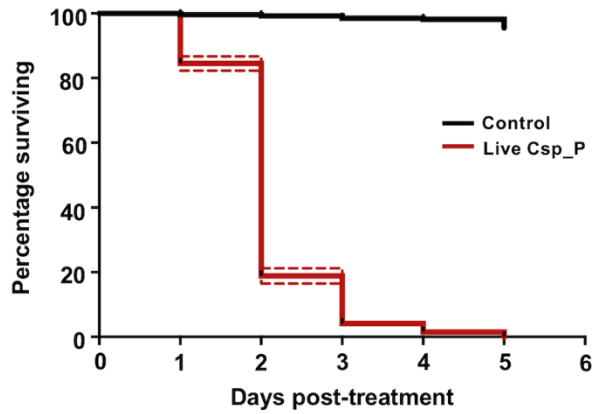961

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964 **Fig. S1: Live *Chromobacterium* Csp_P effectively kills *Ae. aegypti* ROCK larvae when provided in**

965 **a pellet.** Groups of 30 L_2 larvae were provided either a control (black line) or live Csp_P (red line) pellet in

966 300mL of deionized water. Pellets contained fishmeal as an attractant, and 20% gelatin as a stabilizing

967 agent. Pellets containing live Csp_P induced a significantly higher rate of mortality than no bacteria

968 control pellets (Cox Regression: P < 0.0001). Lines depict the percentage of larvae surviving at each day

969 post-treatment (± s.e.m.) for four experimental replicates, with each containing three cages per treatment.

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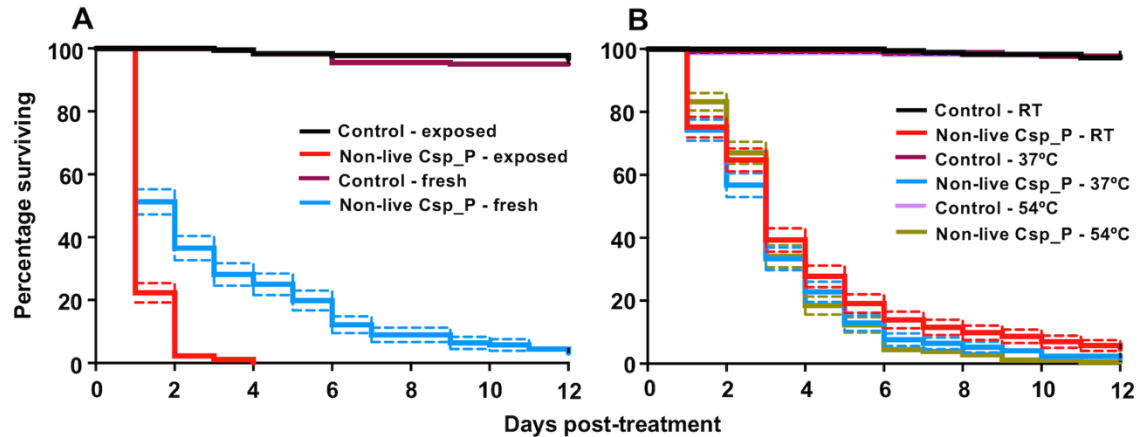
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Fig. S2: Non-live Csp_P agar pellets have lengthy residual activity, and are heat stable. We

developed a revised non-live Csp_P pellet formulation, substituting 1.5% agar for 20% gelatin to improve

pellet stability in water. We examined the efficacy of these pellets against *Ae. aegypti* ROCK larvae where

pellets were either freshly prepared and fed to larvae (fresh), or left sitting in a mosquito breeder and

300mL of water for 2 weeks before larvae were introduced (exposed) **(A)**. We observed that larvae in the

exposed Csp_P agar treatment experienced more rapid death, than those in the fresh Csp_P agar

treatments (Cox Regression: *P* < 0.0001). We then heat-treated whole Csp_P and control agar pellets at

room temperature, 37°C or 54°C for two weeks, and then fed them to larvae **(B)**, where we observed that

heat treatment had no effect on the larvicidal activity of the Csp_P agar pellets (Cox Regression: *P* =

0.929). In all experiments, groups of 30 ROCK L_2 larvae were provided either a control or non-live Csp_P

agar pellet in 300mL of deionized water.

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Experiments assessing attractiveness of fishmeal pellets

20% fishmeal   400mg in 2mL gelatin pellet with 1mL Csp_P

Chambers

1 Attractant
2 Initial chamber
3 No bait

	1	2	3

Rep 1

Chamber	Time (mins)	1	5	10	15	20	25	30
1		3	10	11	14	16	17	19
2		8	3	1	2	2	1	1
3		9	7	8	4	2	2	0

Rep 2

Chamber	Time (mins)	1	5	10	15	20	25	30
1		12	20	24	25	26	27	30
2		16	5	3	4	0	2	0
3		2	5	3	1	4	1	0

Percentages at 30 mins

	R1	R2	Average
1	95	100	97.5
2	5	0	2.5
3	0	0	0

Summary

Responded to bait	97.5
Did not respond	2.5
Moved to empty chamber	0

Supplementary File 1 - Pellet attractiveness (Fish food Pellets)

Experiments assessing attractiveness of fish food pellets

20% fish food   400mg in 2mL gelatin pellet with 1mL Csp_P

Chambers   1 Attractant

1	2	3

2 Initial chamber
3 No bait

Rep 1

Chamber	Time (mins)	1	5	10	15	20	25	30
1		2	11	13	13	13	11	10
2		11	3	2	2	2	1	2
3		7	6	5	5	7	8	8

Rep 2

Chamber	Time (mins)	1	5	10	15	20	25	30
1		0	18	22	22	27	26	28
2		27	8	4	3	0	0	0
3		3	4	4	5	3	4	2

Percentages at 30 mins

	R1	R2	Average
1	50	93.3333333	71.6666667
2	10	0	5
3	40	6.66666667	23.3333333

Summary	71.6666667
Responded to bait	5
Did not respond	23.3333333
Moved to empty chamber	0

Experiments comparing the attractiveness of Fishmeal to Fish Food

Fish Food - ground powder of Tetramin tropical flakes

Chambers	1 Fish Food	1	2	3
	2 Initial chamber			
	3 Fishmeal			

Rep 1

Chamber	Time (mins)	1	5	10	15	20	25	30
1		6	9	7	4	3	7	4
2		3	4	7	14	15	2	1
3		21	17	16	12	12	21	25

Rep 2

Chamber	Time (mins)	1	5	10	15	20	25	30
1		7	6	10	9	4	5	4
2		6	4	1	3	8	4	3
3		17	20	19	18	18	21	23

Percentages at 30 mins

	R1	R2	Average
1	13.3333333	13.3333333	13.3333333
2	3.33333333	10	6.66666667
3	83.3333333	76.6666667	80

Responded to Fish food	13.3333333
Did not respond	6.66666667
Responded to Fishmeal	80

Statistical comparison of attractiveness   **Choice assay - Fishmeal vs Fishfood**

Small boxes - Fishmeal vs Fishfood

	FM	FF		Stats - Chi square test	
				Table Analyzed	Small box - FM v FF
Responded to bait (R)	49	38			
Did not respond (D)	1	2		Chi-square	
Moved to empty chambers (M)	0	10		Chi-square, df	11.72, 2
				P value	**0.0028**
				P value summary	**
				One- or two-tailed	NA
				Statistically significant? (alpha<0.05)	Yes
				Data analyzed	
				Number of rows	2
				Number of columns	3