Applied and Environmental Microbiology

The bHLH transcription factor DevR significantly affects polysaccharide metabolism in *Aspergillus oryzae*

Running title: AoDevR affects the polysaccharide metabolism

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Figure legendsy

Fig. S1 Comparison of the deduced amino acid sequence of AO090026000797 with those of *A. nidulans* and *A. fumigatus*. The enclosed regions indicate a putative bHLH-binding domain. AfDEVR, DevR of *Aspergillus fumigatus*; ANDEVR, DevR of *Aspergillus nidulans*.

Fig. S2 Construction of *AodevR*-disrupted and *AodevR*-overexpressing strains. (A) Construction of the *AodevR* disruptant. The *AodevR* ORF was deleted and replaced by a *pyrG* marker. (B) Confirmation of *AodevR* gene disruption by Southern blot analysis. Genomic DNA was digested with SmaI and hybridized with the indicated probe. The expected DNA sizes are shown by the arrows. Lane 1, parent strain; lanes 2-4, transformants. Band shifts of lane 2 and lane 4 indicate that the *AodevR* ORF was deleted in the two transformants. (C) Construction of the plasmid for *AodevR* overexpression. The full-length *AodevR* ORF was inserted downstream of the *amyB* promoter. (D) Confirmation of the constructed *AodevR*-overexpression strain by Southern blot analysis. Genomic DNAs were digested with PstI and hybridized with the probe (*AodevR* ORF). Lane 1, parent strain; lane 2-7, transformants. The appearance of the second band

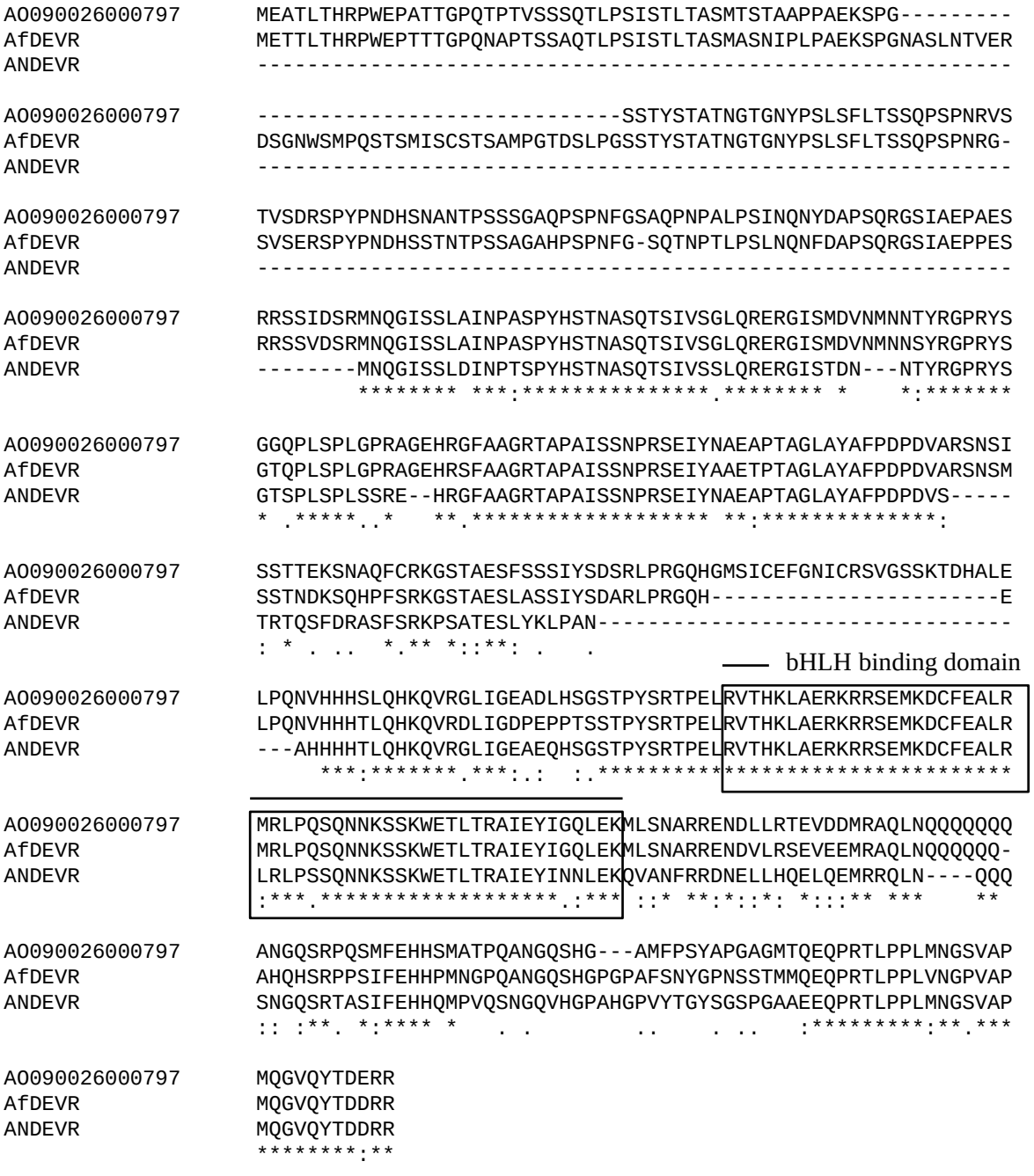
indicates that the plasmid was successfully integrated into the parent strain. (E) Confirmation of *AodevR* overexpression by semi-quantitative RT-PCR analysis. Total RNA was isolated from the strains cultured on dextrin-containing CD media for 2 days. The histone H2A as a positive control and negative-control PCR analysis using each mRNA as a template showed no amplified fragments (data not shown).

