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*An investigation into an in vitro model of post-death metabolism and the cryptic growth phase.*

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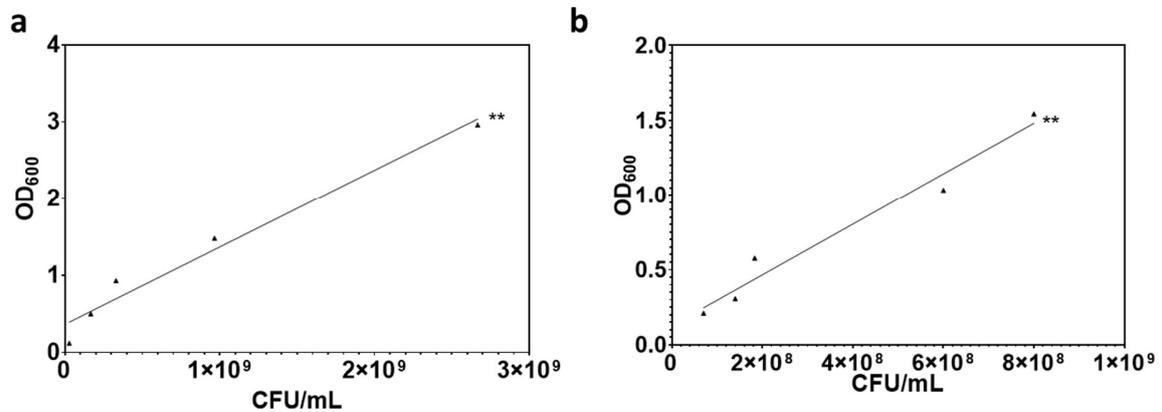
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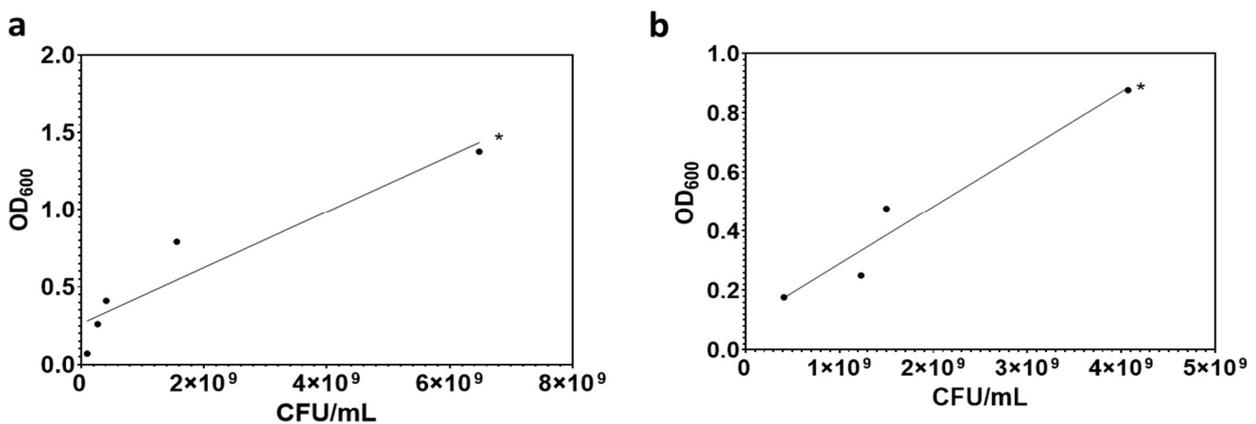
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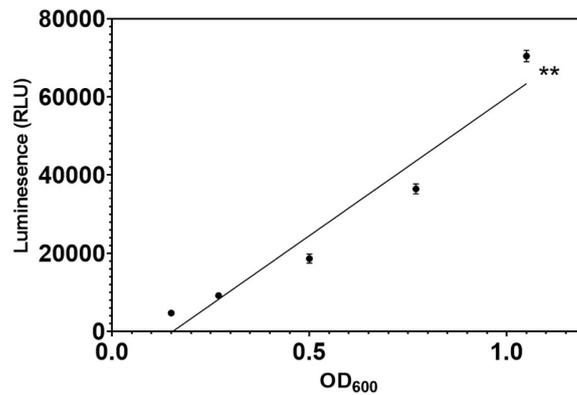
## 11. Supplementary



