Supplementary Information for:

Hydrogen cyanide produced by the soil bacterium Chromobacterium sp. Panama contributes to mortality in Anopheles gambiae mosquito larvae

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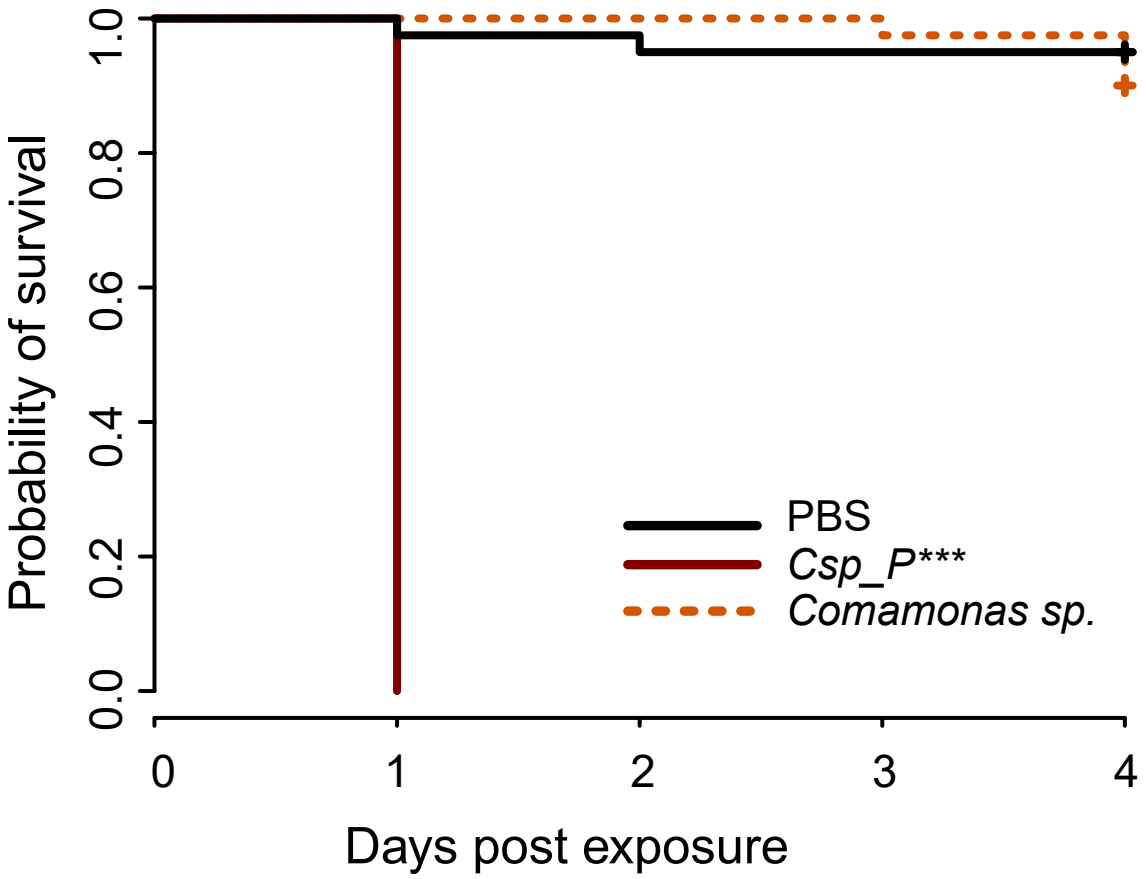

Figure S1: Exposure to *Comamonas sp.*, a common mosquito midgut bacterium does not induce larval mortality. First and second instar *An. gambiae* larvae were treated in groups of ten in 5mL of water containing 10mg of ground larval food. *C.sp_P* or *Comamonas sp.*overnight cultures were diluted to 1.0 (±0.1) OD_{600} and 100µL of each culture was added to the larval breeding water. 100µL of 1X PBS was added as a control and survival of larvae was monitored for four days. Compared to the 1X PBS control, *C.sp_P* treated larvae had significantly lower survival ($p = 2.14 \times 10^{-7}$) while *Comamonas sp.* treated larvae did not (p = 0.435). The experiment was repeated two independent times and for each experimental replicate two pools of ten larvae were measured per treatment. Data were analyzed using a Cox proportional hazards model. Total sample sizes are as follows: PBS = 40, *C.sp_P*= 40, *Comamonas sp.* = 40.

