Supporting Information

Article title: A phytobacterial TIR domain effector manipulates NAD⁺ to promote virulence

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The following Supporting Information is available for this article:

†Deceased.
Fig. S1 HopAM1 contains a putative TIR domain.

(a) A phylogenetic tree of HopAM1 homologs that are moderately distributed in phytobacterial pathogens.
(b) Alignment of HopAM1’s putative TIR-domain with known TIR-domains.
(c) A phylogenetic tree of the TIR domain sequences of the proteins shown in (a).
(d) Immunoblots show expression of HopAM1 and its mutants examined in yeast strains in Fig. 1c and in plants in Fig. 1d, e.
Fig. S2 HopAM1 hydrolyzes NAD\(^+\) \textit{in vitro}.

(a) Time 0 of \textit{in vitro} assays with purified HopAM1-His and HopAM1\(_{E191A}\)-His proteins, and mock preparation of vector control. (b) HPLC chromatograms of \textit{in vitro} reaction with HopAM1 protein. (c) and (d) Mass spectrum of NAD\(^+\), and NAM in an \textit{in vitro} reaction, respectively. (e) Chromatographs of time course enzymatic reactions with purified AbTIR and HopAM1. (f) Time course enzymatic activity showing NAD\(^+\) hydrolysis by HopAM1, HopAM1\(_{E191A}\), and AbTIR. Bars denote standard error (SE).
Fig. S3 HopAM1’s NAD⁺ hydrolysis activity is associated with NAD⁺ depletion in yeast.

(a) Immunoblots detecting expression of HopAM1 and HopAM1E191A galactose-induced yeast cultures. (b) Normalized NAD⁺ levels of the yeast strains upon the expression of HopAM1, HopAM1E191A, HopA₁Psy61, Bax and a vector control. Expression of HopAM1, HopA₁Psy61, and Bax all have result in growth defect in yeast. Bars indicate standard error. (c) The variant v2-cADPR peak was eluted around 4.5 minutes, while the endogenous canonical cADPR at around 3.8 min with both identified to have the same 542 m/z as cADPR. The results here indicated that decrease of NAD⁺ is specifically associated with HopAM1 and dependent on its putative catalytic site of TIR domain and independent of yeast cell death triggered by either HopAM1 or the other PCD inducers in yeast. HopA₁Psy61 is a type III effector from *P. syringae* pv. syringae 61 and Bax is well-known pro-apoptosis protein, both are lethal when expressed in yeast.
Fig. S4 HopAM1-mediated metabolites in Arabidopsis Xan-2

(a) v2-cADPR and (b) NAD⁺ in Xan-2 plants infected with Pto DC3000D28E strains. Pto DC3000D28E strains carrying respective plasmids were infiltrated into Arabidopsis Xan-2 leaves at cell density of 2 x 10⁸ cells ml⁻¹. Bars denote standard error.
Fig. S5 HopAM1 in Pto DC3000 is responsible to produce v2-cADPR in Arabidopsis

(a) HPLC analysis of metabolites in Arabidopsis Col-0 leaves inoculated with Pto DC3000, ΔhopAM1-1, ΔhopAM1-2, ΔhopAM1-1 ΔhopAM11-2 double mutant, and ΔhopAM1-1/1-2(phopAM1).

(b) Quantification of v2-cADPR from samples examined in (a). Leaves infected with Pto DC3000 and complementing strains produced significantly higher amount of v2-cADPR, while v2-cADPR was undetectable in samples infected with hrcC and ΔhopAM1-1/1-2 double mutant. Bars indicate standard error.
Fig. S6 HopAM1’s ETI-like response in Arabidopsis Xan-5 is dependent on its conserved residues in TIR domain.

