀₀ of ~10.0. Then 500 µL samples were
7 inoculated into 50 mL M9 or M9 supplemented with 0.6 M NaCl in 250 mL screw-capped flasks at an
8 OD₆₀₀ of ~0.1, and incubated at 37 °C with shaking (275 rpm). Two technical replicates were made for
9 each sample. When OD₆₀₀ of samples reached exponential phase (OD₆₀₀ ~0.7 - 1), the two technical
10 replicates with a total volume of 100 mL were combined and cells were harvested by centrifugation
11 at 9,000× g, 1 mL supernatant with extracellular metabolites was filtered using 0.2 µm syringe filter
12 (VWR, Radnor, PA USA) and kept at -20 °C. The remaining supernatants were removed completely.
13 The cell pellets were washed with 5 mL fresh media, and then the supernatants were removed
14 completely. Intracellular metabolites were extracted by suspending the cell pellets in 500 µL -20 °C
15 intracellular metabolites extraction buffer (acetonitrile: methanol: water (40:40:20)) [1] and incubated
16 at -20 °C for 30 minutes. Then samples were centrifuged (9,000× g) at 4 °C for 5 minutes, and the
17 supernatant was kept at -20 °C. This extraction step was repeated for another two times using 400 µL
18 and 300 µL intracellular metabolites extraction buffer (acetonitrile: methanol: water (40:40:20))
19 respectively with 15 minutes incubation at -20 °C and then centrifuged (9,000× g) at 4 °C for 5 minutes.
20 The supernatants from the three extraction steps were combined. Then 500 µL water was combined
21 with 1 mL extracted intracellular metabolites, filtered using 0.2 µm syringe filter (VWR, Radnor, PA
22 USA) and kept at -20°C.

23 1.2. HPLC analysis of free amino acids

24 Free amino acids were analyzed using modified Agilent amino acid analysis method [2]. We
25 used high-performance liquid chromatography (HPLC; Agilent Technologies, 1260 Infinity, Santa
26 Clara, CA) and Cogent Bidentate C18™ (4 µm, 100 Å. Dimensions: 4.6 mm i.d. x 150 mm) column
27 (MicroSolv Technology, Eatontown, NJ USA) at 40 °C. Flow rate of mobile phase was 0.6 mL min⁻¹.
28 The mobile phase and reagents are listed in Table S1. Injection program and mobile phase gradients
29 are listed in Table S2 and Table S3. The detection wavelength was 338 nm from 0 - 18 min, and 262
30 nm from 18 - 30 min.
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32 **2. Supplementary Tables**33 **Table S1.** Reagents used in analysis of amino acids.

Reagents	Composition
Mobile phase A	10 mM Na ₂ HPO ₄ : 10 mM Na ₂ B ₄ O ₇ : 5 mM NaN ₃ , pH 8.2
Mobile phase B	acetonitrile: methanol: water (45:45:10, v: v: v)
Borate buffers	0.4 M in water, pH 10.2
FMOc	2.5 mg mL ⁻¹ in acetonitrile
OPA	10 mg mL ⁻¹ Phthaldialdehyd and 3-Mercaptopropionic acids in 0.4 M borate buffer

34 **Table S2.** Injection program.

Steps	Injection program
1	Draw 12.5 µL from borate vial.
2	Draw 5 µL from sample vial.
3	Mix 17.5 µL from air 5 times.
4	Wait 0.2 min.
5	Draw 2.5 µL from OPA vial.
6	Mix 20 µL from air 10 times default speed.
7	Draw 2 µL from FMOc vial.
8	Mix 22 µL from air 10 times default speed.
9	Inject.
10	Wait 0.1 min.
11	Valve bypass.

35 **Table S3.** Mobile phase gradients.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	98	2
0.5	98	2
20	43	57
20.1	0	100
23.5	0	100
23.6	98	2
25	98	2
35	98	2

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Table S4. Growth in micro-aerobic condition in M9 supplemented with 0.9 M NaCl.

Strains	0 h	24 h	48 h	72 h
BW25113	-	+	-	-
EJW3	-	+	-	-
JW1253*	-	+	+	+
EJW4*	-	+	+	+
Hfr-2×SFX-*	-	+	+	+
G3*	-	+	+	+

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* Tryptophan (50 µg mL⁻¹) was supplemented

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- OD₆₀₀ < 0.1, + 0.1 < OD₆₀₀ < 0.5, ++ 0.5 < OD₆₀₀ < 1.0, +++ 1.0 < OD₆₀₀ < 2.0, ++++ OD₆₀₀ > 2.0

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Table S5. Growth in aerobic condition in M9 supplemented with 0.8 M NaCl.