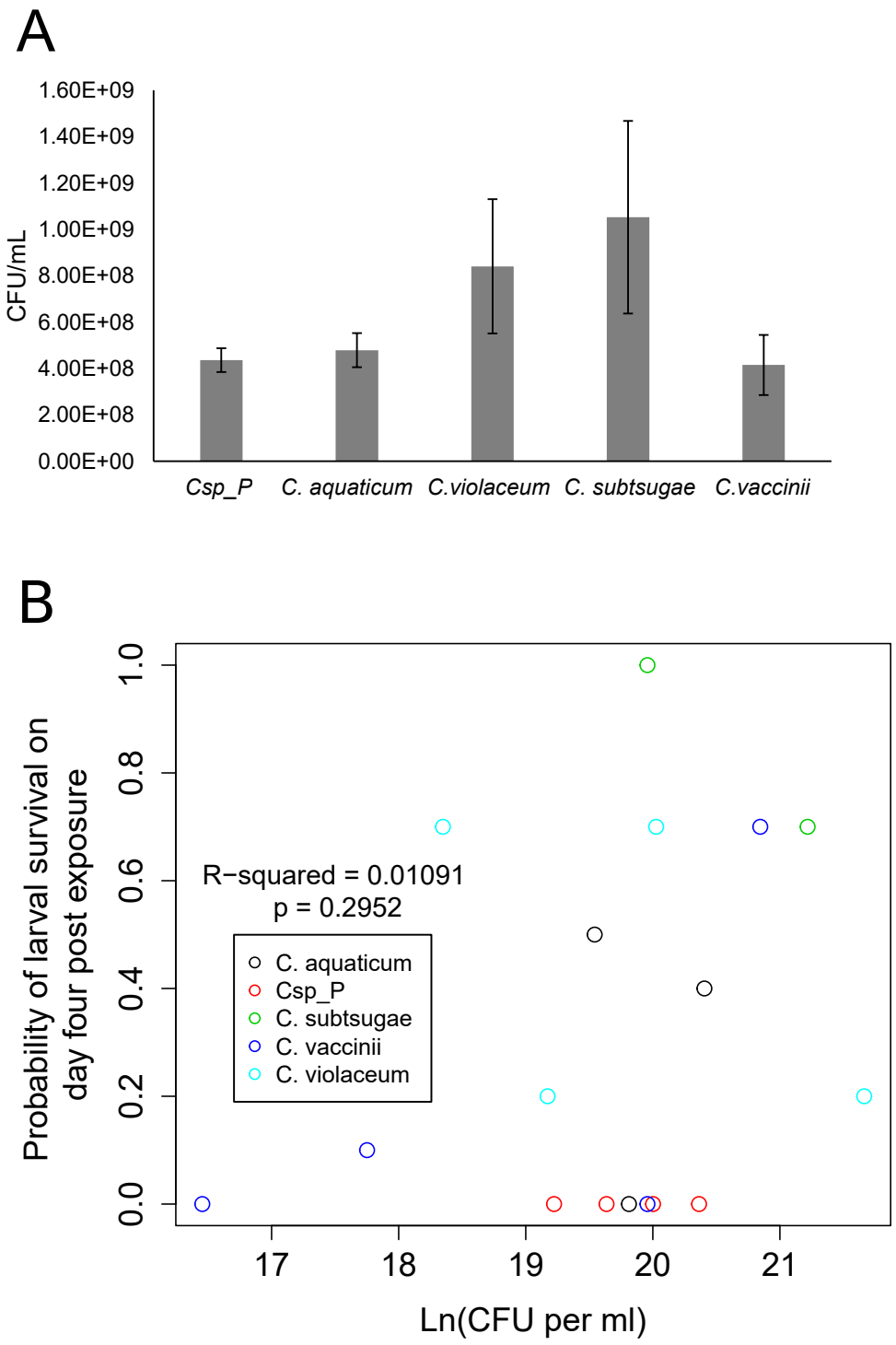

Figure S2: (A) CFU added to larval breeding water for each species of *Chromobacterium*. Live bacteria were added at the above average concentrations to 5mL larval breeding water over three replicate experiments. **(B) CFU across all species of *Chromobacterium* does not predict larval survival.** For the five species of *Chromobacterium* used to treat *An. gambiae* larvae, we performed a linear regression to assess the relationship between CFUs added to the larval water and larval mortality. We found no correlation between these factors (r-squared = 0.01091, p = 0.2952).