(a) Immunoblot detection of expression of HopAM1 and its mutants in the indicated *Pto* DC3000D28E strains. (b) ETI-like response of HopAM1 and its mutants in Arabidopsis Xan-5. *Pto* DC3000D28E strains with indicated plasmids carrying respective plasmids were infiltrated into Arabidopsis Xan-5 leaves at cell density of 2 x 10^8 cells ml^{-1}. Images were taken 3 days post inoculation.
Fig. S7 HopAM1 contributes to *Pto* DC3000 virulence in tomato plants in a manner dependent on its putative catalytic residue. Tomato cv. “Money Maker” plants were spray-inoculated at 2x10^8 cells ml^-1 with *Pto* DC3000, *hrcC* type-III defective mutant, double mutant ∆hopAM1-1 ∆hopAM1-2 (∆hopAM1-1/1-2), double mutant ∆avrPto ∆avrPtoB, and a quadruple mutant ∆avrPto ∆avrPtoB ∆hopAM1-1 ∆hopAM1-2 (∆E4) and the latter complemented with wild type hopAM1 or TIR-domain mutant derivative hopAM1E191A. After 0 and 3 days, leaf tissue was assayed for bacterial growth. Bacterial growth assays were repeated 3 times with similar results. Bars denote standard error. Letters denote statistical significance (p < 0.05).
Fig. S8 Mutation of conserved residues in HopAM1’s TIR domain do not affect its subcellular localization.

