SUPPLEMENTAL INFORMATION 1

Pathogenicity and infection cycle of *Vibrio owensii* in larviculture of ornate spiny lobster (*Panulirus ornatus*)

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SUPPLEMENTAL METHODS

Transconjugation. Donor, helper and recipient strains were cultured in LB broth with 40 µg mL^{-1} kanamycin (for helper and gfp-donor) or LB20 broth (5 g L^{-1} yeast extract, 10 g L^{-1} neutralized peptone, 20 g L^{-1} NaCl; for *V. owensii* DY05) for 24 h at 30°C before subculture in the respective media for a further 16 h. Two hundred and fifty microlitres of each helper, donor and recipient subculture were combined in a microfuge tube, centrifuged (5200 g for 5 min) and the pellet resuspended in 200 µL 30°C LB20 without antibiotics. Fifty microlitres of the mixed culture was spot inoculated onto LB20 agar (5 g L^{-1} yeast extract, 10 g L^{-1} neutralized peptone, 20 g L^{-1} NaCl, 1.5% agar) and incubated for 24 h at 30°C. The mixed bacterial colonies were streak inoculated onto selective LB20 agar plates (supplemented with 40 µg mL^{-1} kanamycin and 50 µg mL^{-1} colistin) and incubated for 24 h at 30°C. Fluorescent transconjugant colonies were detected using a blue light transilluminator and re-streaked on *Vibrionaceae*-selective thiosulfate citrate bile sucrose agar plates (TCBS). TCBS-viable colonies were re-checked under blue light to substantiate gfp expression. Isolates were cryopreserved at -80°C in 30% glycerol with LB20 supplemented with 40 µg mL^{-1} kanamycin.

Expression of gfp. *V. owensii* DY05[gfp] was assessed for stable expression of gfp by continuous subculture in media without antibiotic selection pressure. DY05[gfp] was precultured on selective LB20 agar for 24 h at 28°C, and in triplicate, a single fluorescent colony was inoculated with 5 mL MB without antibiotics and cultured for 24 h (28°C, 170 rpm). The cultures were continuously subcultured in MB by transferring a 100 µL aliquot to 5 mL of MB every 24 h. Subcultures were sampled by spiral plating (Eddy Jet; IUL) on marine agar (MA; Becton, Dickinson and Company), incubation overnight (28°C) and enumeration by an automatic colony counter (Flash and Grow v1.2; IUL).

Growth of transconjugants. The growth curve of DY05[gfp] was compared to wild type *V. owensii* DY05 using a modified microgrowth assay (1). *V. owensii* DY05 and DY05[gfp] were revived on agar media as described previously, colony material was suspended in 2 mL PBS (8 g L^{-1} NaCl; 0.2 g L^{-1} KCl; 1.44 g L^{-1} Na_2HPO_4; 0.24 g L^{-1} KH_2PO_4; pH 7.2) and suspensions were adjusted to absorbance OD_{600nm} 0.1 (Nanodrop ND1000). Total viable counts (expressed as CFU mL^{-1}) were determined for each bacterial strain by spiral plating as described previously. MB was inoculated with PBS suspensions of *V. owensii* DY05 or DY05[gfp] at an initial concentration of 1 x 10^3 CFU mL^{-1} in a Nunc™ (NUN167008) 96-well microtitre plate (final well volume 200 µL). Treatments were performed in hextuplicate well sets. Perimeter rows and columns were loaded with 200 µL sterile milli-Q water to minimize evaporative loss and plate covers were made hydrophilic by treatment with 0.1% Triton X-100 in 20% ethanol to smooth condensation. Plates were sealed with parafilm and incubated at 28^oC with agitation (170 rpm) and growth of the strains was monitored by absorbance (OD_{595nm}) with a Wallac Victor^2 1420 multilabel counter. Measurements were adjusted by subtracting the average background noise generated from a MB control.

REFERENCES

1. Brewster, J.D. 2003. A simple micro-growth assay for enumerating bacteria. J. Microbiol. Meth. **53**: 77-86.