Strains	0 h	24 h	48 h	72 h
BW25113	-	-	-	-
EJW3	-	+	-	-
JW1253*	-	+	+	+
EJW4*	-	+	+	+
Hfr-2×SFX-*	-	+	-	-
G3*	-	+	+	+

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* Tryptophan (50 µg mL⁻¹) was supplemented

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- OD₆₀₀ < 0.1, + 0.1 < OD₆₀₀ < 0.5, ++ 0.5 < OD₆₀₀ < 1.0, +++ 1.0 < OD₆₀₀ < 2.0, ++++ OD₆₀₀ > 2.0

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Table S6. Cell density (OD₆₀₀) in M9 supplemented with 0.65 M NaCl and amino acids.

Amino acid	Concentration (µg mL ⁻¹)	24h		48h	
		BW25113	EJW3	BW25113	EJW3
Control ^a	0	0.22 ± 0.09	0.70 ± 0.31	0.90 ± 0.45	3.03 ± 0.30
Ala	10	0.34 ± 0.09	1.19 ± 0.44	0.80 ± 0.44	2.92 ± 0.29
	100	1.77 ± 0.58*	2.78 ± 0.30*	2.20 ± 0.10*	3.11 ± 0.15
	1000	0.93 ± 0.33	0.22 ± 0.10*	4.13 ± 0.47*	0.45 ± 0.26*
Arg	10	0.14 ± 0.01*	1.76 ± 0.53	0.71 ± 0.16	3.57 ± 0.38
	100	1.70 ± 0.44*	2.84 ± 0.17*	2.90 ± 0.33*	3.77 ± 0.20*
	1000	2.40 ± 0.26*	2.69 ± 0.26*	2.93 ± 0.58*	3.23 ± 0.29
Asn	10	0.41 ± 0.02*	1.90 ± 0.42*	2.63 ± 0.14*	3.49 ± 0.25
	100	3.16 ± 0.30*	3.04 ± 0.19*	3.30 ± 0.10*	4.02 ± 0.15*
	1000	3.04 ± 0.46*	3.45 ± 0.29*	3.44 ± 0.22*	4.12 ± 0.52
Asp	10	0.33 ± 0.08	0.60 ± 0.12	1.02 ± 0.46	2.77 ± 0.10*
	100	1.82 ± 0.29*	2.77 ± 0.21*	2.50 ± 0.09*	3.08 ± 0.32
	1000	2.21 ± 0.52*	3.13 ± 0.09*	2.27 ± 0.31*	3.65 ± 0.22*
Cys	10	2.68 ± 0.25*	2.95 ± 0.25*	3.10 ± 0.24*	4.04 ± 0.25*
	100	0.78 ± 0.22*	1.60 ± 0.12*	2.68 ± 0.05*	2.33 ± 0.04*
	1000	0.10 ± 0.01*	0.09 ± 0.01*	1.90 ± 0.78	1.05 ± 1.47
Glu	10	0.19 ± 0.03	1.16 ± 0.10*	1.46 ± 0.12*	2.84 ± 0.19
	100	1.88 ± 0.44*	2.64 ± 0.11*	2.56 ± 0.18*	3.04 ± 0.19
	1000	2.48 ± 0.39*	2.74 ± 0.12*	3.33 ± 0.51*	3.35 ± 0.31
Gln	10	0.29 ± 0.08	0.77 ± 0.16	0.98 ± 0.38	3.01 ± 0.29
	100	0.94 ± 0.10*	2.76 ± 0.06*	2.69 ± 0.13*	3.30 ± 0.14*
	1000	2.75 ± 0.41*	3.19 ± 0.52*	3.09 ± 0.22*	3.86 ± 0.35*
Gly	10	0.40 ± 0.01*	1.88 ± 0.05*	1.85 ± 0.14*	3.37 ± 0.19
	100	2.01 ± 0.14*	3.17 ± 0.23*	2.79 ± 0.07*	3.83 ± 0.05*
	1000	0.50 ± 0.13	2.17 ± 0.36*	0.28 ± 0.07*	3.52 ± 0.09*
His	10	2.38 ± 0.31*	3.05 ± 0.20*	3.27 ± 0.18*	4.15 ± 0.18*
	100	2.48 ± 0.23*	2.80 ± 0.15*	3.24 ± 0.07*	3.85 ± 0.26*
	1000	2.63 ± 0.21*	2.96 ± 0.10*	3.12 ± 0.30*	3.54 ± 0.12*
Ile	10	0.23 ± 0.04	1.72 ± 0.14*	1.29 ± 0.13*	3.15 ± 0.23
	100	2.04 ± 0.07*	2.97 ± 0.07*	1.81 ± 0.16*	3.69 ± 0.16*
	1000	0.39 ± 0.05*	0.21 ± 0.02*	0.46 ± 0.06*	0.22 ± 0.02*
Leu	10	0.62 ± 0.19	1.51 ± 0.06*	2.28 ± 0.53*	3.15 ± 0.04
	100	1.97 ± 0.14*	1.78 ± 0.21*	2.52 ± 0.14*	2.04 ± 0.10*
	1000	0.40 ± 0.08*	0.27 ± 0.04*	0.36 ± 0.11*	0.27 ± 0.05*
Lys	10	0.27 ± 0.03	0.77 ± 0.10	1.00 ± 0.82	2.92 ± 0.07
	100	1.41 ± 0.27*	2.75 ± 0.24*	2.47 ± 0.15*	3.11 ± 0.21
	1000	2.79 ± 0.15*	2.34 ± 0.06*	2.66 ± 0.37*	3.12 ± 0.50
Met	10	0.23 ± 0.02	0.57 ± 0.06	0.18 ± 0.01*	2.68 ± 0.20