GFP fusion proteins of HopAM1 and the respective mutation derivatives were visualized with a confocal microscope. The GFP fusions were transiently expressed via Agrobacterium-mediated transformation in N. benthamiana. Images were captured at 40x magnification. Scale bars = 50 µm.
| Strains or plasmids | Characteristics | References or sources |
|---------------------|----------------|-----------------------|
| *Pseudomonas syringae* pv. *tomato* DC3000 | Wild type; spontaneous Rif<sup>R</sup> | (Cuppels, 1986) |
| *P. syringae* DC3000 | *hrcC* mutant defective in T3SS, Cm<sup>R</sup> | (Yuan & He, 1996) |
| JSU2 | <i>ΔhopAM1-1 ΔhopAM1-2</i>, Pto DC3000 *hopAM1-1 hopAM1-2* double mutant, Rif<sup>R</sup> | This study |
| JSU7 | <i>ΔavrPto ΔavrPtoB</i>, Pto DC3000 *avrPto avrPtoB* double mutant, Rif<sup>R</sup> | This study |
| JSU8 | <i>ΔhopAM1-1 ΔhopAM1-2 ΔavrPto ΔavrPtoB</i>, Pto DC3000 *hopAM1-1 hopAM1-2 avrPto avrPtoB* quadruple mutant (ΔE4), Rif<sup>R</sup> | This study |
| *Pto DC3000D28E* | *Pto* DC3000 mutant lacking 28 effector genes, Rif<sup>R</sup>, Sp<sup>R</sup>, Gm<sup>R</sup> | (Cunnac et al., 2011) |
| *Pto DC3000D28E hrcU* | *Pto* DC3000 mutant lacking *hrcU* and 28 effector genes, Rif<sup>R</sup>, Sp<sup>R</sup> Gm<sup>R</sup> | (Cunnac et al., 2011) |
| *Pseudomonas fluorescens* 55 | Nx<sup>R</sup> | M. Sasser |
| *E. coli* | | |
| DH5α | *supE44 ΔlacU169(φ80lacZΔM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1, Nal<sup>R</sup> | (Hanahan, 1983) Life Technologies |
| BL21 | *fhuA2 [lon] ompT gal [dcm] ΔhsdS* | Novagen |
| DB3.1 | *F<sup>−</sup> gyrA462 endA1 Δ(sr1-recA) mcrB mrr hsdS20* (rB<sup>−</sup>, mB<sup>−</sup>) supE44 ara-14 galK2 lacY1 proA2 rpsL20 (Sm<sup>R</sup>) xyl-5 λ<sup>−</sup> leu mtl-1* | Invitrogen |
| *Agrobacterium tumefaciens* C58C1 | Rif<sup>R</sup>, Gm<sup>R</sup> | |
| *Yeast* | | |
| *Saccharomyces cerevisiae* EGY48 | MAT<sup>a</sup>, his<sup>−</sup>, trp<sup>−</sup>, ura<sup>−</sup>, LexAop(x6)-LEU2 | Clonetech |
| pER8 | Binary vector with estradiol-inducible expression, Sp<sup>R</sup> | (Zuo et al., 2000) |
| pET28a(+) | HIS-tag protein expression vector, Km<sup>R</sup> | Novagen |
| pGilda | Yeast expression Lex-A fusion vector, Ap<sup>R</sup> His<sup>−</sup> | Clontech |
| pHIR11 | Cosmid carrying a full functional T3SS and effector *hopA1* derived from *P. syringae pv. syringae* 61, Tc<sup>R</sup> | (Alfano et al., 1997) |
| Vectors | Description | References |
|---------|-------------|------------|
| pK18mobsacB | sacB-based vector for bacterial mutagenesis | (Schäfer et al., 1994) |
| pK7FWG2 | Gateway destination binary vector with GFP fusion at C-terminus, Sp<sup>R</sup> Cm<sup>R</sup> | (Karimi et al., 2002) |
| pLN462 | pZP212-derived P35S-gateway-HA-T35S destination vector, Sp<sup>R</sup> Cm<sup>R</sup> | (Jamir et al., 2004) |
| pLN507 | pGilda-hopAM1, pGilda derivative carrying hopAM1, Ap<sup>R</sup> | (Jamir et al., 2004) |
| pLN533 | pGilda-hopA<sub>61</sub>, pGilda derivative carrying hopA<sub>61</sub>, Ap<sup>R</sup> | This study |
| pLN565 | pGilda-Bax, pGilda derivative carrying Bax, Ap<sup>R</sup> | (Jamir et al., 2004) |
| pLN604 | pER8-derived Gateway-HA destination vector, Sp<sup>R</sup> Cm<sup>R</sup> | (Guo et al., 2009) |
| pLN615 | pML123-derived gateway-HA destination vector, Gm<sup>R</sup> Cm<sup>R</sup> | (Guo et al., 2009) |
| pLN1965 | pHIR11 derivative carrying hopA<sub>1</sub> deletion, Tc<sup>R</sup> Sp<sup>R</sup> | (Guo et al., 2009) |
