Supporting Material

Nematicidal Activity and Action Receptor of A Methyl-Accepting Chemotaxis Protein from the Phytopathogen *Pseudomonas syringae* Against *Caenorhabditis elegans*

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Table S1. Bacterial, yeast and nematode strains, plasmids and oligonucleotide primers used in this study.

Strains/plasmids/primers	Phenotypes/sequences [a]	Sources
Bacterial strains		
P. syringae		
MB03	A wild-type strain with high ice-nucleating activity	Li et al. [1]
E. coli		
DH5α	*supE44ΔlacU169(Φ80 lacZΔM15) hdsR17 recA1endA1 gyrA96 thi-1 relA1*	Laboratory stock
BL21(DE3)	F-*ompT hsdS* (rB^{-} mB^{-}) dcm^{+} Tetr *galλ*(DE3) *endA* The [*argU proL* Cam^{r}] [*argU ileY leuW* $Strep/Spe^{cr}$]	Invitrogen
OP50	Amp^{r}; uracil auxotroph; Food source used for *C. elegans*	Laboratory stock
HT115(DE3)	Tet^{r}; an RNase III-deficient *E. coli* strain	CGC [b]
MB830	*E. coli* BL21(DE3) harboring pGEX-6P-1	This study
MB831	*E. coli* BL21(DE3) harboring pMB831	This study
MB833	*E. coli* BL21(DE3) harboring pMB833	This study
MB834	*E. coli* HT115(DE3) harboring pMB834	This study
Yeasts		
Y2Hgold	*MATa, trp1, leu2, ura3, his3, gal4Δ, gal80Δ*	Clontech
Y187	*MATa, ura3, his3, ade2, trp1, Leu2, gal4Δ, met, gal80Δ*	Clontech
MB832	Y2Hgold harboring pMB832	This study
Nematodes		
N2	A wild-type *C. elegans*	CGC
FT63 (*dlg*::*gfp*)	A GFP-labeled transgenic *C. elegans* expressed (*dlg*::*gfp*) in epithelial cell junction	CGC
LG333 (*skn-1b*::*gfp*)	A GFP-labeled transgenic *C. elegans* expressed (*skn-1b*::*gfp*)	CGC
Plasmids		
pET-28a	Kan^{r}; *E. coli* expression vector, 5369 bp	Novagen
pMB831	Kan^{r}; pET28a derivative harboring *mcp03* gene at *Eco*R I/*Xho* I site, 7138 bp	This study
pGBKT7	Amp^{r}; pGEX-6P-1 derivative harboring *csn-5* gene at *Eco*R I site, 6000 bp	TaKaRa, Inc.
pMB832	Kan^{r}; pGBKT7 derivative harboring *mcp03* gene at *Nde* I/*Bam*H I sites, 9100 bp	This study
pGADT7-*csn-5*	Amp^{r}; Y2H expressing csn-5, 9100 bp	This study
pGBKT7-53	Kan^{r}; Y2H expression vector, positive control plasmid, 8300 bp	TaKaRa, Inc.
pGBKT7-lam	Kan^{r}; Y2H expression vector, negative control plasmid, 7900 bp	TaKaRa, Inc.
pGEX-6P-1	Amp^{r}; *E. coli* expression vector, 4900 bp	Novagen
pMB833	Amp^{r}; pGEX-6P-1 derivative harboring *csn5* gene at *Eco*R I site, 6000 bp	This study
pL4440	Amp^{r}; *E. coli* expression vector, 2800 bp	Takara
pMB834	Amp^{r}; pL4440 derivative harboring *csn-5* gene at *Hin*d III site, 3900 bp	This study
Oligonucleotide primers [c]		
F-pET28a-mcp03	5′–CCGGAATTCATGCAGACGTTAAAGGC–3′	

