



S3 Fig. *cbs* deletion does not affect biofilm formation, virulence expression, organic hydroperoxide resistance, and growth in *V. cholerae*.

(A) The biofilm-forming capability of Δcbs at static condition. Overnight cultures of wild-type (WT) or Δcbs were 1:100 sub-cultured into fresh medium. Biofilms formed at solid-liquid interface were analyzed by crystal violet staining after 24 hrs of incubation at 25°C in glass test tubes.

(B) *tcpA* expression in Δcbs compared with wild-type (WT). WT and Δcbs , containing

promoter-*luxCDABE* transcriptional fusion reporter plasmids of virulence gene *tcpA* were grown aerobically and then 1:100 incubated in LB or AKI medium, at 37°C for 4 hrs without shaking. Luminescence was then measured and normalized against OD₆₀₀. Results are the means and S.D. of three independent experiments.

(C) *cbs* expression and the organic hydroperoxide resistance in *V. cholerae*. Bacteria were exposed to 100 μM of Cumene hydroperoxide (CHP) for 30 min at their exponential-phase or left untreated, in M9 minimal medium (M9 salts plus 2 mM MgSO₄, 0.1 mM CaCl₂, and 0.2% casein acid hydrolysate as sole carbon source) containing 200 μM IPTG. Viability was determined by comparing the CFU in the CHP-challenged and the unchallenged samples. Three replicates were sampled for each strain. No significance was detected (ns).

(D) Growth of wild-type (WT/vector), *cbs* deletion mutant (Δcbs /vector), and *cbs*-complemented strain ($\Delta cbs/P_{tac-cbs}$) in Luria–Bertani broth or M9 minimal medium (M9 salts plus 2 mM MgSO₄, 0.1 mM CaCl₂) with different sole carbon sources. Overnight cultures were 1:1,000 sub-cultured in LB and grown to log-phase at 37°C with shaking. Bacterial cultures were pelleted by centrifugation, washed once, and adjusted OD₆₀₀ to 0.1 with fresh medium. Growth kinetics of test strains were determined by OD₆₀₀ measurement in 96-well microplate using microplate-reader (Spark, Tecan). Samples were incubated at 37°C with shaking, and readings were taken every 20 min for 15 hrs. Four individual experiments were taken.