| pLN5841 | pK18mobsacB derived Gateway destination vector; Cm<sup>R</sup>, Km<sup>R</sup> | This study |
| pLN6374 | pML123-hopAM<sub>1</sub><sup>F210A</sup>, pLN615 derivative carrying hopAM<sub>1</sub><sup>F210A</sup>-HA, Gm<sup>R</sup> | This study |
| pLN6375 | pER8-hopAM1, estradiol inducible pLN604 derivative carrying hopAM1-HA, Sp<sup>R</sup> | This study |
| pLN6381 | pML123-hopAM1, pLN615 derivative carrying hopAM1-HA, Gm<sup>R</sup> | This study |
| pLN6382 | pML123-hopAM<sub>1</sub><sup>F177A</sup>, pLN615 derivative carrying hopAM<sub>1</sub><sup>F177A</sup>-HA, Gm<sup>R</sup> | This study |
| pLN6383 | pML123-hopAM<sub>1</sub><sup>F207A</sup>, pLN615 derivative carrying hopAM<sub>1</sub><sup>F207A</sup>-HA, Gm<sup>R</sup> | This study |
| pLN6384 | p35S-hopAM1, pLN462 derivative carrying hopAM1-HA, Sp<sup>R</sup> | This study |
| pLN6385 | p35S-hopAM<sub>1</sub><sup>F177A</sup>, pLN462 derivative carrying hopAM<sub>1</sub><sup>F177A</sup>-HA, Sp<sup>R</sup> | This study |
| pLN6387 | p35S-hopAM<sub>1</sub><sup>F207A</sup>, pLN462 derivative carrying hopAM<sub>1</sub><sup>F207A</sup>-HA, Sp<sup>R</sup> | This study |
| pLN6388 | p35S-hopAM<sub>1</sub><sup>F210A</sup>, pLN462 derivative carrying hopAM<sub>1</sub><sup>F210A</sup>-HA, Sp<sup>R</sup> | This study |
| pLN6572 | p35S-hopAM1-GFP, pK7FWG2-derivative carrying hopAM1-GFP, Sp<sup>R</sup> | This study |
| pLN6573 | p35S-hopAM<sub>1</sub><sup>F177A</sup>-GFP, pK7FWG2-derivative carrying hopAM<sub>1</sub><sup>F177A</sup>, Sp<sup>R</sup> | This study |
| pLN6576 | p35S-hopAM<sub>1</sub><sup>F207A</sup>-GFP, pK7FWG2-derivative carrying hopAM<sub>1</sub><sup>F207A</sup>-GFP, Sp<sup>R</sup> | This study |
| pLN6650 | pET28a(+-)hopAM<sub>1</sub><sup>E191A</sup>-His, pET28a(+-) derivative carrying hopAM<sub>1</sub><sup>E191A</sup>, Km<sup>R</sup> | This study |
| pLN6651 | pET28a(+-)hopAM1-His, pET28a(+) derivative carrying hopAM1, Km<sup>R</sup> | This study |
| pLN6656 | pGilda-hopAM<sub>1</sub><sup>E191A</sup>, pGilda derivative carrying hopAM<sub>1</sub><sup>E191A</sup>, Ap<sup>R</sup> | This study |
pLN6663  pER8-hopAM1_{E191A}, estradiol inducible pLN604 derivative carrying hopAM1_{E191A}-HA, SpR, This study
pLN6664  pML123-hopAM1_{E191A}, pLN615 derivative carrying hopAM1_{E191A}-HA, GmR, This study
pML123   Broad-host-range vector, GmR KmR (Labes et al., 1990)
pMQ1     pLN5841 derivative carrying the upstream/downstream of avrPto, This study
pMQ2     pLN5841 derivative carrying the upstream/downstream of hopAM1-1, This study
pMQ3     pLN5841 derivative carrying the upstream/downstream of hopAM1-2, This study
pMQ4     pLN5841 derivative carrying the upstream/downstream of avrPtoB, This study
pMQ11    pML123-hopAM1_{I168A}, pLN615 derivative carrying hopAM1_{I168A}-HA, GmR, This study
pMQ12    p35S-hopAM1_{E191A}, pLN462 derivative carrying hopAM1_{E191A}-HA, SpR, This study
pMQ13    p35S-hopAM1_{F210A}-GFP, pK7FWG2-derivative carrying hopAM1_{F210A}-GFP, SpR, This study
pMQ14    p35S-hopAM1_{E191A}-GFP, pK7FWG2-derivative carrying hopAM1_{E191A}-GFP, SpR, This study
pZG-19   pGilda-hopAM1_{F207A}, pGilda derivative carrying hopAM1_{F207A}, ApR, This study
pZG-20   pGilda-hopAM1_{F177A}, pGilda derivative carrying hopAM1_{F177A}, ApR, This study
pZG-21   pGilda-hopAM1_{E197A}, pGilda derivative carrying hopAM1_{E197A}, ApR, This study
pZG-22   pGilda-hopAM1_{F210A}, pGilda derivative carrying hopAM1_{F210A}, ApR, This study
pAbTIR   pET30 derivative carrying AbTIR, KmR, This study
Methods S1 Supporting information for Materials and Methods

