Supporting Information

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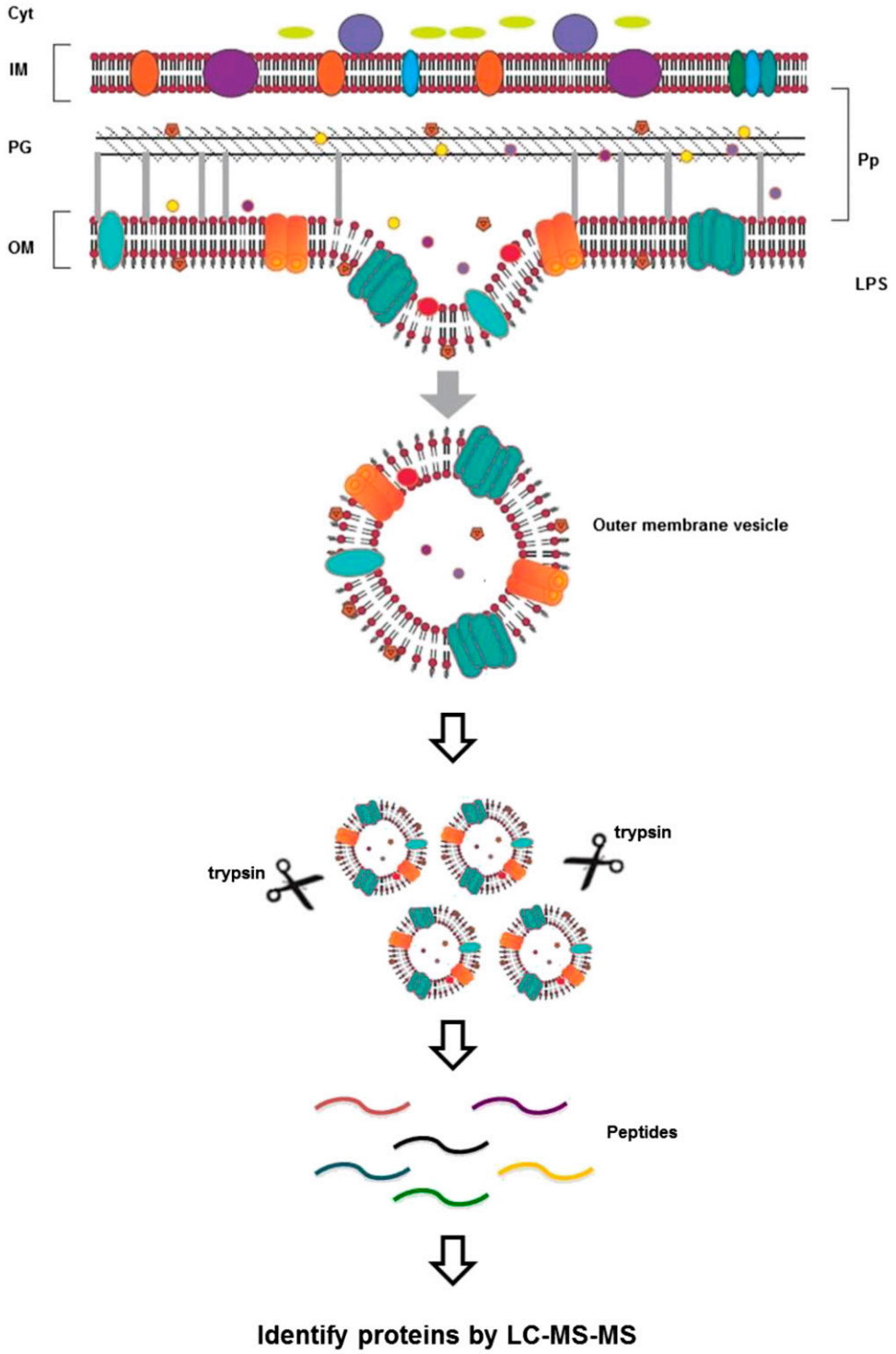

Fig. S1. In-solution digestion approach to characterize outer membrane vesicles (OMVs). OMVs are secreted from the outer membrane and are composed of outer membrane proteins, lipopolysaccharides, and periplasmic proteins (LPS, lipopolysaccharide; Pp, periplasm; OM, outer membrane; PG, peptidoglycan; IM, inner membrane; Cyt, cytosol). OMVs from *Vibrio cholerae* El Tor C6706 were purified from culture media by ultracentrifugation and subjected to overnight trypsin digestion. The resulting peptides were analyzed by LC-MS/MS. Amino acid sequences of peptides were identified by searching obtained spectra against the *V. cholerae* El Tor Inaba N16961 strain database.