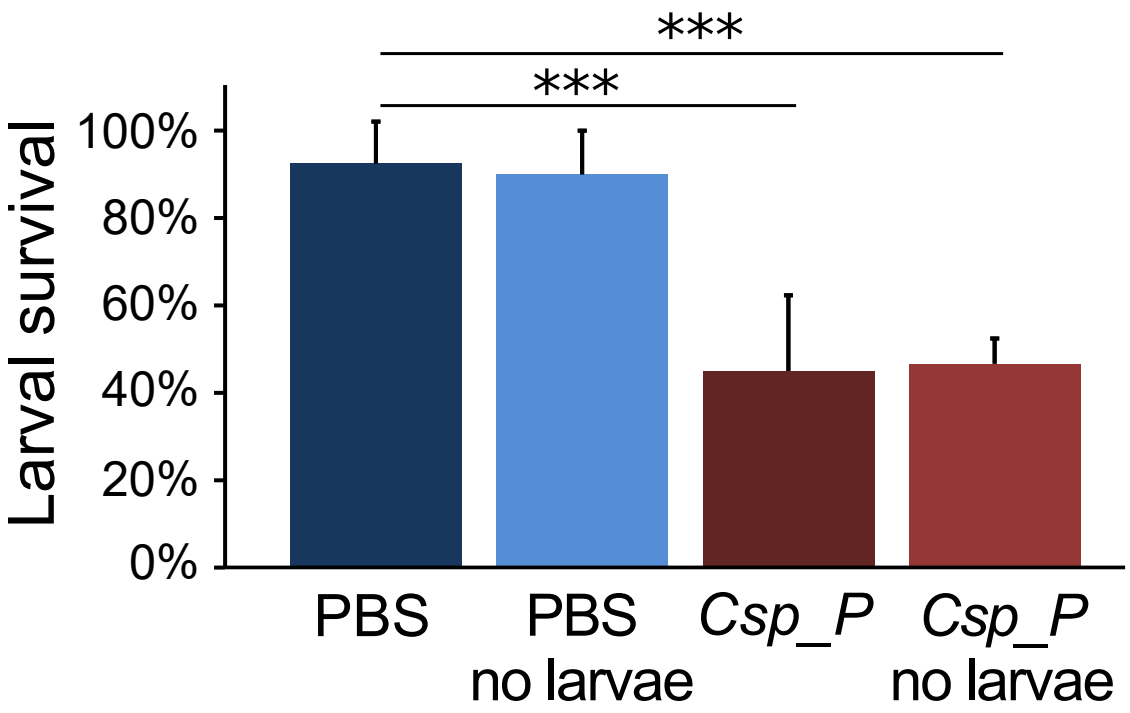

Figure S3: Larvicidal activity not dependent on the presence of larvae. Larval breeding water was treated with either 1X PBS or *C.sp_P*, and incubated for 6-8 hours until all larvae in the *C.sp_P* treatment were dead. In half the treatments, no larvae were added to the larval breeding water. Larval water was then collected and filtered using a 0.2µm filter to remove all live bacterial cells. This filtered water was then used to treat new pools of five *An. gambiae*larvae and survival was recorded after 16-17 hours exposure to the filtered water. The "PBS" and "*C.sp_P*" data presented here are the same as those presented in Figure 3. Larvae exposed to *C.sp_P* treated water suffered significant mortality regardless of whether larvae were co-incubated with *C.sp_P* bacteria before filtration (with larvae, $p = 0.0011$, without larvae, $p = 0.00097$). The graph shows average percent survival across replicate experiments and error bars represent one standard deviation. For each experiment two pools of five larvae were measured per treatment and the experiment was repeated three independent times. For all treatments, total sample size was $n = 30$.

Table S1: Model outputs for Statistical Analyses

Figure 1A:

coxph(formula = Surv(day, status, type = c("right")) ~ treatment + rep + pool)

n= 355, number of events= 97

	coef	exp(coef)	se(coef)	z	Pr(>|z|)
CspP 10^5	0.69003	1.99378	0.41689	1.655	0.0979 .
CspP 10^3	-0.45264	0.63595	0.52716	-0.859	0.3905
CspP 10^1	-0.65412	0.51990	0.55782	-1.173	0.2409
CspP 0.1	-1.58830	0.20427	0.78183	-2.032	0.0422 *