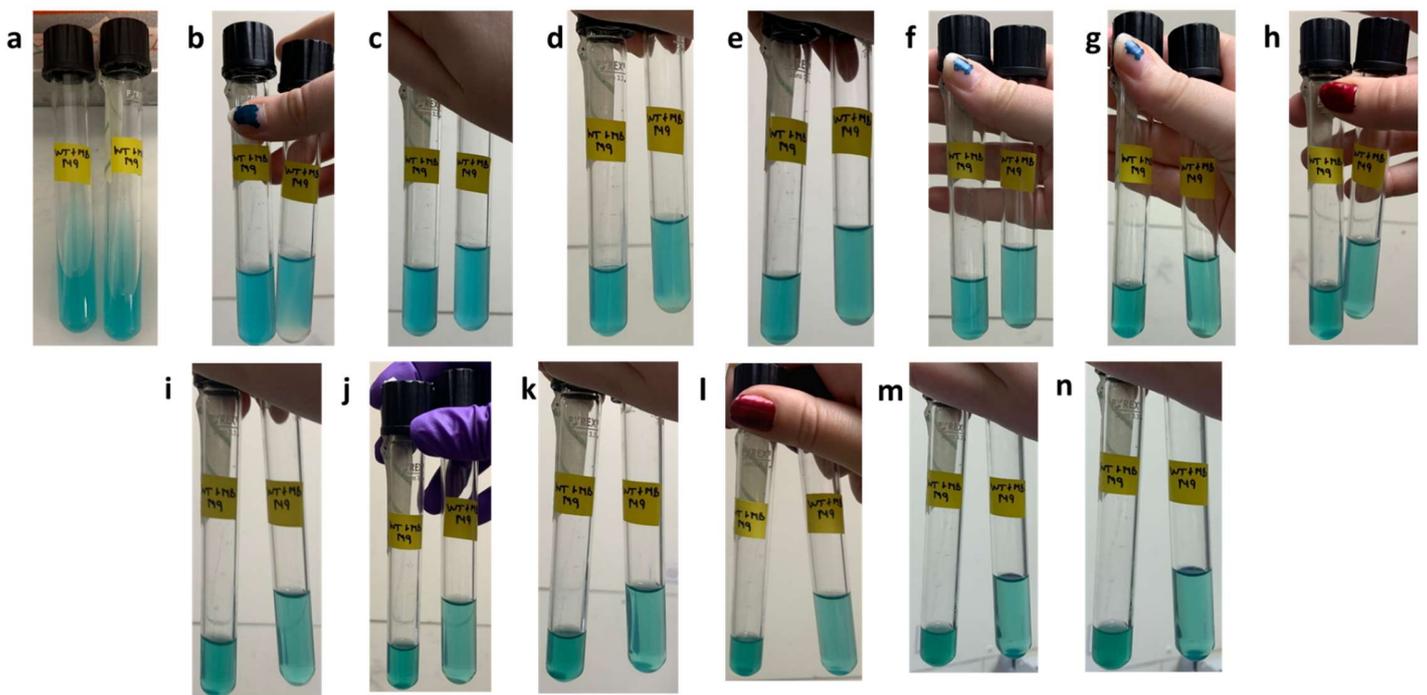
**Figure 1: Additional calibration curves showing how the OD of a culture grown in LB depends on the number of CFU/mL present.** Experimental points denote the average of three technical replicates with error bars representing the S.E.M.  $R^2$  values were calculated through a simple linear regression (a:  $R^2 = 0.9838$ , b:  $R^2 = 0.9836$ ). Correlation is statistically significant and calculated through Pearson's correlation coefficient (a: ( $r(3) = 0.9838$ ,  $p = 0.0025$ ), b: ( $r(3) = 0.9836$ ,  $p = 0.0025$ )).