Fig. S3 Complementation of the *AodevR* deletion strain. (A) Complementation of the *devR* deletion strain by re-integrating the *AodevR* gene into the Δ*devR* strain. The complementation was confirmed by Southern blotting. Genomic DNA was digested with SmaI and hybridized with the indicated probe. The expected DNA sizes are shown by the arrows. Lane 1, Δ*devR* strain; lanes 2-3, transformants. (B) Phenotype of the *devR*-restored strain. The generated strain Δ*devR* (*devR*$^{+}$), *devR* disrupted strain (Δ*devR*), and control strain were spot-cultured on CD+Uridine agar medium at 30°C for 5 days. Their phenotypes were observed and compared.

Fig. S4 Construction of P*thiA*-inducing *AodevR* overexpression strain and phenotypic analysis. (A) Construction of plasmid for P*thiA*-inducing *AodevR* overexpression. *amyB* promoter was replaced by *thiA* promoter to induce *AodevR* expression. (B)

Phenotypic analysis. Strains were cultivated onto the CD(dextrin) agar media at 30 °C for 5 days, and their phenotype was observed and compared. PthiA-*devR*, P*thiA*-inducing *AodevR* overexpression strain; OE-*devR*, P*amyB*-inducing *AodevR* overexpression strain. (C) qPCR analysis. Constructed PthiA-*devR* strain and control strain were cultivated in liquid medium at 30 °C for 2 days and the RNA was extracted. The relative expression levels of *AodevR* and some genes involved in starch degradation were examined and compared by qPCR. The expression levels of the genes were normalized to the expression level of the endogenous control gene histone H2A. The expression value of each gene in the control strain was used as the baseline. For qPCR, three replicates were performed for each sample. The average values and standard deviations were represented as bars and error bars, respectively. Con, Control strain; PthiA-*devR*, *thiA* promoter inducing *AodevR* overexpression strain.