	100	0.31 ± 0.01*	1.87 ± 0.31*	0.25 ± 0.10*	1.84 ± 0.07*
	1000	0.18 ± 0.00	0.27 ± 0.10*	0.09 ± 0.01*	0.21 ± 0.11*
Phe	10	0.36 ± 0.02*	2.19 ± 0.16*	2.25 ± 0.36*	3.32 ± 0.33
	100	2.69 ± 0.20*	2.84 ± 0.06*	3.37 ± 0.37*	3.62 ± 0.04*
	1000	2.88 ± 0.27*	2.66 ± 0.08*	3.83 ± 0.07*	4.08 ± 0.04*
Pro	10	0.39 ± 0.01*	1.00 ± 0.09*	0.89 ± 0.12	3.26 ± 0.24
	100	2.27 ± 0.80*	0.23 ± 0.02*	3.53 ± 0.10*	0.42 ± 0.14*
	1000	1.25 ± 0.65	0.21 ± 0.01*	3.69 ± 0.12*	0.58 ± 0.16*
Ser	10	0.15 ± 0.01*	1.60 ± 0.35*	0.79 ± 0.03	3.21 ± 0.24
	100	2.70 ± 0.33*	2.96 ± 0.28*	3.29 ± 0.20*	3.91 ± 0.06*
	1000	0.27 ± 0.02	0.45 ± 0.08*	0.27 ± 0.02*	0.52 ± 0.11*
Thr	10	0.44 ± 0.07*	1.28 ± 0.31	2.20 ± 0.24*	3.08 ± 0.12
	100	2.80 ± 0.54*	2.92 ± 0.01*	2.59 ± 0.34*	3.43 ± 0.08*
	1000	0.28 ± 0.11	0.16 ± 0.02*	0.51 ± 0.51	0.31 ± 0.15*
Trp	10	0.52 ± 0.06*	2.48 ± 0.26*	2.31 ± 0.12*	3.73 ± 0.41
	100	2.60 ± 0.09*	3.10 ± 0.14*	3.36 ± 0.14*	3.71 ± 0.12*
	1000	1.85 ± 0.23*	2.72 ± 0.09*	2.76 ± 0.18*	3.61 ± 0.27*
Tyr	10	0.73 ± 0.12*	2.67 ± 0.22*	2.79 ± 0.16*	3.76 ± 0.05*
	100	2.78 ± 0.33*	2.73 ± 0.35*	3.74 ± 0.12*	3.29 ± 0.35
	400	2.05 ± 0.41*	1.94 ± 0.03*	3.32 ± 0.06*	3.10 ± 0.11
Val	10	0.21 ± 0.01	0.19 ± 0.00*	0.20 ± 0.01*	0.18 ± 0.06*
	20	0.23 ± 0.01	0.19 ± 0.00*	0.23 ± 0.01*	0.19 ± 0.01*
	40	0.24 ± 0.01	0.20 ± 0.01*	0.25 ± 0.02*	0.20 ± 0.02*

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^a Control is the condition with no addition of amino acid, and includes fifteen biological replicates from five batches, three biological replicates per batch.

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* Statistically significantly different from control (p -value < 0.05). P -values are calculated using a two-tailed student's t -test. Significantly increased values are bolded. Significantly reduced values are bolded and italicized.

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Table S7. Quantification of intracellular and extracellular metabolites in M9.

