High-Level Production of Bacteriotoxic Phospholipase A1 in Bacterial host *Pseudomonas fluorescens* Via ABC-Transporter Mediated Secretion and Inducible xpression

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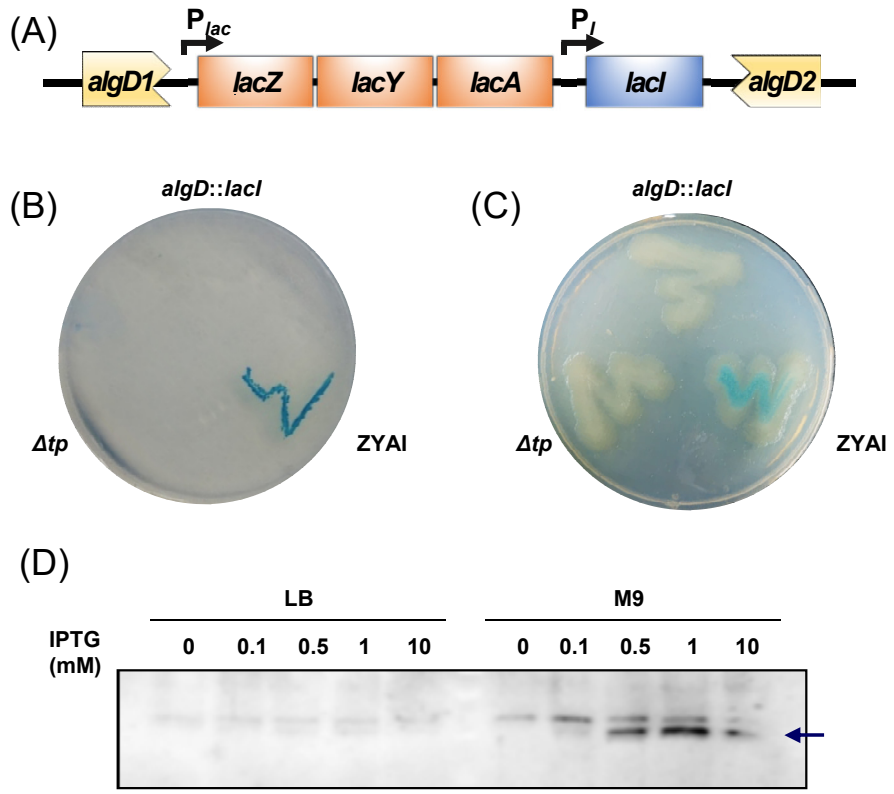

Figure S1. Construction of *P. fluorescens* ZYAI. **(A)** Genomic organization of knocked-in *P. fluorescence* ZYAI. **(B)** Each recombinant *P. fluorescens* was streaked on an M9 agar plate containing 40 µg/ml X-Gal, 1 mM IPTG, and 0.6 % lactose as sole carbon source. *P. fluorescens Δtp* and *algD::lacI* could not grow in lactose supplemented M9 medium. **(C)** It was streaked similarly on an M9 agar plate containing 40 µg/ml X-Gal, 1 mM IPTG, 0.6 % lactose, and 2 % glucose. Only *P. fluorescens* ZYAI could hydrolyze X-gal to show a blue colony. **(D)** Western blot for the analysis of secreted PlaA from *P. fluorescens* ZYAI in the different IPTG concentrations on LB or M9 medium. The arrow indicates PlaA (45 kDa). *Δtp*: *ΔtliA ΔprtA, algD*::*lacI*: *lacI* knocked-in *Δtp, ZYAI: lacZYA* and *lacI* knocked in *Δtp.*

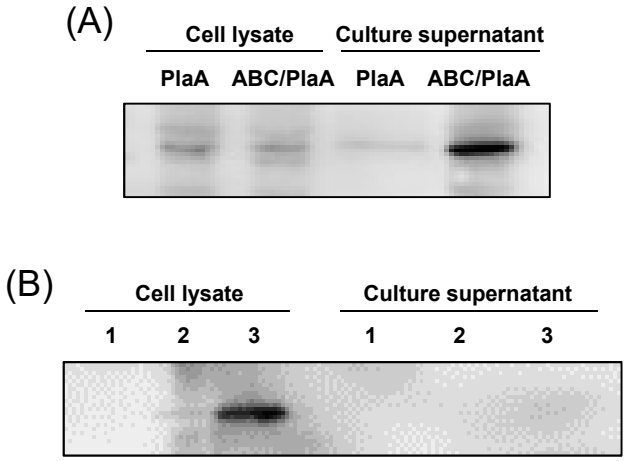

Figure S2. Secretion of PlaA by the ABC transporter system. **(A)** *P. fluorescens* cells harboring the PlaA-encoding gene fused with LARD3 were cultured at 25 °C for 4 days in the presence or absence of the ABC transporter. Western blot analysis was performed in the cell and in the supernatant. **(B)** PlaA expression without LARD3 was analyzed. Three plasmids were used for this purpose: pDSK (1), pDSK-PlaA (2), and pDSK-PlaA/PlaS (3), with control, PlaA, and PlaA/PlaS in pDSK519, respectively. Culture conditions and analyses used were the same as above.