Fig. S1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ANDEVR ----------
AO090026000797 ----------SSTYSTATNGTGNYPSLSFLTSSQPSPNRVS
AfDEVR DSGNWSMPQSTSMISCSTSAMPGTDSLPGSSTYSTATNGTGNYPSLSFLTSSQPSPNRG-
ANDEVR ----------
AO090026000797 TVSDRSPYPNDHSNANTPSSSGAQPSPNFGSAQPNPALPSINQNYDAPSQRGSIAEPAES
AfDEVR SVSERSPYPNDHSSTNTPSSAGAHPSPNFG-SQTNPTLPSLNQNFDAPSQRGSIAEPPES
ANDEVR ----------
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---AHHHHTLQHKQVRGLIGEAEQHSGSTPYSRTPELRVTHKLAERKRRSEMKDCFEALR
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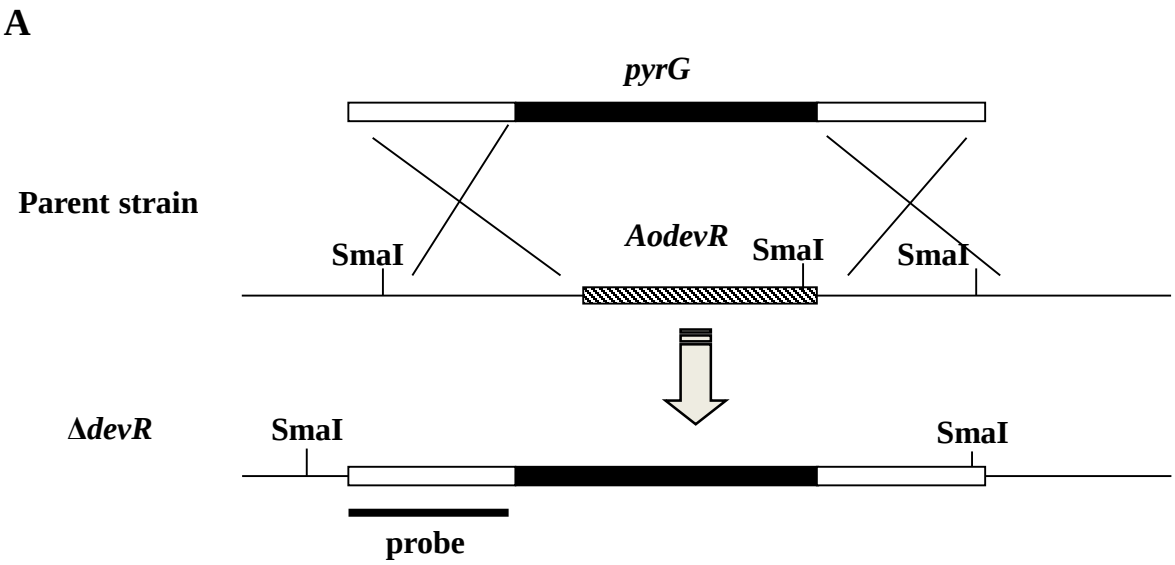
MQGVQYTDDRR

Fig. S2


A
pyrG
Parent strain
SmaI
AodevR
SmaI
SmaI
ΔdevR
SmaI
SmaI
probe

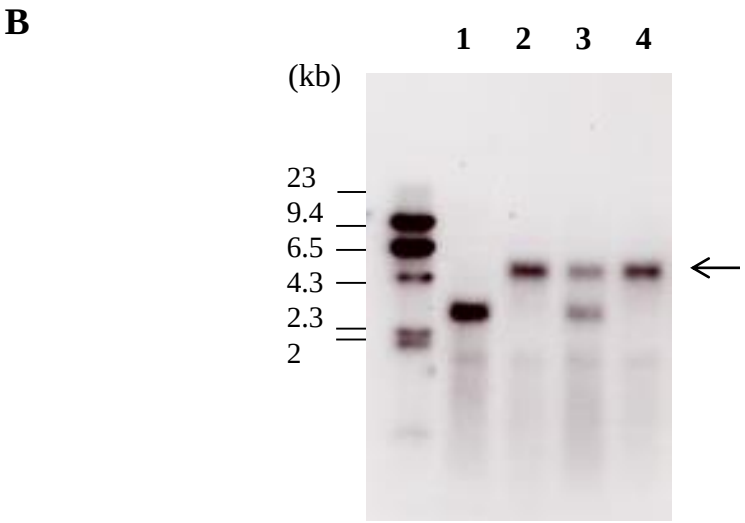
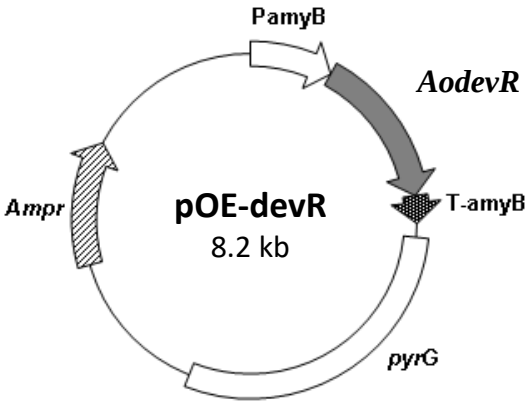