	Metabolite	BW25113	EJW3	P-value	
Intracellular	Trehalose ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	-	-	-	
	Glucose ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	770 \pm 140	800 \pm 110	0.706	
	Acetic acid ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	650 \pm 90	390 \pm 80	0.000*	
	Asp ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	-	-	-	
	Glu ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	187.72 \pm 35.94	212.37 \pm 27.2	0.210	
	Asn Ser ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	9.45 \pm 0.75	10.11 \pm 0.94	0.204	
	Gln Gly His Thr ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	10.82 \pm 0.67	10.14 \pm 2.49	0.534	
		1)			
	Ala ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	81.18 \pm 16.55	83.71 \pm 3.15	0.721	
	Arg ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	-	4.78 \pm 7.41	0.145	
	Tyr ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	-	4.10 \pm 10.04	0.341	
	Val ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	-	-	-	
	Met ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	55.11 \pm 6.01	74.52 \pm 10.88	0.003*	
	Trp ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	-	-	-	
	Phe Ile ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	-	-	-	
	Leu ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	3.83 \pm 9.38	17.56 \pm 13.81	0.072	
	Lys ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	6.95 \pm 10.78	7.08 \pm 11.24	0.983	
	Pro ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	61.64 \pm 6.12	75.06 \pm 1.95	0.000*	
	Extracellular	Trehalose ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	-	-	-
Glucose ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)		5280 \pm 300	5380 \pm 140	0.466	
Acetic acid ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)		280 \pm 20	370 \pm 10	0.000*	
Asp ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)		-	-	-	
Glu ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)		-	6.42 \pm 12.41	0.234	
Asn Ser ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)		-	-	-	
Gln Gly His Thr ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)		-	-	-	
		1)			
Ala ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)		10.86 \pm 6.33	1.93 \pm 4.72	0.020*	
Arg ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)		2.95 \pm 7.21	-	0.341	
Tyr ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)		26.01 \pm 14.8	45.14 \pm 5.72	0.014*	
Val ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)		-	-	-	
Met ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)		3.92 \pm 9.60	41.35 \pm 11.43	0.000*	
Trp ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)		24.85 \pm 60.86	131.60 \pm 90.56	0.038*	
Phe Ile ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)		89.07 \pm 45.53	20.37 \pm 49.90	0.032*	
Leu ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)		-	-	-	
Lys ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)		-	-	-	
Pro ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)		516.13 \pm 35.09	776.02 \pm 117.02	0.000*	

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- Not detectible. * Statistically significantly different between BW25113 and EJW3 (p -values < 0.05).

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Values bolded are significantly higher in EJW3. Values bolded and italicized are significantly lower

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in EJW3. P -values are calculated by using a two-tailed student's t -test with six biological replicates.

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57**Table S8.** Quantification of intracellular and extracellular metabolites in M9 supplemented with 0.6 M NaCl.

	Metabolite	BW25113	EJW3	P-value
Intracellular	Trehalose ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	1190 \pm 190	1630 \pm 220	0.005*
	Glucose ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	1050 \pm 80	1030 \pm 180	0.797
	Acetic acid ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	270 \pm 140	370 \pm 50	0.137
	Asp ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	-	-	-
	Glu ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	565.50 \pm 44.39	698.75 \pm 82.47	0.006*
	Asn Ser ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	1.02 \pm 2.50	6.74 \pm 3.71	0.011*
	Gln Gly His Thr ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	8.64 \pm 1.59	9.69 \pm 4.83	0.623
	Ala ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	38.49 \pm 9.03	71.58 \pm 13.51	0.001*
	Arg ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	28.79 \pm 2.73	44.87 \pm 6.42	0.000*
	Tyr ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	-	-	-
	Val ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	-	0.44 \pm 1.08	0.341
	Met ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	21.29 \pm 6.35	30.55 \pm 2.53	0.008*
	Trp ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	-	-	-
	Phe Ile ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	-	3.24 \pm 5.27	0.164
	Leu ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	-	-	-
	Lys ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	-	9.08 \pm 10.05	0.051
Pro ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	94.31 \pm 6.14	107.31 \pm 18.37	0.131	
Extracellular	Trehalose ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	-	-	-
	Glucose ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	4950 \pm 770	4600 \pm 1000	0.518
	Acetic acid ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	260 \pm 20	360 \pm 20	0.000*
	Asp ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	-	-	-
	Glu ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	234.82 \pm 15.44	88.05 \pm 19.38	0.000*
	Asn Ser ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	-	-	-
	Gln Gly His Thr ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	-	-	-
	Ala ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	-	-	-
	Arg ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	-	7.90 \pm 12.34	0.148
	Tyr ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	41.73 \pm 6.31	16.05 \pm 24.95	0.035*
	Val ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	-	-	-
	Met ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	40.03 \pm 8.39	38.80 \pm 18.26	0.884
	Trp ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	88.49 \pm 30.23	20.22 \pm 49.52	0.016*
	Phe Ile ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	0.86 \pm 2.10	85.07 \pm 46.36	0.001*
	Leu ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	-	-	-
	Lys ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	-	-	-
Pro ($\mu\text{g mL}^{-1} \text{OD}_{600^{-1}}$)	747.75 \pm 120.88	728.90 \pm 156.43	0.820	

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- Not detectible. * Statistically significantly different between BW25113 and EJW3 (p -values $<$ 0.05). Values bolded are significantly higher in EJW3. Values bolded and italicized are significantly lower in EJW3. P -values are calculated by using a two-tailed student's t -test with six biological replicates.

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Table S9. Upregulated genes selected for validation for their roles in osmotic tolerance.