Bacterial cultures and growing conditions

_E. coli_ strains DH5α, DB3.1, and BL21 were grown on LM medium amended with selective antibiotics at 37°C. _A. tumefaciens_ C58C1 strains were maintained on LM medium with antibiotics at 30°C. _P. syringae_ and _P. fluorescens_ strains were maintained on King’s B (KB) (King _et al._, 1954) medium with antibiotics at 30°C. All strains used in this study are listed in supplementary Table S1. Antibiotics were used at the following concentrations (mg ml⁻¹): ampicillin, 100; Chloramphenicol, 20; gentamycin, 10; kanamycin, 50; nalidixic acid, 20; rifampicin, 100; spectinomycin 50; and tetracycline, 20.

DNA manipulation

Desired DNA regions were amplified from _Pto_ DC3000 genomic DNA using DreamTaq polymerase (Thermo Fisher Scientific). Standard protocols were followed for conventional ligation or gateway cloning. For conventional cloning, PCR products were digested with restriction enzymes and ligated to vectors with T4 DNA Ligase (New England Biolabs). For gateway cloning, amplicons were cloned into a pENTR vector (Invitrogen) and the resulting pENTR plasmids with insertion were recombined into gateway destination vectors with LR clonase to create recombinant constructs. Site-directed mutagenesis of the _hopAM1_ gene was achieved by amplifying two overlapping fragments of _hopAM1_ by PCR using primers introduced with specific mutation, then fusing the fragments in a second round of PCR with _hopAM1_ specific forward and reverse primers. The resulting PCR products were cloned into pENTR and the constructs containing desired mutation were confirmed by sequencing. All constructs generated for this study are listed in supplementary Table S1.

Purification of recombinant protein

Overnight _E. coli_ BL21 cultures carrying pET28a(+) plasmids that express _hopAM1_, _hopAM1_E191A_, as well as empty vector (mock) were diluted at a ratio of 1:50 into 100 ml LM and grown with shaking at 37°C till an optical density (OD₆₀₀nm) of 0.6 (~4 hours). Cultures were induced with 1 mM IPTG (Invitrogen) for 2.5 hours at 37°C. The induced cultures were pelleted by centrifugation at 16,000 g and resuspended with 10 ml CelLytic B (Sigma-Aldrich) buffer amended with Benzonase (50 U ml⁻¹) (Sigma-Aldrich), Lysozyme (200 µg ml⁻¹) (Sigma-Aldrich), and Protease Inhibitor Cocktail for His-tagged protein purification (10 µl ml⁻¹) (Sigma-Aldrich). Cells were agitated at room temperature (25°C) for 15 minutes, then centrifuged at 16,000 g for 10 minutes. The supernatant cell lysates were mixed with 200 µl HIS-select Nickle Affinity resin (Sigma-Aldrich) for 15 minutes at room temperature (25°C) with agitation. The resin was washed three times with 2 ml 50 mM sodium phosphate, pH 8.0, 0.3 M sodium chloride and then eluted in 4 ml 50 mM sodium phosphate, pH 8.0, 0.3 M sodium chloride, and 250 mM imidazole. Eluate was concentrated for 45 minutes at 4,000 g using an Amicon Ultracel 3K Centrifugal Filter (Millipore) and then mixed with 2 ml of 0.64x PBS and re-concentrated two more times to a final volume of 100 µL.

Proteins and immunoblot analysis

For plant protein extraction, 48 hours after infiltration leaves were sampled with a 16 mm diameter cork borer. Samples were frozen in liquid nitrogen and ground with a plastic pestle, then resuspended in 200 µL x1.5 sample buffer, vortexed and boiled for 10 minutes before storage at -20°C until SDS-PAGE.

For yeast protein extraction, 1 ml of culture was spun down and resuspended in 0.1 M NaOH for five minutes as described by (Kushnirov, 2000), then spun down for 30 seconds at
15,000 g and resuspended in 100 µL x1.5 sample buffer, vortexed and boiled for 10 minutes before storage at -20°C until SDS-PAGE.

Extracted proteins were separated by SDS-PAGE and transferred to PVDF membrane using a TransBlot Turbo transfer system (Bio-RAD) with the standard setting. Immunoblots were performed with appropriate antibodies following standard protocols. The following primary antibodies were used: Rabbit anti-Lex (Millipore), Rat anti-HA (Roche), and Mouse anti-HIS (Sigma-Aldrich). All secondary antibodies were conjugated with alkaline phosphatase (Sigma-Aldrich). Immunoblots were visualized using CDP-Star (Roche).

**Unmarked mutagenesis of* P. syringae* DC3000**

Upstream and downstream DNA regions of *avrPto*, *avrPtoB*, *hopAM1-1*, or *hopAM1-2* were PCR-amplified using DreamTaq or Phire II DNA polymerase (Thermo Fisher Scientific) and cloned into a gateway pENTR vector (Invitrogen). The resulting pENTR constructs were recombined with pLN5841 by LR clonase creating sacB-based constructs for unmarked mutagenesis. The resulting mutagenesis constructs were conjugated using tri-parental or bi-parental mating and integrated into the chromosome of *Pto* DC3000 by homologous recombination. Desired deletions were screened on KB medium containing sucrose (5%) to counter-select for the survived colonies that lack of antibiotic resistance. The final mutants were further verified by PCR.