B
1
2
3
4
(kb)
23
9.4
6.5
4.3
2.3
2

Fig. S2

C



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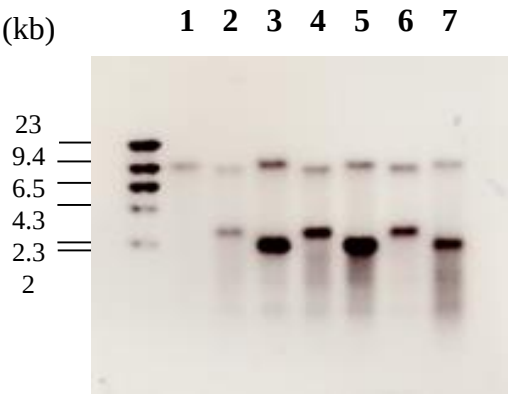

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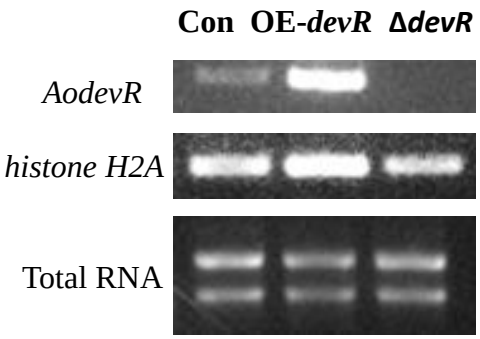


CD (dextrin)