CspP 10^7	3.95136	52.00587	0.42587	9.278	<2e-16 ***
rep2	0.47306	1.60490	0.26506	1.785	0.0743 .
rep3	0.26788	1.30719	0.27574	0.971	0.3313
pool2	0.05954	1.06135	0.20336	0.293	0.7697

Figure 1B:

coxph(formula = Surv(day, status, type = c("right")) ~ treatment + rep + pool)

n= 240, number of events= 102

	coef	exp(coef)	se(coef)	z	Pr(>|z|)
CspP 10^5	-0.33149	0.71785	0.39705	-0.835	0.404
CspP 10^3	0.36408	1.43919	0.34165	1.066	0.287
CspP 10^1	0.15112	1.16314	0.35436	0.426	0.670
CspP 0.1	-0.02647	0.97388	0.37166	-0.071	0.943
CspP 10^7	1.35025	3.85838	0.32948	4.098	4.17e-05 ***
rep2	-0.03130	0.96918	0.20013	-0.156	0.876
pool2	-0.02145	0.97878	0.20064	-0.107	0.915

Figure 2:

coxph(formula = Surv(day, status, type = c("right")) ~ treatment + rep)

n= 260, number of events= 141

	coef	exp(coef)	se(coef)	z	Pr(>|z|)
C. aquaticum	1.73922	5.69291	0.31997	5.436	5.46e-08 ***
CspP	3.01887	20.46817	0.31796	9.494	< 2e-16 ***
C. subtsugae	-0.12424	0.88317	0.64027	-0.194	0.846
C. vaccinii	2.32464	10.22296	0.31987	7.268	3.66e-13 ***
C. violaceum	1.36668	3.92232	0.33550	4.074	4.63e-05 ***
rep2	0.04868	1.04988	0.23295	0.209	0.834
rep3	0.09991	1.10507	0.23457	0.426	0.670
rep4	-0.45661	0.63343	0.25110	-1.818	0.069 .

Figure 3:

glm(formula = status ~ rep + treat/pool, family = binomial(link = logit))

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-2.9892	0.9428	-3.170	0.00152 **
rep2	0.8844	0.8837	1.001	0.31697
rep3	-0.1025	0.8416	-0.122	0.90303
treatCspP	3.6174	0.8678	4.169	3.06e-05 ***
treatPBS:poo2	-17.0511	2364.3872	-0.007	0.99425
treatCspP:pool2	2.0318	1.1475	1.771	0.07662 .