**Bioinformatics tools**

BLAST at NCBI was routinely used for searching homologs of HopAM1. Structural similarity of HopAM1 was predicted using 3-D the threading online tool PHYRE2 ([http://www.sbg.bio.ic.ac.uk/~phyre2](http://www.sbg.bio.ic.ac.uk/~phyre2)) (Kelley *et al.*, 2015). Alignments of multiple proteins were made with Clustal Omega with manual correction ([https://www.ebi.ac.uk/Tools/msa/clustalo/](https://www.ebi.ac.uk/Tools/msa/clustalo/)) (Madeira *et al.*, 2019). MEGA-X was used to build phylogenetic trees (Kumar *et al.*, 2018).

**Yeast cell death assay**

Yeast strain EGY48 carrying pGilda derivatives were grown overnight at 30 °C in synthetic dropout glucose media lacking histidine (glucose-His). The cells were washed and resuspended in ddH2O to an OD600nm of 0.1. A 10-fold dilution series was plated on glucose-His agar or galactose-His and grown at 30 °C. Colonies were assessed for survival after three days.

**Plant materials**

All Arabidopsis plants were grown at 24 °C with a 10 hour light/14 hour dark cycle in micro-climate controlled growth chambers. *N. benthamiana* and *N. tabacum* cv. Xanthi plants were grown in standard greenhouses.

**Agrobacterium-mediated transient assays**

For assays with tobacco, *Agrobacterium tumefaciens* C58C1 strains carrying binary vectors were grown overnight, centrifuged at 4,696 g for 10 min and resuspended in induction medium (0.5 g L⁻¹ sodium citrate, 10 mM MES, 1 g L⁻¹ ammonium sulfate, 250 µM magnesium sulfate, 0.1% glycerol, 0.2% glucose, 10.5 g L⁻¹ potassium phosphate dibasic, 4.5 g L⁻¹ potassium phosphate monobasic) containing 150 µM acetosyringone (Fisher Scientific) and incubated with shaking at 30 °C for 6 hours. The induced *Agrobacterium* cultures were pelleted by centrifugation at 4,696 g for 10 min and resuspended in infiltration medium (2.15 g L⁻¹ MS basal salts, 10 mM MES, pH5.6) containing 300 µM acetosyringone adjusted to an OD600nm of 0.8. Leaves of *N. tabacum* cv. Xanthi or *N. benthamiana* were infiltrated with needless syringe and plants were kept at room temperature. For inducible-expression constructs, twenty-four hours after infiltration infiltrated leaves were sprayed with 20 µM estradiol (Sigma-Aldrich).
containing 0.02% Silwet-L77 (Lehle Seeds) using a spray bottle. Samples were harvested at indicated time points for metabolite analysis and immunoblot analyses.

**Confocal microscopy**

GFP fusion proteins were transiently expressed in *N. benthamiana* via *Agrobacterium tumefaciens* infiltration. After 48 hours infiltrated leaf discs were detached to monitor green fluorescence and imaged at 40x magnification with a Nikon A1-NiE confocal microscope. Images were visualized using NIS-Elements software with internal ruler.

**Plant hypersensitivity response assays**

For assays in tobacco, the development of a hypersensitive response was assessed and photographed 72 hours after infiltration with *Agrobacterium* cultures. For assays with Arabidopsis, overnight cultures of *Pto* DC3000 D28E strains were resuspended in 10 mM MgCl₂ and adjusted to an OD₆₀₀nm of 0.1 (equivalent to a cell density of 1x10⁸ CFU ml⁻¹). Leaves of four-week-old *Arabidopsis* accessions Xan-2, Xan-5, or Col-0 plants were infiltrated with a needleless syringe. Plants were covered with a plastic lid and maintained for moisture at room temperature (25°C). The development of a hypersensitive response or symptoms was assessed and photographed after 2-4 days.

