Summer 8-10-2020

Plant defensin antibacterial mode of action against Pseudomonas species

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Plant defensin antibacterial mode of action against *Pseudomonas* species

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Abstract

Many plant defensins exhibit antibacterial activity, but little is known about their antibacterial mode of action (MOA). Antimicrobial peptides with characterized MOAs induce the expression of multiple outer membrane modifications, which are required for resistance to these membrane-targeting peptides. Mini-Tn*5*-*lux* mutant strains of *Pseudomonas aeruginosa* with Tn insertions disrupting outer membrane protective modifications were assessed for sensitivity against plant defensin peptides. Defensins displayed specific and potent antibacterial activity against strains of *P. aeruginosa*. These transcriptional *lux* reporter strains were also evaluated for *lux* gene expression in response to sublethal plant defensin exposure. A defensin from *Medicago truncatula*, MtDef4, induced dose-dependent gene expression of the aminoarabinose modification of LPS and surface polycation spermidine production operons. The ability for MtDef4 to damage bacterial outer membranes was visually verified with fluorescent microscopy. Another defensin from *M. truncatula*, MtDef5, appears to have a different antibacterial MOA. MtDef5 treatments failed to induce *lux* gene expression and limited outer membrane damage was detected with fluorescent microscopy. A plant pathogen, *P. syringae* pv. *syringae* was modified through transposon mutagenesis to create mutants that are resistant to MtDef4 treatments. The transposon insertion site on defensin resistant bacterial mutants was sequenced, and modifications of ribosomal genes were identified to contribute to enhanced resistance to defensin treatments.

Objectives

- Assess the ability of plant defensins to inhibit strains of *Pseudomonas* species
- Characterize plant defensin antibacterial MOA using the mini-Tn*5*-*luxCDABE* mutant library in *P. aeruginosa*
- Generate tools to explore plant defensin MOA against bacterial plant pathogens

Methods

In vitro antibacterial activity assays

A spread-plate assay was used to monitor growth inhibition of wild-type and antimicrobial peptide sensitive mutants of *P. aeruginosa* as previously described (1). From these assays, the amount of γ-core motif defensin peptides needed to inhibit growth of the pathogens strains by 50% (IC_{50}) was calculated.

Lux-reporter gene expression assay

Transcriptional lux reporters of the *P. aeruginosa pmr* operon (*PA3553*) and spermidine synthesis genes *speD2E2* (*PA4774*) are induced by the presence of antimicrobial peptides at a sublethal concentration (2). We used these lux reporters under non-inducing conditions to determine if exposure of these reporters to sub-MIC concentrations of plant defensins causes induced expression. The lux-reporter strains of *P. aeruginosa* were grown overnight in diluted LB broth, treated with plant defensin γ-core motif peptides, and monitored for bioluminescence in a microplate reader, where bioluminescence would indicate the induction of the inactivated bacterial membrane modification genes.

LIVE/DEAD *Bac*Light staining

Strains of *P. aeruginosa* were treated with γ-core defensin peptides and stained using a LIVE/DEAD *Bac*light kit, which includes two stains. Green-fluorescent SYTO 9 labels bacterial cells with both intact and damaged membranes, but red-fluorescent propidium iodine can only penetrate and label bacteria with damaged membranes. Bacteria were visualized and counted using fluorescent microscopy (3).

Results

Plant defensins displayed specific and potent antibacterial activity against strains of *P. aeruginosa* (Table 1). A defensin from *Medicago truncatula*, MtDef4, induced dose-dependent gene expression of the aminoarabinose modification of LPS and surface polycation spermidine production operons (Fig. 1). The ability for MtDef4 to damage bacterial outer membranes was also verified visually through fluorescent microscopy (Table 2). Another defensin from *M. truncatula*, MtDef5, failed to induce *lux* gene expression and limited outer membrane damage was detected with fluorescent microscopy (Fig. 2, Table 2). The transposon insertion site on MtDef4 resistant *P. syringae* pv. *syringae* mutants was sequenced, and modifications of ribosomal genes were identified to contribute to enhanced resistance to plant defensin treatments.

Table 1 Activity of core motif plant defensin peptides against *Pseudomonas aeruginosa* strains. The mean IC_{50} (μM) values are reported ± SE of three independent experiments (n=3).

Pseudomonas aeruginosa strains	MtDef4 core	MtDef5A core	So-D2 core
PAO1	4.2 ± 0.4	11.8 ± 1.4	11.6 ± 0.6
PA3553:lux	2.7 ± 0.3	8.5 ± 0.8	3.0 ± 0.3
PA4774:lux	1.7 ± 0.2	14.6 ± 1.0	5.2 ± 0.5

Table 2 Membrane permeating activity of the γ-core motif defensin peptides against *Pseudomonas aeruginosa* strains.