**Figure 2: Additional calibration curves showing how the OD of a culture grown in M9 depends on the number of CFU/mL present.** Experimental points denote the average of three technical replicates with error bars representing the S.E.M.  $R^2$  values were calculated through a simple linear regression (a:  $R^2 = 0.8916$ , b:  $R^2 = 0.9740$ ). Correlation is statistically significant and calculated through Pearson's correlation coefficient (a: ( $r(3) = 0.9442$ ,  $p = 0.0157$ ), b: ( $r(3) = 0.9740$ ,  $p = 0.026$ )).



**Figure 3: Luminescence given off by BacTiter-Glo™ Microbial Cell Viability Assay kit (Promega) is dependent on the OD of the sample.** Experimental points denote the average of three technical replicates with error bars representing S.E.M.  $R^2$  values were calculated through a simple linear regression ( $R^2 = 0.9439$ ) Correlation is statistically significant and calculated through Pearson's correlation coefficient ( $r(3) = 0.9715$ ,  $p = 0.0057$ ).



**Figure 4: Appearance of WT cultures with the addition of Methylene Blue.** The tubes were imaged at the following time points: Day 0 (a), Day 2 (b), Day 3 (c), Day 7 (d), Day 9 (e), Day 10 (f), Day 15 (g), Day 16 (h), Day 17 (i), Day 18 (j), Day 21 (k), Day 23 (l), Day 24 (m), and Day 25 (n). The open system is on the LHS and the closed system is on the RHS with the exception of a which is reversed. The blue colour became less intense in the closed system by day 7 however remained present throughout. The volume dropped in the open system due to evaporation losses.

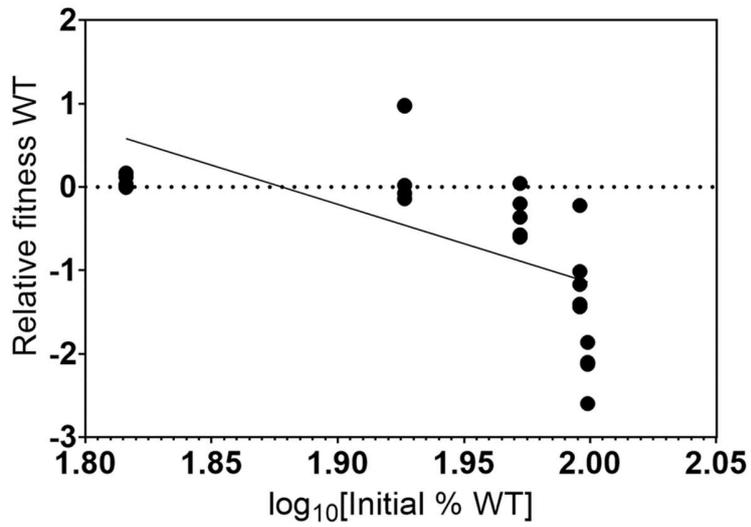
**Table 1: Tukey's multiple comparison was performed to analyse if there was a difference in the number of *E. coli* (determined through CFU/mL tests) present in WT,  $\Delta lon$ , and  $\Delta lonR$  cultures when grown for various times.**