Fig. S3

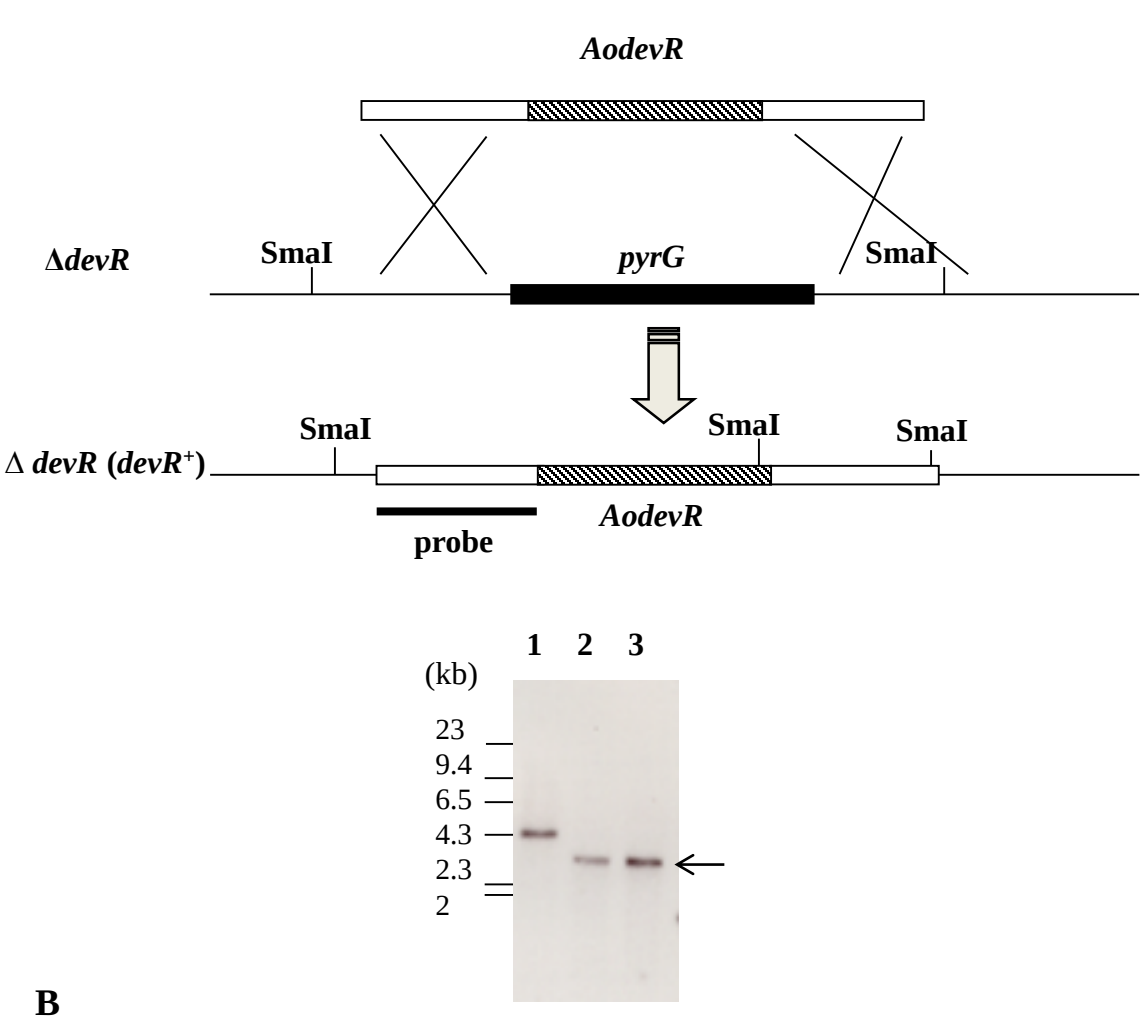
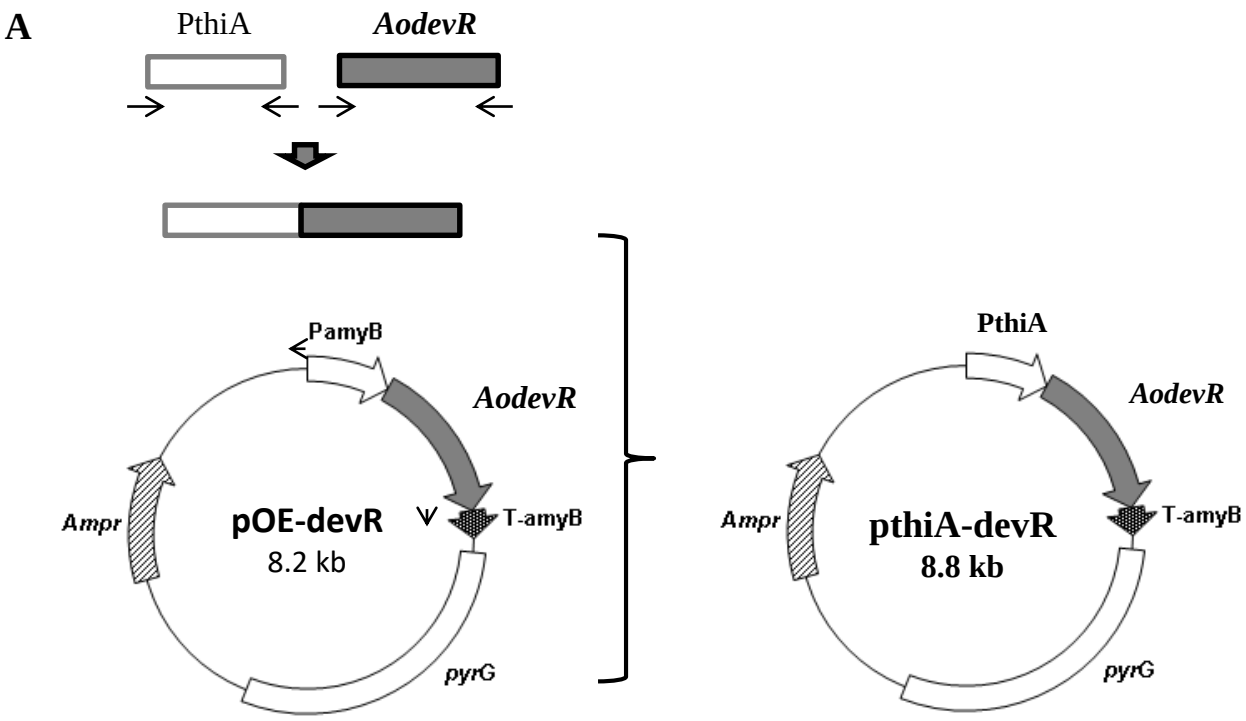

A
AodevR
ΔdevR
SmaI
pyrG
SmaI
SmaI
SmaI
SmaI
Δ devR (devR+)
probe
AodevR
1 2 3
(kb)
23
9.4
6.5
4.3
2.3
2
B
Control
ΔdevR
Δ devR (devR+)
CD+Uridine