Treatment	*PA3553:lux*		*PA4774:lux*	
	% Live[a]	% Dead[b]	% Live	% Dead
MtDef4 core	71.6 ± 5.4	28.4 ± 2.5	49.4 ± 3.8	50.8 ± 1.3
MtDef5A core	99.0 ± 0.8	0.9 ± 0.3	95.4 ± 1.7	4.6 ± 2.1

[a]Live cells have intact cell membranes and were stained fluorescent green
[b]Dead cells are permeable to propidium iodine and were stained fluorescent red, which indicates membrane disruption

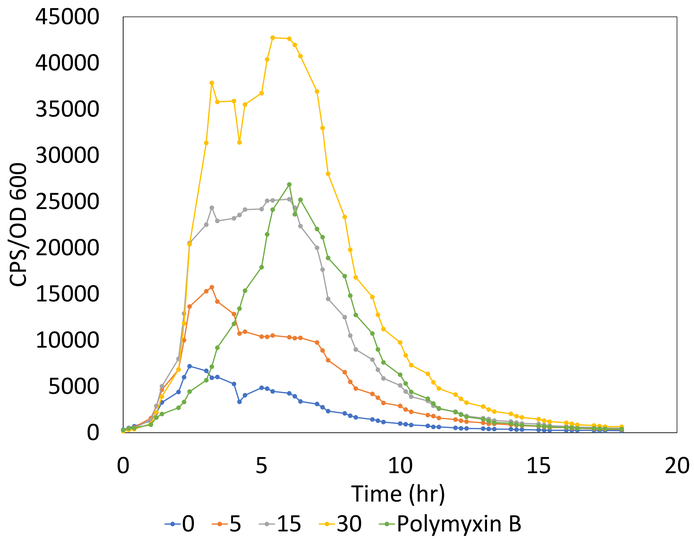

Figure 1. MtDef4 γ-core motif peptide induces *PA4774* gene expression. Effects of MtDef4 γ-core peptide at sub-minimal inhibitory concentrations of 0, 5, 15, or 30 μg/mL or polymyxin B at 0.5 μg/mL on the expression of the *PA4774::lux* transcriptional fusion in planktonic cultures in LB broth.

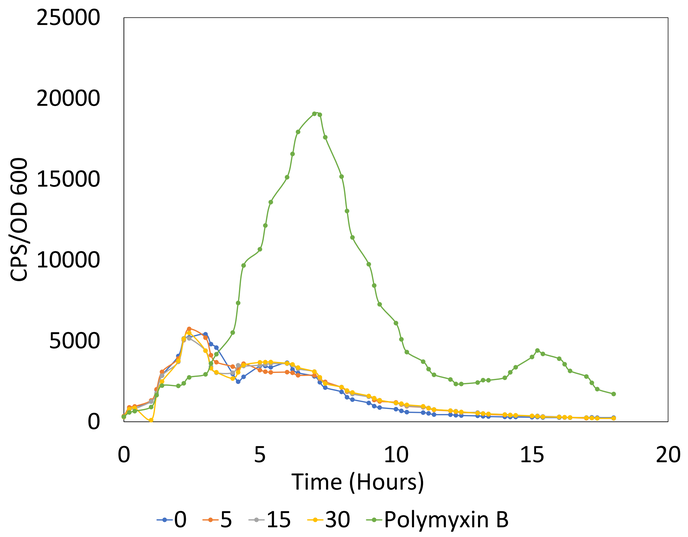

Figure 2. MtDef5 γ-core motif peptides fail to induce *PA4774* gene expression. Effects of MtDef5 γ-core peptide at sub-minimal inhibitory concentrations of 0, 5, 15, or 30 μg/mL or polymyxin B at 0.5 μg/mL on the expression of the *PA4774::lux* transcriptional fusion in planktonic cultures in LB broth.

Conclusions

- Against *P. aeruginosa*, MtDef4 and So-D2 interact with the bacterial outer membrane and possibly create pores leading to bacterial cell death
- MtDef5 appears to have a different antibacterial MOA where outer membrane binding is not as vital and may have an intracellular target
- Plant defensins seem to have a different MOA than polymyxin B.
- Plant defensin γ-core motif peptides can be utilized for the development of treatments against both plant and human bacterial pathogens

References

1. Kim, M., Chen, Y., Xi, J., Waters, C., Chen, R., and Wang, D. 2015. An antimicrobial peptide essential for bacterial survival in the nitrogen-fixing symbiosis. Proc. Natl. Acad. Sci. USA 112:15238-15243.
2. Mulcahy, H., Charron-Mazenod, L., and Lewenza, S. 2008. Extracellular DNA chelates cations and induces antibiotic resistance in *Pseudomonas aeruginosa* biofilms. PLoS Pathog. 4:e1000213.
3. Berney, M., Hammes, F., Bosshard, F., Weilenmann, H. U., and Egli, T. 2007. Assessment and interpretation of bacterial viability by using the LIVE/DEAD *Bac*Light kit in combination with flow cytometry. Appl. Environ. Microbiol. 73:3283–90.