Supporting information

The *Xanthomonas oryzae* pv. *oryzae* PilZ-domain proteins function differentially in cyclic di-GMP binding, and regulation of virulence and motility.

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Table S1. Primers used in this study.

Primer	Sequences(5′- 3′)	Restriction enzyme/use
49PF1	CG**GGATCC**GTGCTTGTGCCCATGTCCCA	*Bam*HI/Protein expression
49PR1	CC**AAGCTT**TCAGAACATGCCGCTCTTGC	*Hin*dIII/Protein expression
49PF2	CGGCGCGAATCATACGACCTGGAAA	Protein expression
49PR2	TCGGTGATCGGCGTTTCCAGGTCGTATGATTC	Protein expression
2374PF1	CG**GGATCC**ATGCAGGACACCCGCCG	*Bam*HI/Protein expression
2374PR1	CC**AAGCTT**TTAGCCAGCCATGTGCTCTTCG	*Hin*dIII/Protein expression
2374PF2	CG**GGATCC**ATGCAGGACACCCGCCGCGCACCGCGCGACCAG CCGA	*Bam*HI/Protein expression
GmF	CC**GAATTC**GACGCACACCGTGGAAA	*Bam*HI/Gene deletion
GmR	GC**TCTAGA**GCGGCGTTGTGACAATTT	*Xba*I/Gene deletion
49MF1	CC**GAATTC**GGCTCATTCCTTCATCGTCCA	*Eco*RI/Gene deletion
49MR1	CG**GGATCC**AATAGAAGCGGGAAGGCGC	*Bam*HI/Gene deletion
49MF2	GC**TCTAGA**ACCCGCCCGCACCGCGC	*Xba*I/Gene deletion
49MR2	CC**AAGCTT**GCCGATGCCGGAGATGAAGCGG	*Hin*dIII/Gene deletion
2374MF1	AAGTCACCTGCCGCAGCGA	Gene deletion
2374MR1	CG**GGATCC**CATGGCTGGGCCTCGGTAAC	*Bam*HI/Gene deletion
2374MF2	GC**TCTAGA**GCGCGGCGTGCACCGTG	*Xba*I/Gene deletion
2374MR2	CC**AAGCTT**CGCCTCGCCAAGAATCGCG	*Hin*dIII/Gene deletion
49CF	GG**GGTACC**CCTTCCCGCTTCTATTGTGCTTGTG	*Kpn*I
2374CF	GG**GGTACC**CCGAGGCCCAGCCATGCAGGA	*Kpn*I
gyrBF	GGCGAGCACAATGGCATT	qRT-PCR
gyrBR	CCATCCTTCTGCGGGATGT	qRT-PCR
hrpGF	TGTCCACCTGATGAACGACCCT	qRT-PCR
hrpGR	GGCGAATGCCGCAACGAA	qRT-PCR
hrpXF	AGGCACTGACCCACTTTC	qRT-PCR
hrpXR	ATCGGAAGCACCACTCTC	qRT-PCR
hpa1F	AAGCCAGGACACAACGTTCG	qRT-PCR
hpa1R	GAAGCAGGGCCGAGATGAG	qRT-PCR
GFPF	GC**TCTAGA**AGTAAAGGAGAACTTTT	*Xba*I /Subcelluar localization
GFPR	C**GAGCTC**TTATTTGTATAGTTCATCCAT	*Sac*I /Subcelluar localization
49LR	CC**AAGCTT**GAACATGCCGCTCTTGCG	*Hin*dIII/Subcelluar localization
2374LR	CC**AAGCTT**GCCAGCCATGTGCTCTG	*Hin*dIII/Subcelluar localization
2715LF	GG**GGTACC**GAACTCGATGAGTGCAATGA	*Kpn*I/Subcelluar localization
2715LR	CC**AAGCTT**CATCGTGTGCGTCGGCTT	*Hin*dIII/Subcelluar localization

Restriction enzyme sites are in bold text.

