Supplementary material

The construction and evaluation of engineered bacteria for the stable inheritance trans-Cry3Aa-T-HasA fusion protein against the *Monochamus alternatus*

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Table S1. Plasmids involved in this experiment.

Figure S1. The pMCSW plasmid and digestion electrophoresis.

Figure S2. Principal component analyses (PCA) of above- and belowground functional traits, soil properties.

Table S1. Plasmids involved in this experiment.

Plasmids	Characteristic	References/Source
pBAM1	Amp^+, Kan^+, *tnpA* gene, *R6KOriV* replicon, *OriT* gene	Martinez-Garcia *et al.* (2011)
pCHKW	Amp^+, Kan^+, *Ori* replicon, *nptII* promoter, *Cry3Aa-T-HasA* gene	Han *et al.* (2024)
pMCSW	$Spec^+$, Kan^+, *tnpA* gene, *R6KOriV* replicon, *OriT* gene, *nptII* promoter, *gfp* gene	This work
pCHSW	$Spec^+$, Kan^+, *tnpA* gene, *R6KOriV* replicon, *OriT* gene, *nptII* promoter, *Cry3Aa-T-HasA* gene	This work

Note: Amp^+: ampicillin resistance gene; Kan^+: kanamycin resistance gene; $Spec^+$: spectinomycin resistance gene

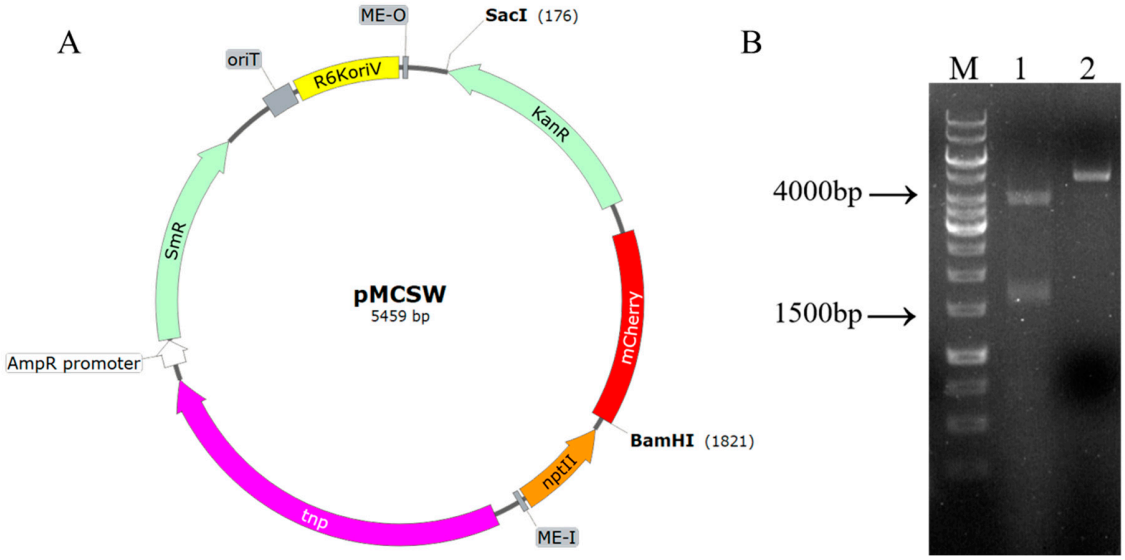

A
ME-O
SacI (176)
oriT
R6KoriV
KanR
SmR
pMCSW
5459 bp
mCherry
AmpR promoter
BamHI (1821)
nptII
tnp
ME-I
B
M
1
2
4000bp
1500bp

Figure S1. The pMCSW plasmid and digestion electrophoresis. A: The pMCSW plasmid; B: plasmid digestion electrophoresis. mCherry: red fluorescent protein; KanR: kanamycin resistance gene; *Sac*I/ *BamH*I: restriction endonuclease; SmR: spectinomycin resistance gene; oriT: origin of transfer for conjugation; R6KoriV: replication origin; ME-O/ME-I: recognition sites for transposase; tnp: transposase; nptII: promoter. M: 1kb DNA Marker; 1: pMCSW plasmid digestion products (*SacI and BamH*I); 2: pMCSW plasmid.