Figure 4A:

glm(formula = status ~ rep + treat, family = binomial(link = logit))

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-2.7030	0.6940	-3.895	9.82e-05 ***
rep2	0.4258	0.5328	0.799	0.424
rep3	0.1479	0.5382	0.275	0.784
rep4	0.1479	0.5382	0.275	0.784
treatcsp	2.7246	0.6808	4.002	6.27e-05 ***
treatcsp10k	-16.0520	1029.4223	-0.016	0.988
treatcsp3-10k	-1.1525	1.1778	-0.979	0.328
treatcsp3k	2.7246	0.6808	4.002	6.27e-05 ***

```
treatpbs10k     -0.4328     0.9423  -0.459    0.646
treatpbs3-10k   -0.4328     0.9423  -0.459    0.646
treatpbs3k      -1.1736     1.1776  -0.997    0.319
```

Figure 4B:

Heat treatment experiment:

Room temperature:

```
glm(status~rep+treatment/well,data=rt, family=binomial(link=logit))

               Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL                           59     74.920
rep             2   2.9435        57     71.976 0.2295194
treatment       1  14.8347        56     57.142 0.0001174 ***
treatment:well  2   0.9822        54     56.159 0.6119638
```

37 degrees:

```
glm(status~rep+treatment/pool,data=ts, family=binomial(link=logit))

               Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL                           59     73.304
rep             2   1.4919        57     71.812 0.474286
treatment       1   8.5075        56     63.304 0.003537 **
treatment:pool  2   3.4841        54     59.820 0.175158
```

55 degrees:

```
glm(status~rep+treatment/pool,data=ff, family=binomial(link=logit))

               Df Deviance Resid. Df Resid. Dev  Pr(>Chi)
NULL                           59     73.304
rep             2   1.3964        57     71.907   0.49747
treatment       1  24.2913        56     47.616 8.281e-07 ***
treatment:pool  2   5.1472        54     42.469   0.07626 .
```

70 degrees:

```
glm(status~rep+treatment/pool,data=s, family=binomial(link=logit))

               Df Deviance Resid. Df Resid. Dev  Pr(>Chi)
NULL                           59     81.503
rep             2   0.9590        57     80.544 0.6191014
treatment       1  12.2854        56     68.259 0.0004565 ***
treatment:pool  2   2.1969        54     66.062 0.3333882
```

90 degrees:

```
glm(status~rep+treatment/pool,data=n, family=binomial(link=logit))

               Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL                           59     73.304
rep             2    1.933        57     71.371  0.38049
treatment       1   34.443        56     36.929 4.39e-09 ***
treatment:pool  2    5.951        54     30.977  0.05101 .
```

Figure 4C:

Initial model:

```
glm(status~rep+waterharvest+treat*vac_cent/pool,data=og, family=binomial(link=logit))
```

Final model:

```
glm(status~rep+treat*vac_cent,data=og, family=binomial(link=logit))
```

Anova:

```
            Df Deviance     Resid. Df Resid. Dev  Pr(>Chi)
NULL                            238     313.42
rep             1   11.087       237     302.33   0.0008692 ***
treat           1   23.458       236     278.88   1.277e-06 ***
vac_cent        1   15.764       235     263.11   7.174e-05 ***
treat:vac_cent  1   27.467       234     235.65   1.598e-07 ***
```

Figure 6:

Original model:
glm(status~waterharvest+exptrep+bact*antidote/pool, family=binomial(link=logit))
Final model:
glm(status~bact*antidote, family=binomial(link=logit))

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)	
NULL			159	154.43		
bact	1	4.1680	158	150.26	0.0411951	*
antidote	1	14.4453	157	135.81	0.0001443	***
bact:antidote	1	5.0559	156	130.76	0.0245421	*

Figure 7A:

survdiff(formula = Surv(day, status) ~ treatment)

	N	Observed	Expected	(O-E)^2/E	(O-E)^2/V
treatment=csp	262	140	159	2.38	5.78
treatment=pbs	476	377	358	1.06	5.78

Chisq= 5.8 on 1 degrees of freedom, p= 0.0162