

Supplemental Material:

Antisocial *luxO* mutants provide a stationary-phase survival advantage in *Vibrio fischeri* ES114

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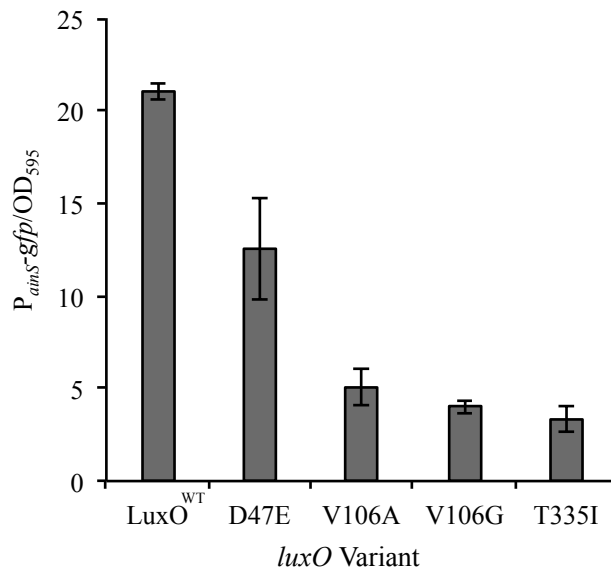


FIG S1. The inactivation of *luxU* does not eliminate the effect of different LuxO* variants on $P_{ainS-gfp}$ reporter activity. GFP expression in strains harboring the $P_{ainS-gfp}$ transcriptional reporter pHK12, grown in SWTO, and assayed at $OD_{595} \sim 2.5$. Strains and their respective *luxO* alleles are JHK068 (wt LuxO), JHK069 (D47E), JHK065 (V106A), JHK087 (V106G), and JHK066 (T335I). Data are from a single representative experiment of three independent experiments. Error bars indicate standard errors ($n = 3$).

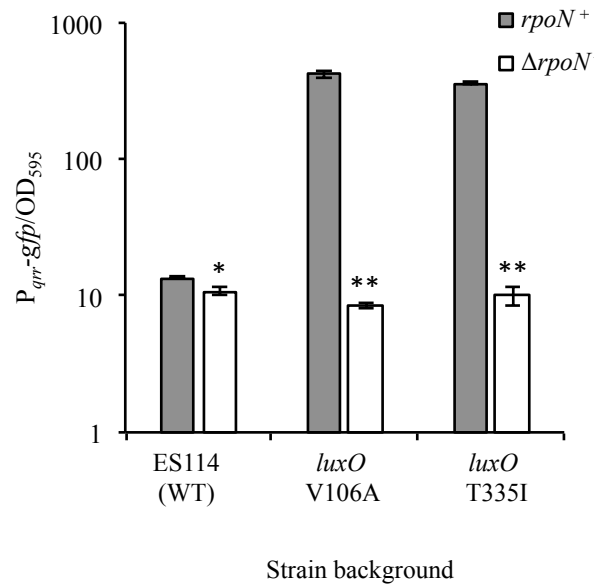


FIG S2. The activity of LuxO* variants at the *qrr* promoter is *rpoN*-dependent. GFP activity from strains harboring the P_{qrr} -*gfp* reporter pTM268 were grown in SWTO and assayed at $OD_{595} \sim 2.5$. Strains containing the reporter plasmid are (left to right) ES114, KV5005, JHK057, JHK062, JHK061, and JHK063. Data are from a single representative experiment of three independent experiments. Error bars indicate standard error ($n = 3$). Asterisks indicate significantly different reporter activity between the $\Delta rpoN$ derivative and its parent strain, as determined using Student's *t*-test; * $P < 0.05$, ** $P < 0.01$.