Days	Strains Compared	Predicted Mean Difference	95% Confidence Interval of difference	Significant	P Value
0	WT vs $\Delta lon$	12411111	-301369994 to 326192216	ns	0.9949
	WT vs $\Delta lonR$	-34766667	-385584607 to 316051274	ns	0.9685
	$\Delta lon$ vs $\Delta lonR$	-47177778	-397995718 to 303640162	ns	0.9427
1	WT vs $\Delta lon$	-902476190	-1237922301 to -567030079	****	<0.0001
	WT vs $\Delta lonR$	-116309524	-486632003 to 254012956	ns	0.7267
	$\Delta lon$ vs $\Delta lonR$	786166667	435348726 to 1136984607	****	<0.0001
0	WT vs $\Delta lon$	44391667	-219888119 to 308671452	ns	0.9133
	WT vs $\Delta lonR$	-16508333	-340183646 to 307166979	ns	0.9917
	$\Delta lon$ vs $\Delta lonR$	-60900000	-384575312 to 262775312	ns	0.8925
5	WT vs $\Delta lon$	-331987000	-614834846 to -49139154	*	0.0178
	WT vs $\Delta lonR$	40575000	-337396333 to 418546333	ns	0.9636
	$\Delta lon$ vs $\Delta lonR$	372562000	-10415794 to 755539794	ns	0.0581
0	WT vs $\Delta lon$	476515	-67045661 to 67998691	ns	0.9998
	WT vs $\Delta lonR$	-34573485	-102095661 to 32948691	ns	0.4396
	$\Delta lon$ vs $\Delta lonR$	-35050000	-101087991 to 30987991	ns	0.4140
10	WT vs $\Delta lon$	-105157875	-183758806 to -26556944	**	0.0059
	WT vs $\Delta lonR$	-17070625	-90903344 to 56762094	ns	0.8437
	$\Delta lon$ vs $\Delta lonR$	88087250	16758067 to 159416433	*	0.0119
0	WT vs $\Delta lon$	-4153333	-161712050 to 153405384	ns	0.9977
	WT vs $\Delta lonR$	-42036667	-199595384 to 115522050	ns	0.7882
	$\Delta lon$ vs $\Delta lonR$	-37883333	-188109676 to 112343009	ns	0.8083
15	WT vs $\Delta lon$	-131678000	-289236717 to 25880717	ns	0.1150
	WT vs $\Delta lonR$	-166769167	-324327884 to -9210450	*	0.0364
	$\Delta lon$ vs $\Delta lonR$	-35091167	-185317509 to 115135176	ns	0.8329
0	WT vs $\Delta lon$	-30166667	-196730465 to 136397131	ns	0.8963
	WT vs $\Delta lonR$	-72333333	-238897131 to 94230465	ns	0.5393
	$\Delta lon$ vs $\Delta lonR$	-42166667	-208730465 to 124397131	ns	0.8081
20	WT vs $\Delta lon$	-335953333	-502517131 to -169389535	****	<0.0001
	WT vs $\Delta lonR$	-72053333	-238617131 to 94510465	ns	0.5418
	$\Delta lon$ vs $\Delta lonR$	263900000	97336202 to 430463798	**	0.0014
0	WT vs $\Delta lon$	-17677778	-97167117 to 61811562	ns	0.8512
	WT vs $\Delta lonR$	-61411111	-140900451 to 18078228	ns	0.1573
	$\Delta lon$ vs $\Delta lonR$	-43733333	-123222673 to 35756006	ns	0.3818
25	WT vs $\Delta lon$	28650000	-106548658 to 163848658	ns	0.8639
	WT vs $\Delta lonR$	-60209222	-192027379 to 71608935	ns	0.5121
	$\Delta lon$ vs $\Delta lonR$	-88859222	-173836901 to -3881544	*	0.0387

**Table 2: Tukey's multiple comparison was performed to analyse the differences between peptide concentrations present in the supernatant taken from either WT,  $\Delta lon$  or  $\Delta lonR$  cultures grown for 1, 5, 10, 15, 20 and 25 days.**