R-pET28a-mcp03	5′–CCGCTCGAGTTATTTACCCAACAGCAGC–3′
F-pGBKT7-mcp03	5′–CGCCATATGATGCAGACGTTAAAGGCTTTG–3′
R-pGBKT7-mcp03	5′–CGCGGATCCTTATTTACCCAACAGCAGCGC–3′
F-pGEX-6P-1-csn5	5′–CCCCTGGGATCCCCGGAAATGGAAGTTGATACGTC–3′
R-pGEX-6P-1-csn5	5′–CCGCTCGAGTCGACCCGGGTTAAGCATCGGCCATCTC–3′
F-pL4440-csn5	5′–CAGGAATTCGATATCAAGATGGAAGTTGATAACGTC–3′
R-pL4440-csn5	5′–TCGACGGTATCGATAAGTTAAGCATCGGCCATCTC–3′
For qRT-PCR	
F-GAPDH	5′–TAACTTCGGTATCATCGAAGGACTC–3′
R-GAPDH	5′–GACGGAAACATCTGGTGTAGGGA–3′
F-csn5	5′–TTCGCTCTTCCAGTTGAGGG–3′
R-csn5	5′–CGACCTTCCGTATCGCACAT–3′
F-col-117	5′–GGTGTGCGTCACTCTTCCAA–3′
R-col-117	5′–ACGGTTTCCAGATGGGATGG–3′
F-mpk-1	5′–TGGACTGGGTGCAAGTGAAG–3′
R-mpk-1	5′–GCAACGAGCCATCTGAAACC–3′
F-kgb-1	5′–GACGATGAGGTAAACGCCCC–3′
R-kgb-1	5′–GTGAAAATGTCGTGGTCGGC–3′
F-unc-98	5′–AGCAAGAAGCGAGTCCTACC–3′
R-unc-98	5′–TGCTTCGGCTCGTATCCTTC–3′

Note: [a] Amp^r, ampicillin resistance; Kan^r, kanamycin resistance; Tet^r, tetracycline resistance. [b] CGC, *Caenorhabditis* Genetics Centre, College of Biological Sciences, University of Minnesota. [c] The underlined sequences indicate the restriction enzyme sites.

REFERENCES

1 Li, Q.; Yan, Q.; Chen, J.; He, Y.; Wang, J.; Zhang, H.; Yu, Z.; Li, L., Molecular characterization of an ice nucleation protein variant (InaQ) from *Pseudomonas syringae* and the analysis of its transmembrane transport activity in *Escherichia coli*. *Int J Biol Sci* **2012,** *8*, 1097–1108.