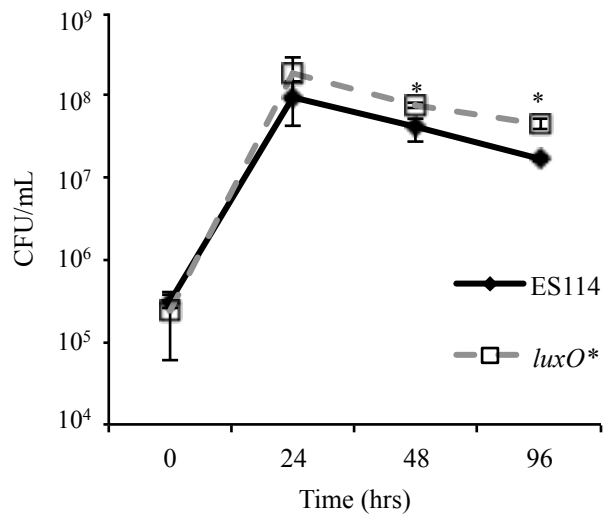


FIG S3. *luxO** mutant JHK057 survives better than wild type in prolonged static culture. ES114 (wild type; solid black line and filled diamonds) and JHK057 (*luxO** V106A; grey dashes and open squares) were grown to $OD_{595} \sim 2.5$ and inoculated into fresh LBS medium in 24-well microtiter plates and cultured without shaking for 96 hr. At the time of initial inoculation, as well as 24, 48, and 96 hr post-inoculation, wells were mixed thoroughly and dilution plated to determine CFU. Data are from a single representative experiment of three independent experiments. Error bars indicate standard error ($n = 3$). * indicates a significant difference from wild-type ES114 ($P < 0.05$). CFU/mL was 1.9-fold and 2.7-fold higher for JHK057 relative to ES115 at 48 and 96 hours, respectively.

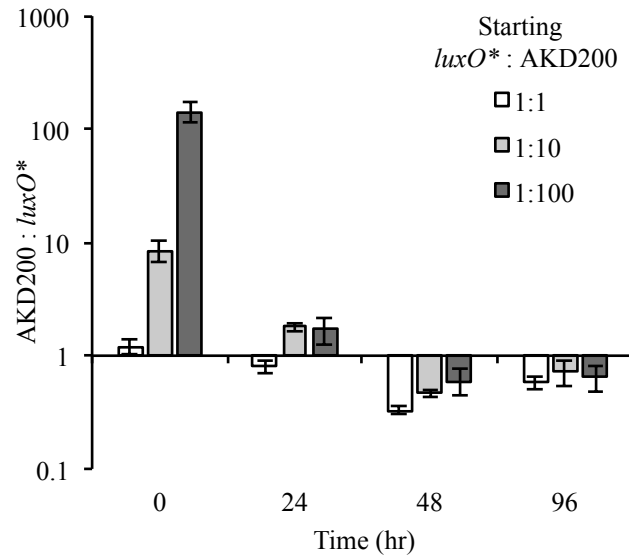


FIG S4. $luxO^*$ (T335I) mutant JHK061 displays a competitive advantage during prolonged co-culture. AKD200 (a chloramphenicol-resistant derivative of ES114 – see main text) and JHK061 ($luxO^*$ T335I) were grown overnight, subcultured into fresh LBS medium, and grown to $OD_{595} \sim 2.0$ at which time they were mixed in JHK061 to AKD200 ratios of 1:1, 1:10, or 1:100. Mixes were outgrown to $OD_{595} \sim 2.5$ and then diluted 1:1000 in fresh LBS medium in 24-well microtiter plates and cultured statically for 96 hr. At intervals of 0, 24, 48, and 96 hr, wells were thoroughly mixed and dilution plated to determine the ratios of viable cells of each strain based on chloramphenicol resistance. Data are from a single representative experiment of two independent experiments. Error bars indicate standard error ($n = 3$).

FIG S5. Strains bearing *litR* or *luxO** mutations have different phenotypes with respect to growth rate (A), luminescence (B), or P_{qrr} -*gfp* reporter activity (C). In Panels A and B, ES114 (solid black line with filled diamonds), JHK057 (*luxO** V106A, black dashed line and filled squares), and JB18 (*litR*, solid grey line and triangles) were grown with shaking at 24°C in SWTO medium and assessed at regular intervals for OD₅₉₅ (A), and specific luminescence (B). Although starting at the same OD₅₉₅ (A), after 480 min JHK057 (*luxO**) consistently had higher OD₅₉₅ than did JB18 (*litR* mutant), by about 8% ($P < 0.05$). By contrast, JB18 achieved 5-fold higher luminescence ($P < 0.01$) than did JHK057 (B). Panel (C) shows GFP expression in strains harboring the P_{qrr} -*gfp* reporter pTM268, grown in SWTO, and assayed at OD₅₉₅ ~2.5. Each panel shows a single representative experiment of three independent trials. Error bars indicate standard error ($n = 3$). In panel C, ** indicates the *luxO* mutant was significantly different from wild-type ES114 and the *litR* mutant JB18 ($P < 0.01$).