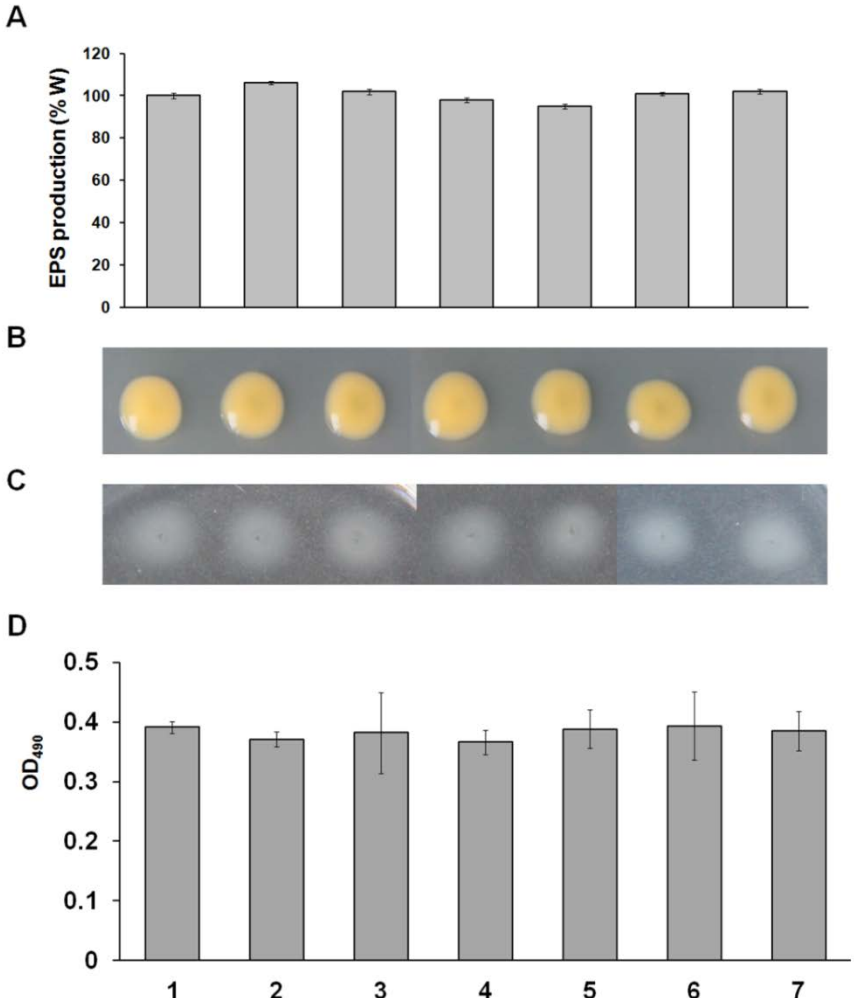


Fig. S1. Assays for EPS production, motility and biofilm formation of Δ*PXO_00049*, Δ*PXO_02374* and Δ***PXO_02715****.*

(A and B) No significant differences in EPS production were observed between PXO99^{A} and relevant mutants. (C) Δ*PXO_00049*, Δ*PXO_02374* and Δ*PXO_02715* displayed no change in swimming motility compared with PXO99^{A}. (D) Δ*PXO_00049*, Δ*PXO_02374* and Δ*PXO_02715* showed no change in biofilm formation compared with PXO99^{A}. 1, Wildtype PXO99^{A}; 2, Δ*PXO_00049*; 3, Δ*PXO_00049* (pB49); 4, Δ*PXO_02374*; 5, Δ*PXO_02374* (pB2374); 6, Δ*PXO_02715*; 7, Δ*PXO_02715* (pB2715).

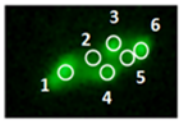
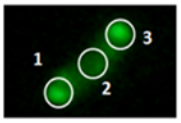
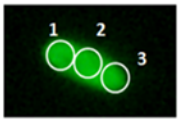
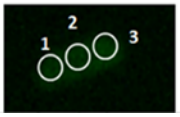
Subcellular Location	Bacterial cell with fluorescence	The average brightness value of the selected area in bacterial cell
Multisite		1: 177.91±5.85 2: 101.99±1.74 3: 166.95±6.46 4: 162.57±4.59 5: 105.11±1.03 6: 176.93±6.48
bipolar		1: 189.52±3.13 2: 104.16±6.24 3: 172.71±5.11
Nonpolar		1: 466.81±6.84 2: 460.99±8.72 3: 463.2±8.18
low level fluorescence		1: 60.81±1.14 2: 58.99±3.0 3: 59.20±4.16

Fig. S2. Definition of the subcellular location of PilZ-domain proteins.

The subcellular localization of proteins in *Xoo* strains was detected by using Fluorescence Microscope and the CellSens Dimension software was used for analysis the average brightness value of the selected area in bacteria cell. The average brightness value >160 were defined as the fluorescence location of the protein. Multisite location contains three or four fluorescence sites in the cell.The cell containing the fluorescence sites in bipolar were defined as bipolar location. The whole cell with high level fluorescence were defined as nonpolar location.