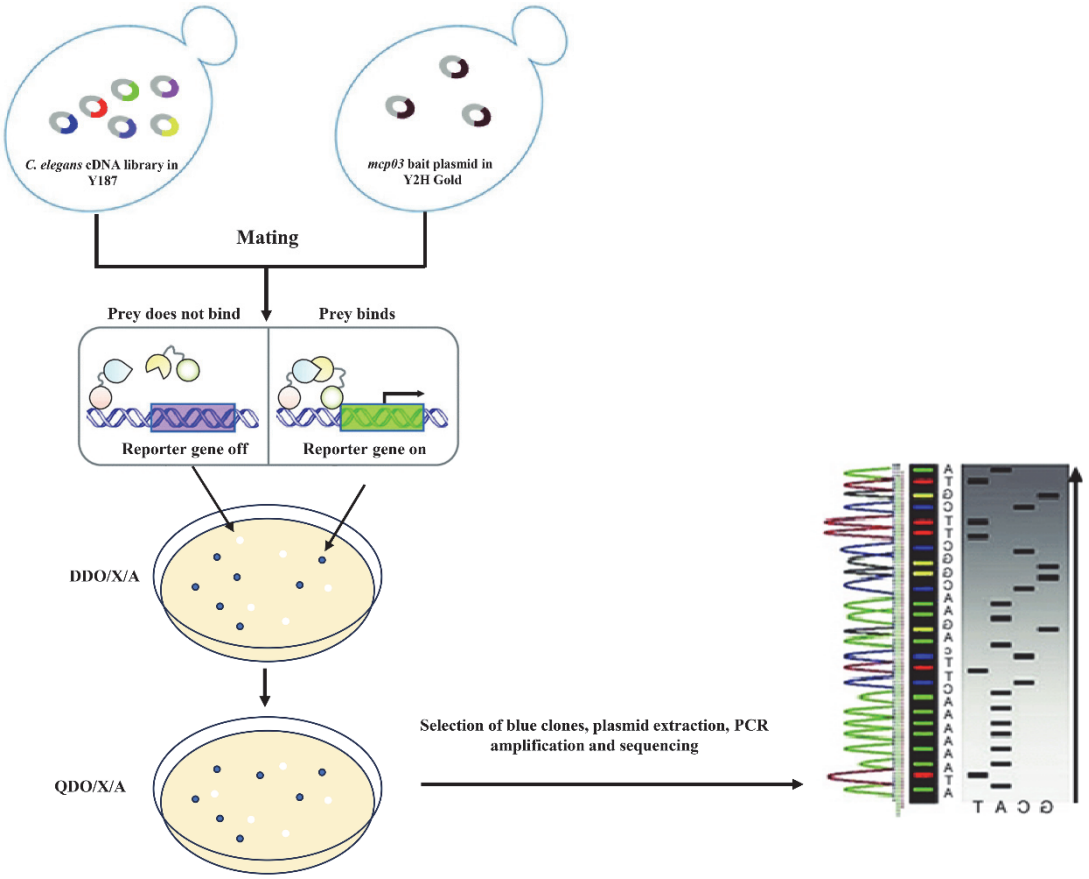

Figure S1. Schematic illustration of Yeast two-hybrid system used for identification of MCP03-binding receptor protein.

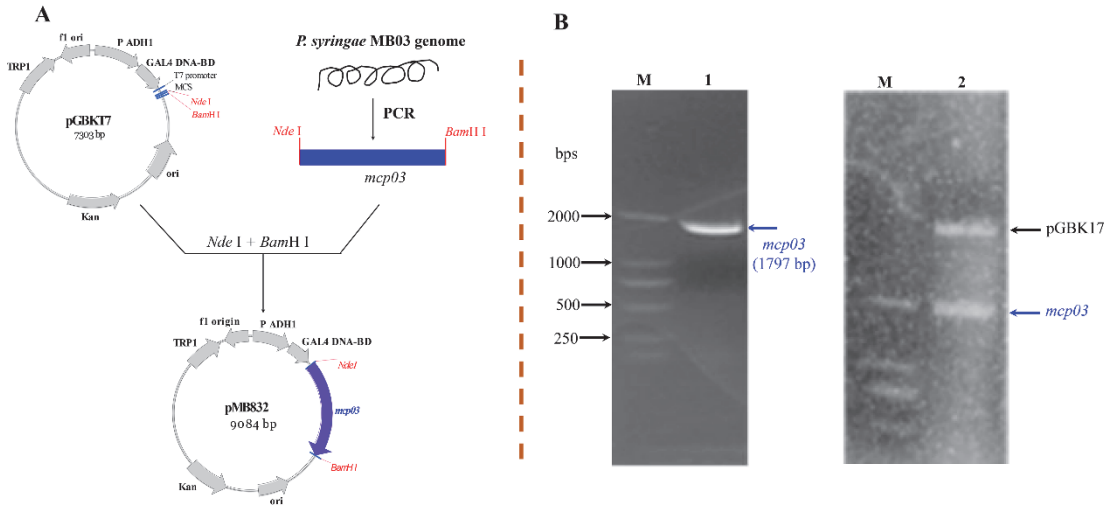

Figure S2. The construction and verification of recombinant plasmid pMB832 expressing the *mcp03* gene. (A) Schematic representation of the construction of pMB832; In (B), left: electrophoresis profile of PCR-amplified *mcp03*. right: electrophoresis profile of *Nde* I/*Bam*H I-digested pMB832. Lane M, DNA Mw marker; lane 1, PCR-amplifed *mcp03* from *P. syirngae* MB03 genome; lane 2, *Nde* I/*Bam*H I-digested pMB832.