Comparison	Mean Difference	95% Confidence Interval of difference	Significant	P Value
LGA-1 WT vs. LGA-1 $\Delta lon$	35.26	-206.7 to 277.2	ns	>0.9999
LGA-1 WT vs. LGA-1 $\Delta lonR$	-36.74	-278.7 to 205.2	ns	>0.9999
LGA-1 WT vs. LGA-5 WT	-223.1	-465.0 to 18.82	ns	0.0985
LGA-1 WT vs. LGA-10 WT	-178.2	-420.1 to 63.70	ns	0.3748
LGA-1 WT vs. LGA-15 WT	84.00	-157.9 to 325.9	ns	0.9964
LGA-1 WT vs. LGA-20 WT	36.15	-205.8 to 278.1	ns	>0.9999
LGA-1 WT vs. LGA-25 WT	105.3	-136.7 to 347.2	ns	0.9668
LGA-1 $\Delta lon$ vs. LGA-1 $\Delta lonR$	-72.00	-313.9 to 169.9	ns	0.9994
LGA-1 $\Delta lon$ vs. LGA-5 $\Delta lon$	-303.2	-545.1 to -61.26	**	0.0041
LGA-1 $\Delta lon$ vs. LGA-10 $\Delta lon$	-232.2	-474.1 to 9.704	ns	0.0715
LGA-1 $\Delta lon$ vs. LGA-15 $\Delta lon$	-74.44	-344.9 to 196.0	ns	0.9998
LGA-1 $\Delta lon$ vs. LGA-20 $\Delta lon$	-173.6	-415.5 to 68.37	ns	0.4184
LGA-1 $\Delta lon$ vs. LGA-25 $\Delta lon$	-111.7	-353.6 to 130.2	ns	0.9456
LGA-1 $\Delta lonR$ vs. LGA-5 $\Delta lonR$	-13.56	-255.5 to 228.4	ns	>0.9999
LGA-1 $\Delta lonR$ vs. LGA-10 $\Delta lonR$	-2.518	-244.4 to 239.4	ns	>0.9999
LGA-1 $\Delta lonR$ vs. LGA-15 $\Delta lonR$	-111.0	-353.0 to 130.9	ns	0.9482
LGA-1 $\Delta lonR$ vs. LGA-20 $\Delta lonR$	-121.2	-363.1 to 120.7	ns	0.8998
LGA-1 $\Delta lonR$ vs. LGA-25 $\Delta lonR$	-181.9	-423.9 to 60.00	ns	0.3420
LGA-5 WT vs. LGA-5 $\Delta lon$	-44.81	-286.7 to 197.1	ns	>0.9999
LGA-5 WT vs. LGA-5 $\Delta lonR$	172.8	-69.11 to 414.7	ns	0.4255
LGA-5 WT vs. LGA-10 WT	44.89	-197.0 to 286.8	ns	>0.9999
LGA-5 WT vs. LGA-15 WT	307.1	65.18 to 549.0	**	0.0034
LGA-5 WT vs. LGA-20 WT	259.3	17.33 to 501.2	*	0.0256
LGA-5 WT vs. LGA-25 WT	328.4	86.44 to 570.3	**	0.0013
LGA-5 $\Delta lon$ vs. LGA-5 $\Delta lonR$	217.6	-24.30 to 459.6	ns	0.1187
LGA-5 $\Delta lon$ vs. LGA-10 $\Delta lon$	70.96	-171.0 to 312.9	ns	0.9995
LGA-5 $\Delta lon$ vs. LGA-15 $\Delta lon$	228.7	-41.74 to 499.2	ns	0.1807
LGA-5 $\Delta lon$ vs. LGA-20 $\Delta lon$	129.6	-112.3 to 371.6	ns	0.8434
LGA-5 $\Delta lon$ vs. LGA-25 $\Delta lon$	191.5	-50.45 to 433.4	ns	0.2654
LGA-5 $\Delta lonR$ vs. LGA-10 $\Delta lonR$	11.04	-230.9 to 253.0	ns	>0.9999
LGA-5 $\Delta lonR$ vs. LGA-15 $\Delta lonR$	-97.48	-339.4 to 144.4	ns	0.9835
LGA-5 $\Delta lonR$ vs. LGA-20 $\Delta lonR$	-107.6	-349.6 to 134.3	ns	0.9599
LGA-5 $\Delta lonR$ vs. LGA-25 $\Delta lonR$	-168.4	-410.3 to 73.56	ns	0.4692
LGA-10 WT vs. LGA-10 $\Delta lon$	-18.74	-260.7 to 223.2	ns	>0.9999