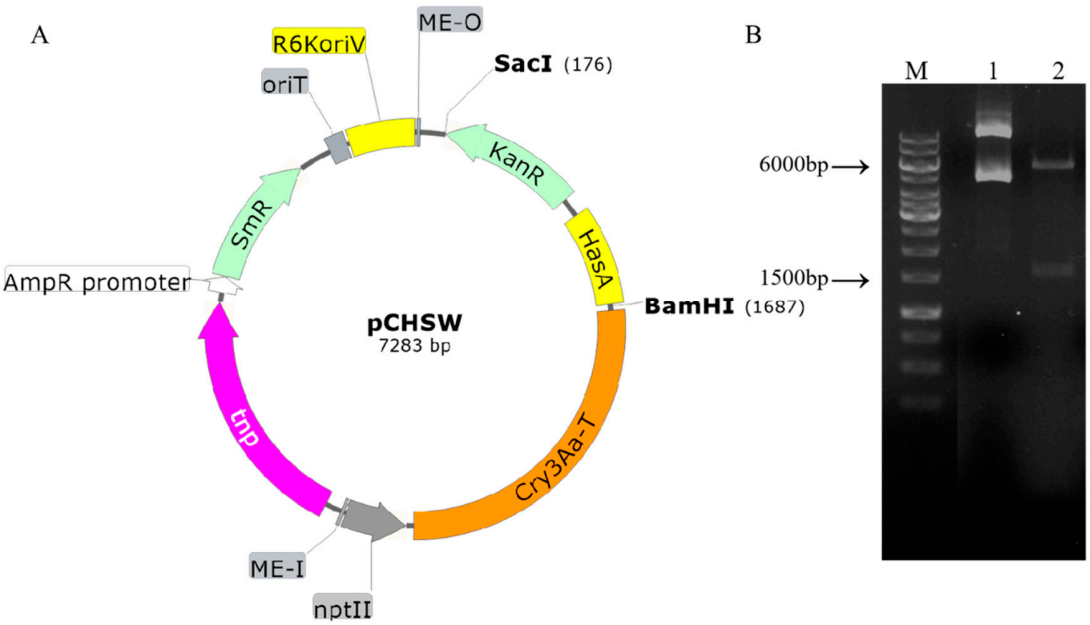

A
R6KoriV
ME-O
oriT
SacI (176)
KanR
HasA
SmR
AmpR promoter
pCHSW
7283 bp
BamHI (1687)
tnp
Cry3Aa-T
ME-I
nptII
B
M
1
2
6000bp
1500bp

Figure S2. The pCHSW plasmid and digestion electrophoresis. A: The pCHSW plasmid; B: plasmid digestion electrophoresis. Cry3Aa-T: insecticidal toxin gene; *Sac*I/ *BamH*I: restriction endonuclease; SmR: spectinomycin resistance gene; oriT: origin of transfer for conjugation; R6KoriV: replication origin; ME-O/ME-I: recognition sites for transposase; HasA: secrete signal; KanR: kanamycin resistance gene; tnp: transposase; nptII: promoter. M: 1kb DNA Marker; 1: pMCSW plasmid; 2: pMCSW plasmid digestion products (*Sac*I and *BamH*I).

Han, X., Huang, C., Qi, H., Zhu, Y., Hu, X., Wen, Y. *et al.* (2024). The construction and evaluation of secretory expression engineering bacteria for the trans-Cry3Aa-T-HasA fusion protein against the Monochamus alternatus vector. *Frontiers in Cellular and Infection Microbiology*, 14.

Martinez-Garcia, E., Calles, B., Arevalo-Rodriguez, M. & de Lorenzo, V. (2011). pBAM1: an all-synthetic genetic tool for analysis and construction of complex bacterial phenotypes. *Bmc Microbiology*, 11.