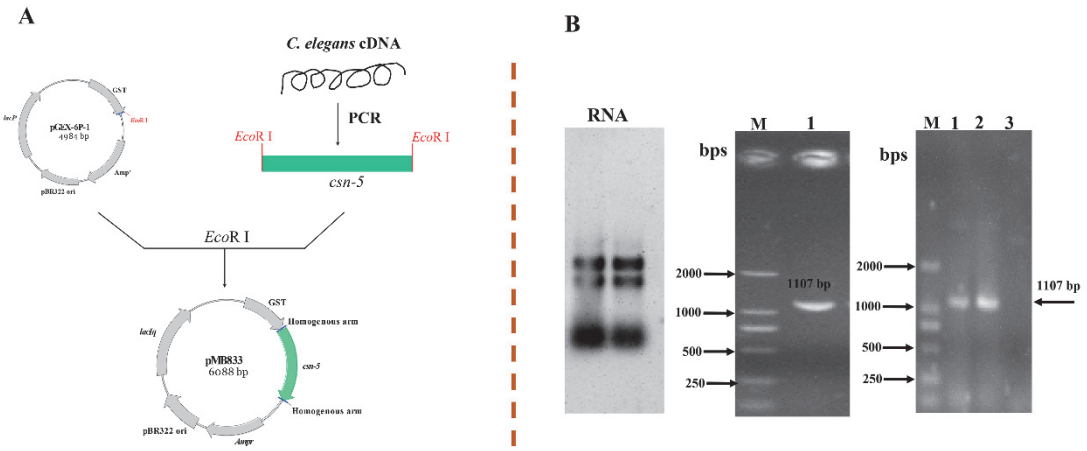

Figure S3. The construction and verification of recombinant plasmid pMB833 expressing the *csn-5* gene. (A) Schematic representation of the construction of pMB833. (B) Electrophoresis profiles of RNA extracted from *C. elegans* and PCR-amplified *csn-5* fragment. Left: Total RNAs extracted from *C. elegans* N2; Middle: electrophoresisi of PCR amplified *csn-5*. Lane M, DNA Mw marker, lane 1, PCR-amplified *csn-5* fragment; Right: PCR amplification verification of *csn-5* from pMB833. Lane M, DNA Mw marker, lane 1/2, PCR-amplified *csn-5*, lane 4, the negative control.