LGA-10 WT vs. LGA-10 $\Delta lonR$	139.0	-103.0 to 380.9	ns	0.7656
LGA-10 WT vs. LGA-15 WT	262.2	20.30 to 504.1	*	0.0227
LGA-10 WT vs. LGA-20 WT	214.4	-27.56 to 456.3	ns	0.1323
LGA-10 WT vs. LGA-25 WT	283.5	41.55 to 525.4	**	0.0095
LGA-10 $\Delta lon$ vs. LGA-15 $\Delta lon$	157.8	-112.7 to 428.3	ns	0.7457
LGA-10 $\Delta lon$ vs. LGA-20 $\Delta lon$	58.67	-183.3 to 300.6	ns	>0.9999
LGA-10 $\Delta lon$ vs. LGA-25 $\Delta lon$	120.5	-121.4 to 362.4	ns	0.9036
LGA-10 $\Delta lonR$ vs. LGA-15 $\Delta lonR$	-108.5	-350.4 to 133.4	ns	0.9570
LGA-10 $\Delta lonR$ vs. LGA-20 $\Delta lonR$	-118.7	-360.6 to 123.3	ns	0.9137
LGA-10 $\Delta lonR$ vs. LGA-25 $\Delta lonR$	-179.4	-421.3 to 62.52	ns	0.3641
LGA-15 WT vs. LGA-15 $\Delta lon$	-123.2	-393.7 to 147.3	ns	0.9513
LGA-15 WT vs. LGA-15 $\Delta lonR$	-231.8	-473.7 to 10.15	ns	0.0726
LGA-15 WT vs. LGA-20 WT	-47.85	-289.8 to 194.1	ns	>0.9999
LGA-15 WT vs. LGA-25 WT	21.26	-220.7 to 263.2	ns	>0.9999
LGA-15 $\Delta lon$ vs. LGA-15 $\Delta lonR$	-108.6	-379.1 to 161.9	ns	0.9840
LGA-15 $\Delta lon$ vs. LGA-20 $\Delta lon$	-99.11	-369.6 to 171.4	ns	0.9936
LGA-15 $\Delta lon$ vs. LGA-25 $\Delta lon$	-37.26	-307.7 to 233.2	ns	>0.9999
LGA-15 $\Delta lonR$ vs. LGA-20 $\Delta lonR$	-10.15	-252.1 to 231.8	ns	>0.9999
LGA-15 $\Delta lonR$ vs. LGA-25 $\Delta lonR$	-70.89	-312.8 to 171.0	ns	0.9995
LGA-20 WT vs. LGA-20 $\Delta lon$	-174.4	-416.4 to 67.48	ns	0.4099
LGA-20 WT vs. LGA-20 $\Delta lonR$	-194.1	-436.0 to 47.85	ns	0.2468
LGA-20 WT vs. LGA-25 WT	69.11	-172.8 to 311.0	ns	0.9996
LGA-20 $\Delta lon$ vs. LGA-20 $\Delta lonR$	-19.63	-261.6 to 222.3	ns	>0.9999
LGA-20 $\Delta lon$ vs. LGA-25 $\Delta lon$	61.85	-180.1 to 303.8	ns	>0.9999
LGA-20 $\Delta lonR$ vs. LGA-25 $\Delta lonR$	-60.74	-302.7 to 181.2	ns	>0.9999
LGA-25 WT vs. LGA-25 $\Delta lon$	-181.7	-423.6 to 60.22	ns	0.3439
LGA-25 WT vs. LGA-25 $\Delta lonR$	-323.9	-565.9 to -82.00	**	0.0016
LGA-25 $\Delta lon$ vs. LGA-25 $\Delta lonR$	-142.2	-384.1 to 99.70	ns	0.7353



**Figure 5: Relative fitness of the WT E. coli is dependent on the initial proportion of the WT in the population.** Relative fitness is calculated through comparing the growth rates of the WT relative to that of the  $\Delta lon$ . Data ( $n = 5$  except for 70% initial percentage where  $n = 4$ ) is fitted with a simple linear regression.