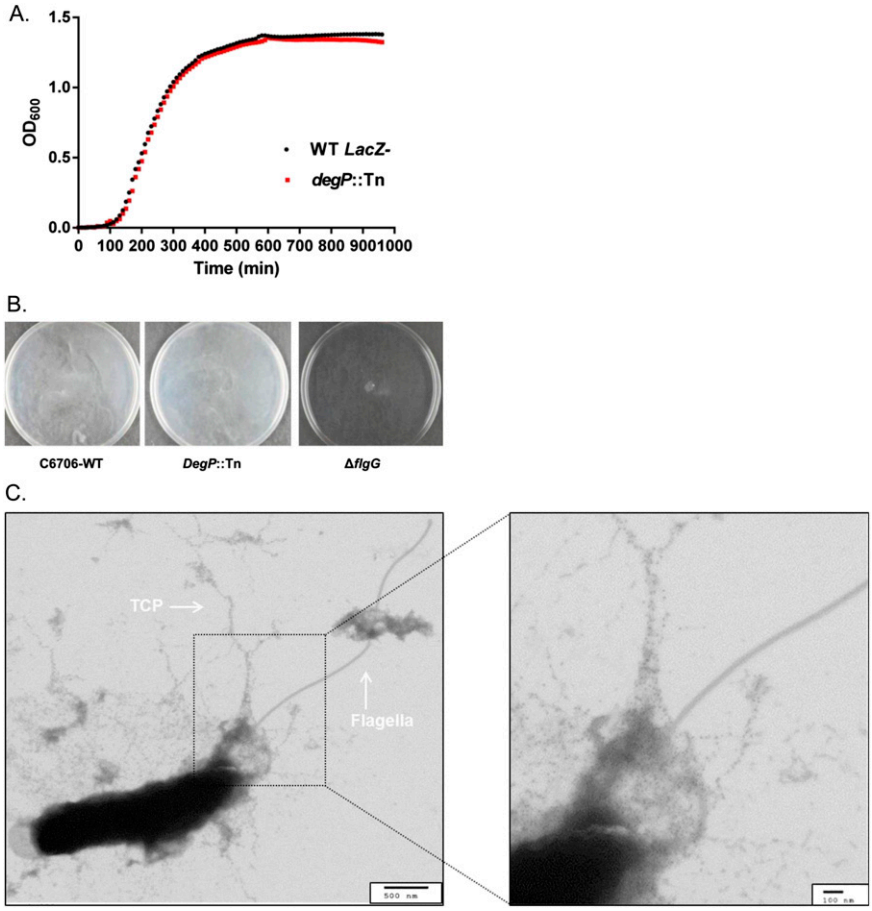

Fig. S2. In vitro growth, motility, and pili formation of *degP* mutant (EC956) mutant. (*A*) Growth curves of C6706 wild-type (WT) compared with *degP* mutant (EC956). (*B*) Evaluation of chemotactic mobility of *degP* mutant (EC956) was tested on 0.3% bactoagar plates. C6706 and *flgG* serve as the wild-type and nonmotile controls, respectively. (*C*) Transmission electron microscopy analysis of α-TcpA monoclonal antibodies binding to *degP* mutant (EC956). The white arrow indicates toxin-coregulated pili (TCP) and flagella. (*Right*) Magnification (5×) of the selected region.

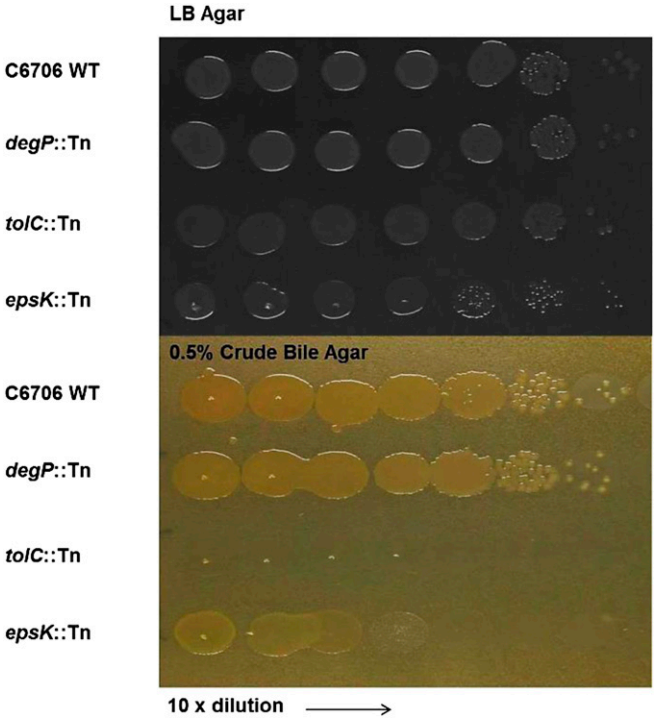

Fig. S3. Sensitivity of *degP* mutant (EC956) on LB agar containing 0.5% crude bile. *V. cholerae* strains were grown in LB until OD_{600} reached 1.0. Cells were serially diluted and then daubed onto both LB agar (row 1–4) or LB agar containing 0.5% crude bile (row 5–8) for colony counting. All TnFGL3 mutants and WT bacteria grew normally on LB agar. *degP* mutant (EC956) grew as well as WT on the bile agar whereas *tolC* mutant (EC2859) did not survive in the presence of bile. The *epsK* mutant (EC903) had severe defects on 0.5% crude bile agar (4 logs). tolC, outer membrane protein TolC; epsK, general secretion pathway protein K.

Other Supporting Information Files

Table S1 (DOCX)
Table S2 (DOCX)
Table S3 (DOCX)
Table S4 (DOCX)
Table S5 (DOCX)
Table S6 (DOCX)
Table S7 (DOCX)