**In vitro NAD hydrolase and enzyme kinetics assays**

Ten microliters of concentrated protein extract were mixed with 3 µL of 250 µM NAD⁺ (Selleck Chemicals) in 0.64x PBS and incubated at room temperature (25°C). Samples at 0 and 60 minutes were mixed with 250 µL 50% methanol at -40°C and vortexed, then mixed with 250 µL chloroform at -40°C. Samples were then centrifuged at 15,000 g for 5 minutes at -10°C. The aqueous/methanol layer was removed, lyophilized, and stored at -80°C for HPLC analysis.

To calculate HopAM1 enzyme kinetics, *in vitro* reactions consisting of 10 microliters of protein laden bead suspension and 40 µl of 1, 10, 50, 100, 400, or 600 µM NAD⁺ in 25 mM HEPES buffer pH=7.5 at room temperature with constant agitation. At 1 hour, reactions were quenched by pulling the beads to the side and transfer 40 microliters of the reaction mixture to a new tube containing 160 microliters of ice cold 0.5 M HClO₄. Ten microliters of 2x Laemmli buffer (BioRad 1610737) supplemented with β-mercaptoethanol were added to the beads and boiled for ten minutes and subjected to gel electrophoresis on a 4-12% gradient Bis-TRIS polyacrylamide gel (Invitrogen NW04125). Protein concentration was approximated by staining the SDS-page gel with Coomassie blue stain (instant blue, AbCam AB119211) and application of densitometry using ImageJ with comparison to a set of BSA standards. NAD⁺ consumption rates for HopAM1 were calculated by finding NAD⁺ peak area reduction compared to empty vector control and using NAD⁺ calibration curve to convert peak area reduction to change in number of moles of NAD⁺. Average change in NAD moles per minute of reaction was then calculated. The Curve Fitting Tool on MATLAB (Version R2020a) was used to fit NAD⁺ consumption kinetics data to the Michaelis-Menten equation, V = Vmax * (NAD⁺ Concentration) / (Km + NAD⁺ Concentration) where parameters Vmax and Km are constants. Optimal values were output for the parameters Vmax and Km as well as an R² value for the data's fit to the model.

**ROS assay**

ROS production was determined following a previously described protocol (Asai et al., 2008). Briefly, *Pseudomonas fluorescens* 55 (*Pf*) (pLN1965) strains were grown overnight, suspended in 10 mM MgCl₂ and infiltrated with a needleless syringe into leaves of five-week-old *Arabidopsis* plants at a density of 1x10⁷ CFU ml⁻¹. After 24 hours leaf discs were excised using a 4 mm diameter cork borer and incubated in H₂O in white 96 well plate overnight. H₂O is then replaced with 0.5 mM L-012 and 1 µM flg22 in 10 mM MOPS-KOH at pH 7.4. The production of
ROS was determined by counting photons using with a Synergy 5 luminometer (BioTek, Winooski, VT, USA).

**Callose deposition assay**

*Pf*(pLN1965) strains were grown overnight, suspended in 10 mM MgCl$_2$ and infiltrated with a needleless syringe into leaves of five-week-old *Arabidopsis* Col-0 plants at a density of 1x10$^7$ CFU ml$^{-1}$. After 48 hours, callose deposits in infiltrated leaves were stained with aniline blue and visualized with a Zeiss Axioplan 2 microscope. The numbers of callose deposits were quantified using ImageJ (http://imagej.nih.gov/ij/) following a procedure previously reported (Guo et al., 2016).

**In planta bacterial growth assay**

*P. syringae* strains were grown overnight, resuspended in 10 mM MgCl$_2$ and infiltrated with a needleless syringe into leaves of five-week-old *Arabidopsis* at 1x10$^5$ cells ml$^{-1}$ or spray-inoculated onto tomato cultivar ‘Moneymaker’ plants with 0.02% Silwet-L77 at a density of 1x10$^8$ cells ml$^{-1}$. Plants were maintained with moisture in covered trays at room temperature. A 6 mm diameter cork borer was used to sample inoculated leaves at the indicated time points. The samples were ground in 250 µl sterile ddH$_2$O using a plastic pestle and 20 µl each of a 10-fold serial dilution series was plated on KB agar and incubated at 30°C. Bacterial colonies were numerated after 2-3 days.

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