Fig. S4


A
PthiA
AodevR
PamyB
AodevR
Ampr
pOE-devR
8.2 kb
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Ampr
pthiA-devR
8.8 kb
T-amyB
pyrG

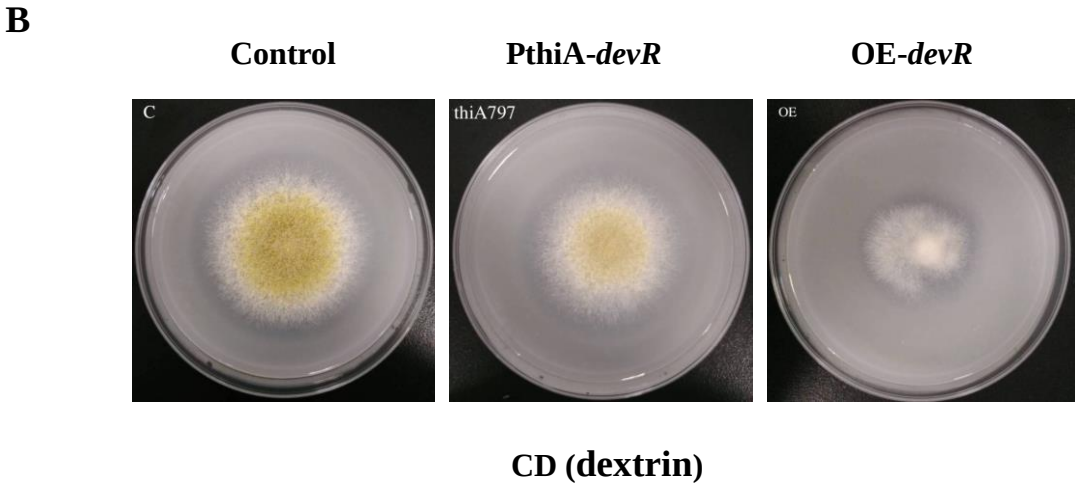
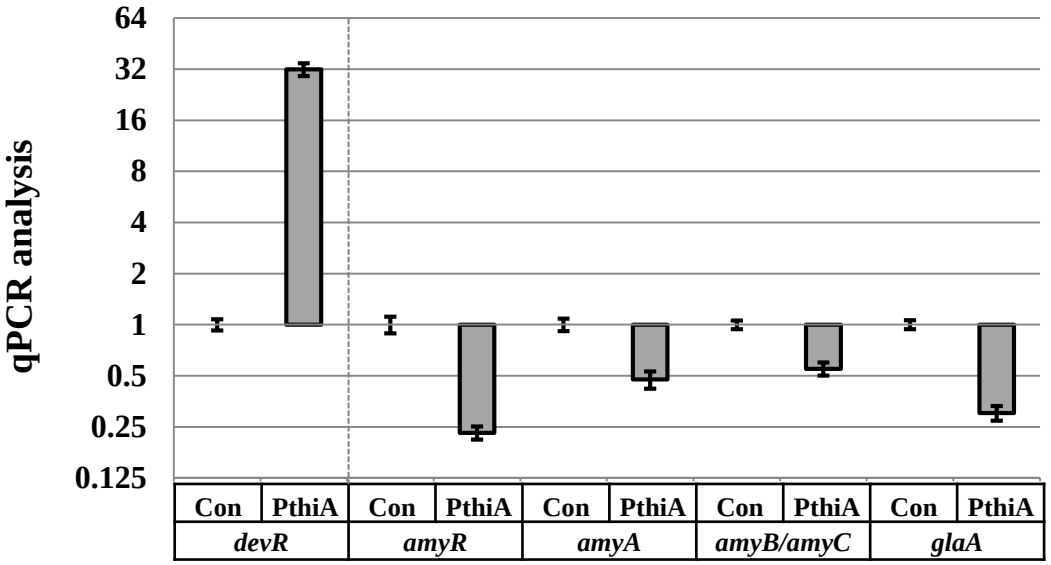

B
Control
PthiA-devR
OE-devR
C
thiA797
OE
CD (dextrin)

Fig. S4

C


qPCR analysis
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32
16
8
4
2
1
0.5
0.25
0.125
Con
PthiA
Con
PthiA
Con
PthiA
Con
PthiA
Con
PthiA
devR
amyR
amyA
amyB/amyC
glaA