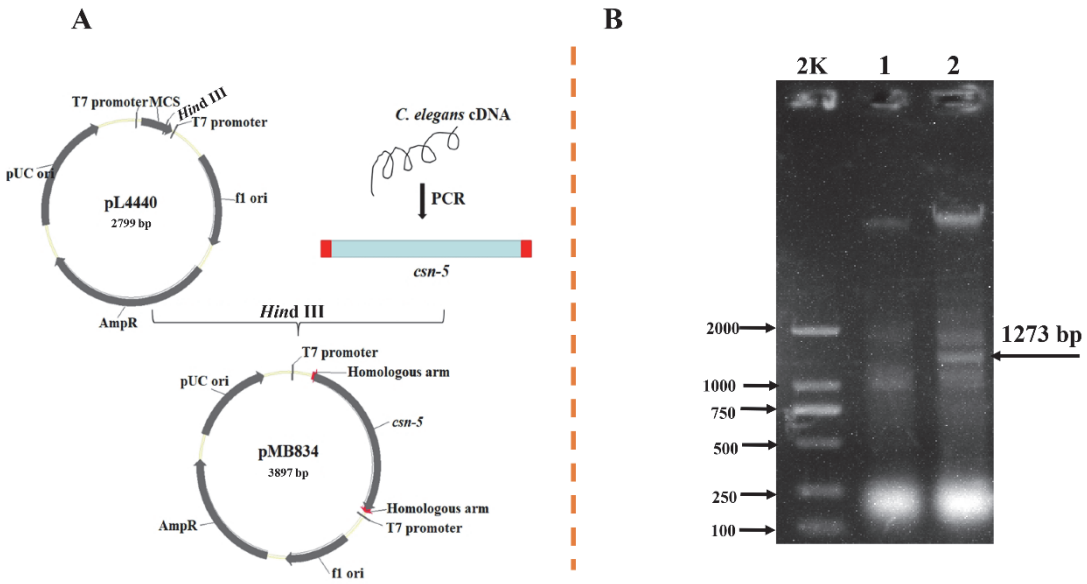

Figure S4. The construction of pMB834 and verification of the expression of dsRNA in yeast MB834. (A) Schematic representation of the construction of pMB834. (B) The expression of dsRNA in MB834. Lane 2K, DNA Mw marker; lanes 1, IPTG-induced pL4440/HT115; lane 2, 1 mM IPTG-induced MB834, arrow indicates the generated dsRNA (1273 bp).

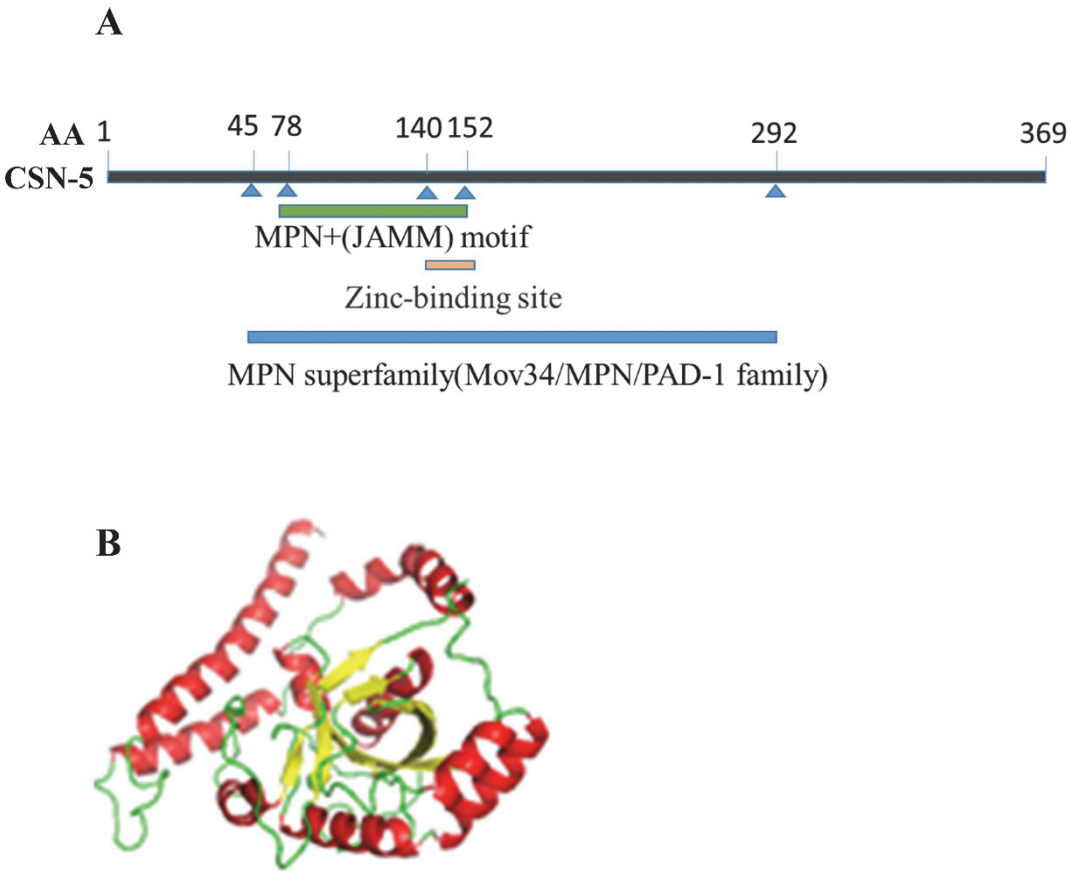

Figure S5. Structural illustration of conserved domains (A) and the predicted three-dimensional configuration (B) of CSN-5 protein.