ID	Function
b0260	CP4-6 prophage; putative S-methylmethionine transporter (<i>mmuP</i>)
b3939	cystathionine gamma-synthase, PLP-dependent (<i>metB</i>)
b4013	homoserine O-transsuccinylase (<i>metA</i>)
b3828	methionine biosynthesis regulon transcriptional regulator (<i>metR</i>)
b2942	S-adenosylmethionine synthetase (<i>metK</i>)
b2421	cysteine synthase B (O-acetylserine sulfhydrylase B) (<i>cysM</i>)
b2366	D-serine dehydratase (<i>dsdA</i>)
b4131	lysine decarboxylase, acid-inducible (<i>cadA</i>)
b0402	proline-specific permease (<i>proY</i>)
b1386	tyramine oxidase, copper-requiring (<i>tynA</i>)

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Table S10. Gene ontology analysis in M9 supplemented with 0.6 M NaCl.

	Category	Term	Count	%	p-value
Upregulated (BW25113)	GOTERM_BP_DIR	GO:0009086~methionine	4	5.405	0.001
	ECT	biosynthetic process			
	UP_KEYWORDS	Methionine biosynthesis	4	5.405	0.002
	UP_KEYWORDS	Pyridoxal phosphate	6	8.108	0.004
Downregulated (BW25113)	UP_KEYWORDS	Transmembrane helix	21	41.176	0.001
	UP_KEYWORDS	Cell membrane	23	45.098	0.001
	UP_SEQ_FEATUR	transmembrane region	21	41.176	0.001
	E				
	UP_KEYWORDS	Transmembrane	21	41.176	0.001
	UP_SEQ_FEATUR	topological	16	31.373	0.001
	E	domain:Cytoplasmic			
	UP_SEQ_FEATUR	topological	16	31.373	0.002
	E	domain:Periplasmic			
	UP_KEYWORDS	Membrane	23	45.098	0.004
	UP_KEYWORDS	Cell inner membrane	19	37.255	0.005
	COG_ONTOLOGY	Inorganic ion transport and metabolism	4	7.843	0.006
	Upregulated (MG1655)	GOTERM_BP_DIR	GO:0006814~sodium ion	3	5.882
ECT		transport			
GOTERM_CC_DIR		GO:0005886~plasma membrane	21	41.176	0.012
Downregulated (MG1655)	KEGG_PATHWAY	eco00780:Biotin metabolism	4	3.636	0.001
	KEGG_PATHWAY	eco00190:Oxidative phosphorylation	19	13.571	0.000
		UP_KEYWORDS	Quinone	10	7.143
	UP_KEYWORDS	Ubiquinone	10	7.143	0.000
	GOTERM_CC_DIR	GO:0045272~plasma membrane respiratory chain complex I	10	7.143	0.000
	ECT				
	GOTERM_CC_DIR	GO:0030964~NADH dehydrogenase complex	10	7.143	0.000
	ECT				
	GOTERM_BP_DIR	GO:0009060~aerobic respiration	11	7.857	0.000
	ECT				
	GOTERM_MF_DIR	GO:0048038~quinone binding	9	6.429	0.000
	ECT				

GOTERM_MF_DIR	GO:0003954~NADH	10	7.143	0.000
ECT	dehydrogenase activity			
UP_KEYWORDS	Ligase	15	10.714	0.000
GOTERM_MF_DIR	GO:0008137~NADH	8	5.714	0.000
ECT	dehydrogenase (ubiquinone) activity			
UP_KEYWORDS	Oxidoreductase	28	20	0.000
UP_KEYWORDS	Enterobactin biosynthesis	5	3.571	0.000
GOTERM_BP_DIR	GO:0006099~tricarboxylic	8	5.714	0.000
ECT	acid cycle			
UP_KEYWORDS	Tricarboxylic acid cycle	7	5	0.000
KEGG_PATHWAY	eco00020:Citrate cycle (TCA cycle)	9	6.429	0.000
UP_KEYWORDS	Transport	43	30.714	0.000
GOTERM_BP_DIR	GO:0009239~enterobactin	5	3.571	0.000
ECT	biosynthetic process			
GOTERM_BP_DIR	GO:0042773~ATP synthesis	5	3.571	0.000
ECT	coupled electron transport			
GOTERM_BP_DIR	GO:0015990~electron	5	3.571	0.000
ECT	transport coupled proton transport			
KEGG_PATHWAY	eco02040:Flagellar assembly	10	7.143	0.000
KEGG_PATHWAY	eco01053:Biosynthesis of siderophore group nonribosomal peptides	5	3.571	0.000
UP_KEYWORDS	Cell inner membrane	49	35	0.000
KEGG_PATHWAY	eco00250:Alanine, aspartate and glutamate metabolism	9	6.429	0.000
UP_KEYWORDS	Pyrimidine biosynthesis	5	3.571	0.000
GOTERM_MF_DIR	GO:0030976~thiamine	5	3.571	0.000
ECT	pyrophosphate binding			
UP_KEYWORDS	Cell membrane	54	38.571	0.000
UP_KEYWORDS	Membrane	58	41.429	0.000
UP_KEYWORDS	NAD	14	10	0.001
GOTERM_MF_DIR	GO:0015421~oligopeptide-	4	2.857	0.001
ECT	transporting ATPase activity			
GOTERM_BP_DIR	GO:0044205~'de novo'	4	2.857	0.002
ECT	UMP biosynthetic process			

