

Fig. S1 Sequence alignment of the GGDEF standalone proteins GdpXs

Multiple sequence alignments were carried out by using DNAMAN software (version 6.0, Lynnon BioSoft, Canada). The amino acids highlighted with red, blue, green, and yellow mean 100%, ≥75%, ≥50% and ≥33% identities, respectively. Black lines showed the corresponding sites of GTP/Mg²⁺ binding, I-site, GGDEF motif and GTP binding sites, respectively.

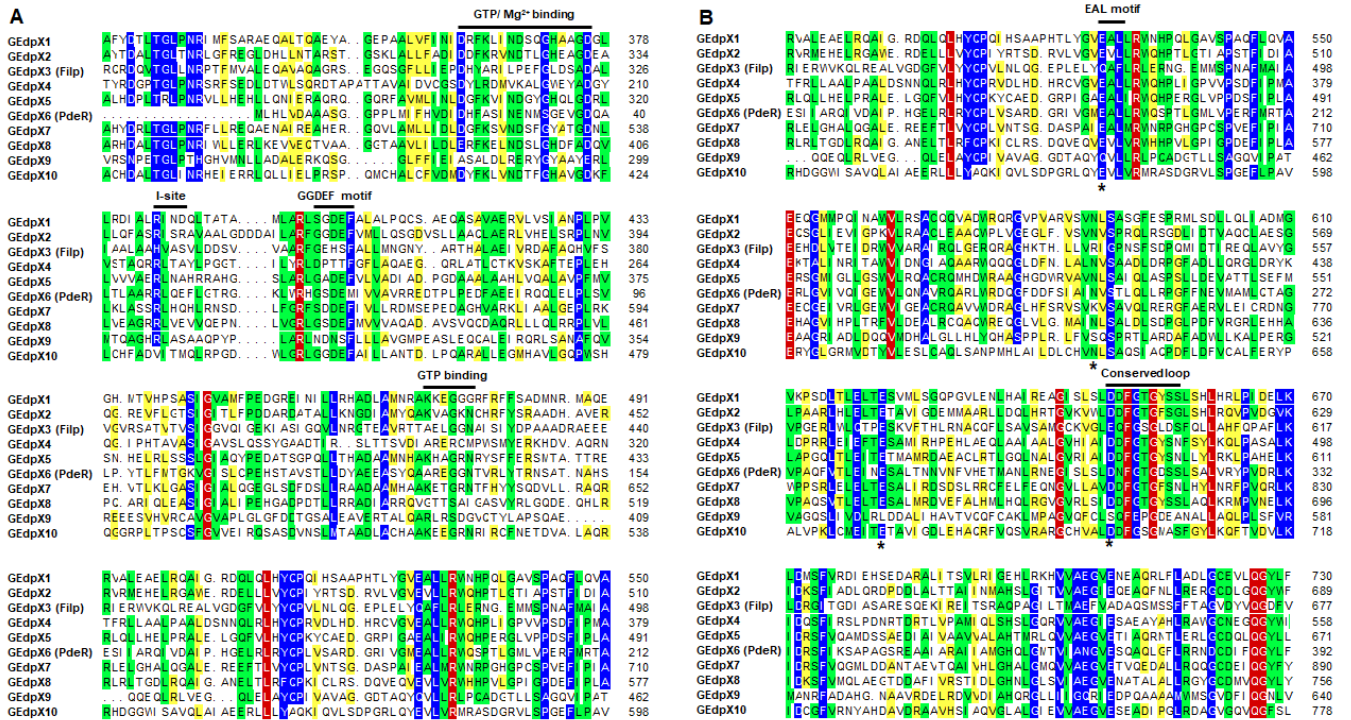


Fig. S2 Sequence alignment of the GGDEF and EAL hybrid proteins GEdpXs

Multiple sequence alignments were carried out by using DNAMAN software (version 6.0, Lynnon BioSoft, Canada). The amino acids highlighted with red, blue, green, and yellow mean 100%, ≥75%, ≥50% and ≥33% identities, respectively. **(A) The alignment of GGDEF domain.** Black lines showed the corresponding sites of GTP/Mg²⁺ binding, I-site, GGDEF motif, and GTP binding sites, respectively. **(B) The alignment of EAL domain.** Asterisks showed the critical metal cation and substrate binding sites. Black lines showed EAL and Loop 6 residues, respectively.

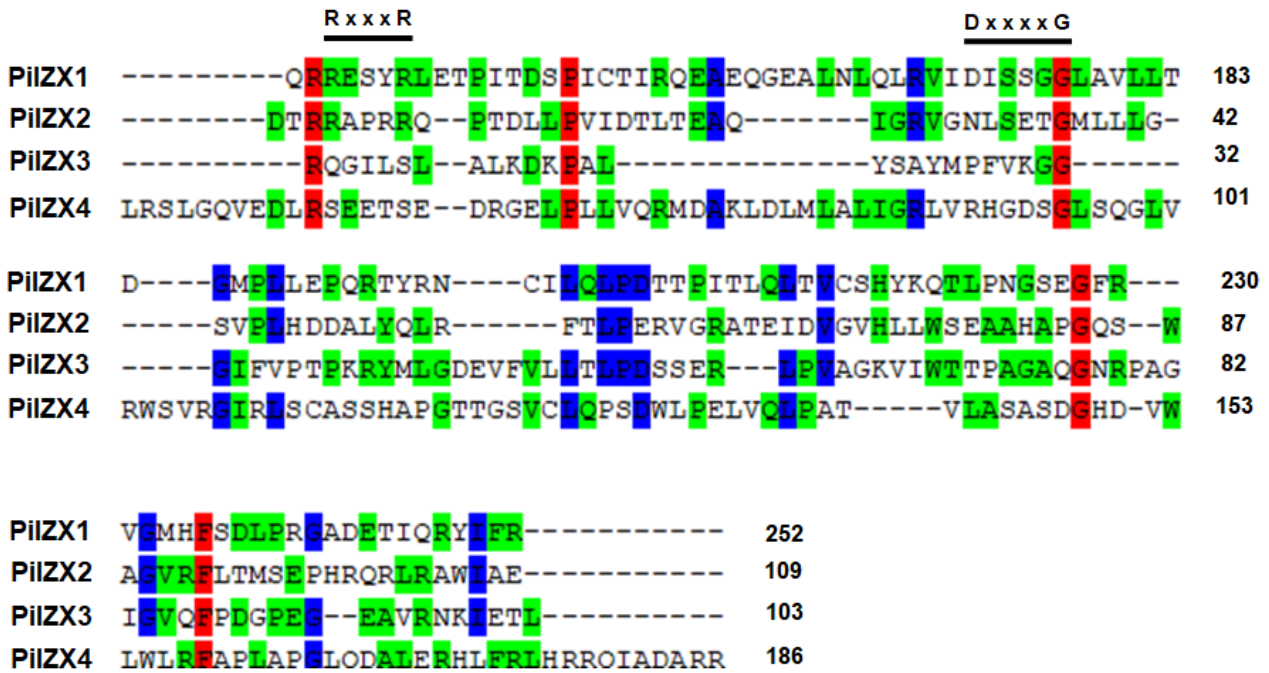


Fig. S5 Sequence alignment of the PilZ domain proteins PilZXs

Multiple sequence alignments were carried out by MEGA Muscle software analysis (version 7.0.21) and edited by using Genedoc software. The amino acids highlighted with red, blue, and green mean 100%, 75%, and 50% identities, respectively. Black lines showed the conserved motifs that were involved in c-di-GMP binding in the PilZ domain.