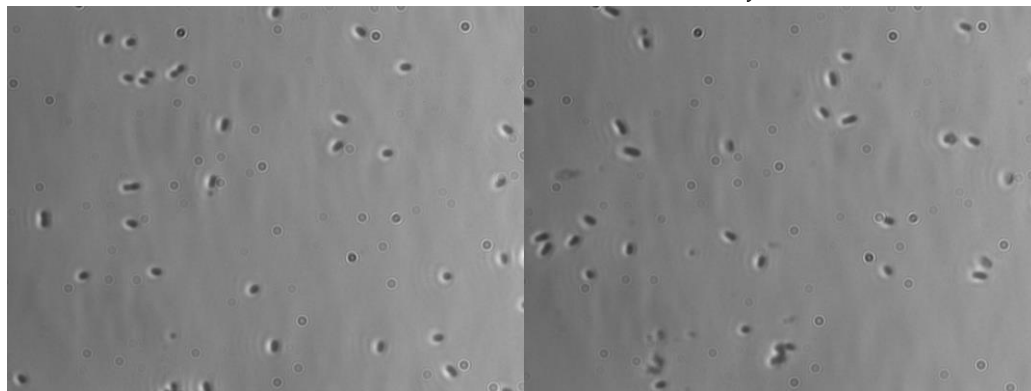
UP_KEYWORDS	Bacterial flagellum biogenesis	5	3.571	0.002
UP_KEYWORDS	Purine biosynthesis	5	3.571	0.002
UP_KEYWORDS	Nucleotide-binding	29	20.714	0.002
KEGG_PATHWAY	eco01100:Metabolic pathways	55	39.286	0.003
GOTERM_BP_DIR	GO:0019646~aerobic electron transport chain	4	2.857	0.004
UP_KEYWORDS	Bacterial flagellum	5	3.571	0.004
GOTERM_MF_DIR	GO:0042936~dipeptide transporter activity	4	2.857	0.004
UP_KEYWORDS	ATP-binding	25	17.857	0.004
GOTERM_MF_DIR	GO:0047527~2,3-dihydroxybenzoate-serine ligase activity	3	2.143	0.004
GOTERM_BP_DIR	GO:0044781~bacterial-type flagellum organization	4	2.857	0.005
GOTERM_BP_DIR	GO:0042938~dipeptide transport	4	2.857	0.005
UP_KEYWORDS	Thiamine pyrophosphate	4	2.857	0.006
UP_KEYWORDS	Glutamine amidotransferase	4	2.857	0.006
GOTERM_CC_DIR	GO:0009424~bacterial-type flagellum hook	4	2.857	0.006
UP_SEQ_FEATUR	topological domain:Cytoplasmic	31	22.143	0.007
UP_SEQ_FEATUR	topological domain:Periplasmic	31	22.143	0.007
GOTERM_BP_DIR	GO:0015796~galactitol transport	3	2.143	0.008
GOTERM_MF_DIR	GO:0090584~protein-phosphocysteine-galactitol-phosphotransferase system transporter activity	3	2.143	0.008

65 **3. Supplementary Figures**

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BW25113 M9 0h

EJW3 M9 0h

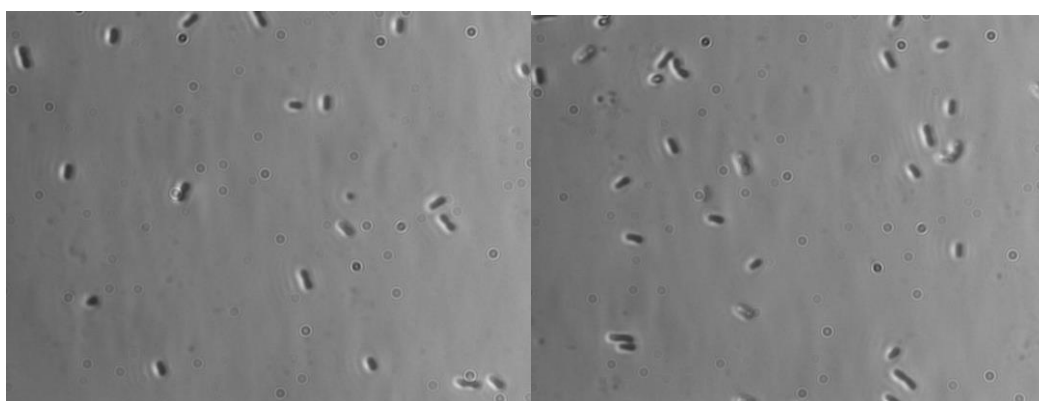


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BW25113 M9 3h

EJW3 M9 3h

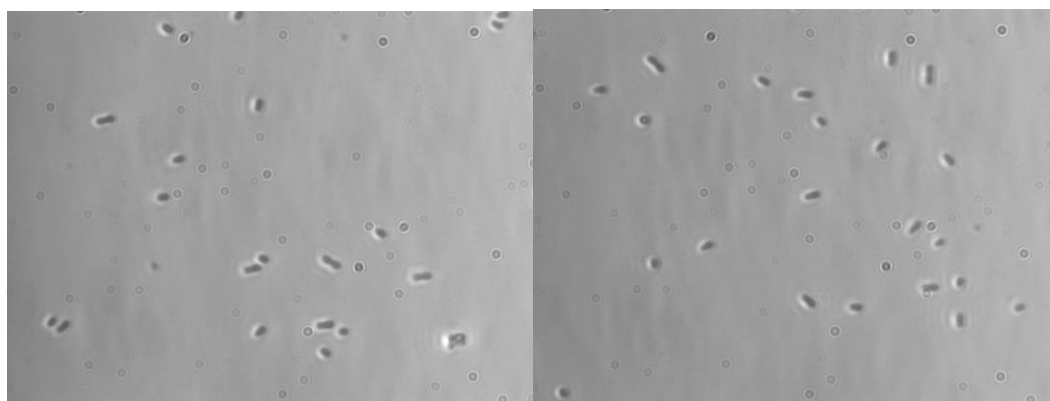


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BW25113 M9 6h

EJW3 M9 6h

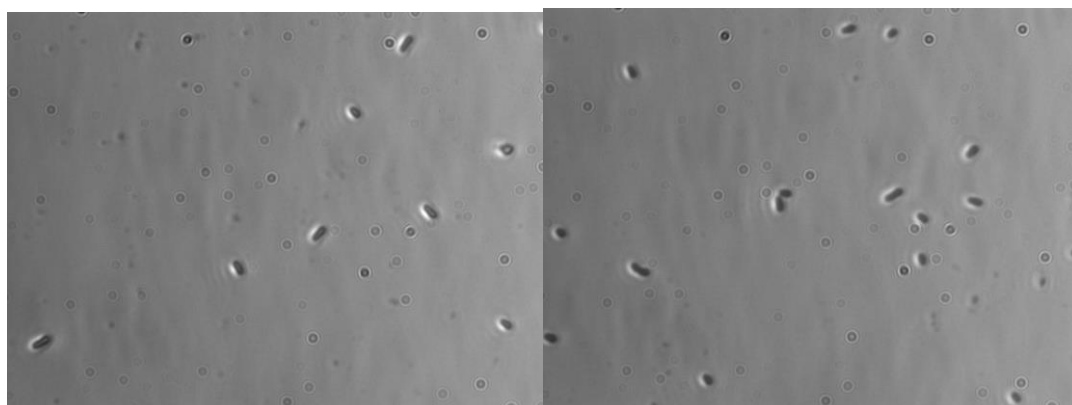


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BW25113 0.6 M NaCl 3h

EJW3 0.6 M NaCl 3h

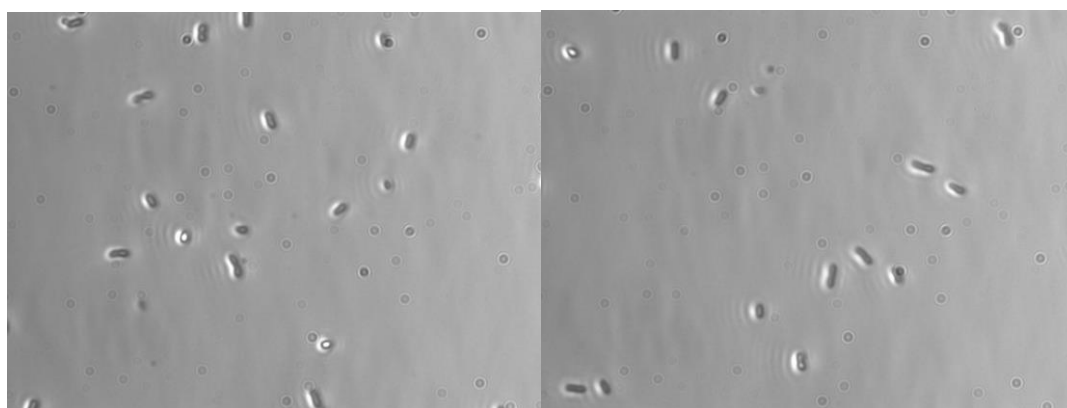


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BW25113 0.6 M NaCl 6h

EJW3 0.6 M NaCl 6h

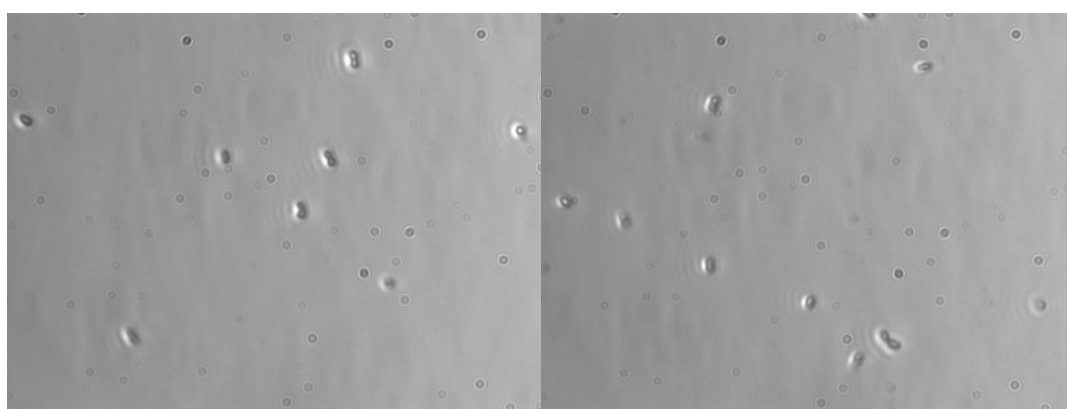


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BW25113 0.6 M NaCl 12h

EJW3 0.6 M NaCl 12h

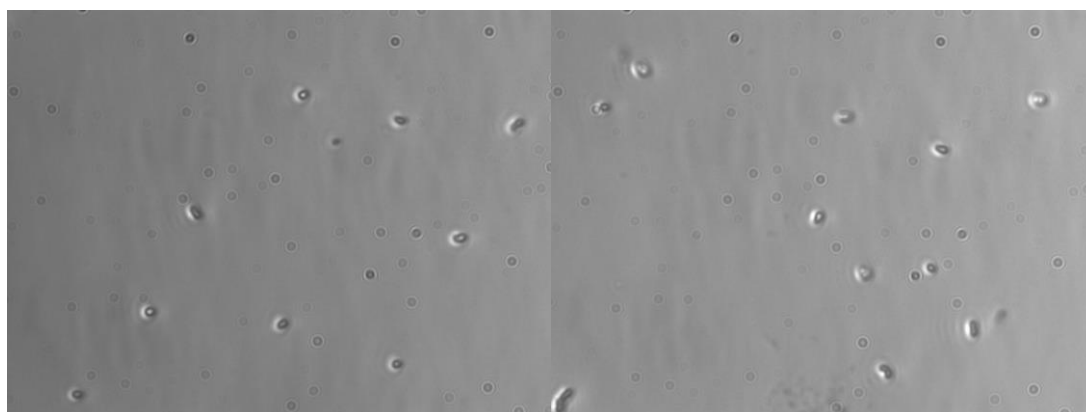


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BW25113 0.6 M NaCl 24h

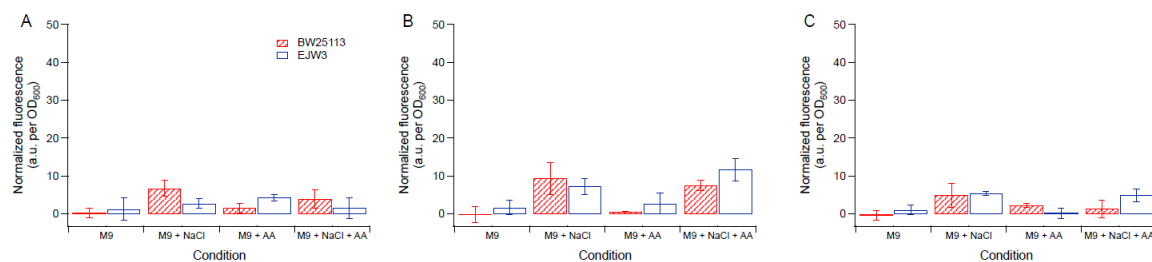
EJW3 0.6 M NaCl 24h



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Figure. S1. Light microscopy of cells in the presence or absence of hyperosmotic stress.



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Figure. S2. Membrane integrity assay using PI staining. (A) lag phase cells. (B) Early stationary phase cells. (C) Late stationary phase cells. NaCl: 0.7 M NaCl. AA: 10 mM acetic acid. Error bars are standard deviations.

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86 **References**

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1. Rabinowitz, J.D.; Kimball, E. Acidic acetonitrile for cellular metabolome extraction from *Escherichia coli*. *Anal Chem* 2007, 79, 6167-6173.

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2. Henderson, J.W.; Brooks, A. Improved amino acid methods using Agilent ZORBAX Eclipse Plus C18 columns for a variety of Agilent LC instrumentation and separation goals. Agilent Technologies 2010.

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