

Antibacterial Activity of Plant Defensins Against Alfalfa Crown Rot Pathogens



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Abstract

Alfalfa (*Medicago sativa*) is the fourth most widely grown crop in the United States. Alfalfa crown rot is a disease complex that severely limits alfalfa stand density and productivity in all alfalfa-producing areas. Currently, there are no viable methods of disease control. Plant defensins are small cationic antimicrobial peptides with a conserved signature of cysteines. The *in vitro* and *in planta* antifungal activity of plant defensins has been extensively studied. However, their antibacterial activity has been less well characterized. Defensins have a γ -core motif, a cluster of cationic and hydrophobic residues, which is essential for antimicrobial activity. The γ -core motifs of four synthetic defensins were tested for antibacterial activity against the bacterial pathogens in the alfalfa crown rot disease complex. Full-length defensins, expressed using a *Pichia pastoris* expression system, were tested to compare antibacterial activity. Using a spread plate assay, the amount of defensin needed to inhibit growth of pathogen strains by 50% (IC_{50}) was calculated. The core motif of MtDef4 was shown to be the most effective truncated peptide with IC_{50} values of 3.4 μ M against *Pseudomonas syringae* pv. *syringae* and 4.52 μ M against *Xanthomonas alfalfae*. In addition, the corresponding full length MtDef4 peptide was found to be active against *P. syringae* pv. *syringae* and *X. alfalfae* with IC_{50} values of 0.43 μ M and 0.68 μ M, respectively.

Introduction

- Crown rot occurs to some extent in every alfalfa stand that is over one year old.
- Resistance has not been identified for developing crown rot resistant cultivars.
- Plant defensins are cationic peptides that have broad antimicrobial activity, but there are few reports of plant defensins with antibacterial activity.
- Full-length defensins are difficult to synthesize because peptide generation is primarily restricted to a *Pichia pastoris* expression system, but the γ -core motif, which has antimicrobial activity, can be created synthetically.

Objectives

- Assess the ability of plant defensins to inhibit bacterial growth.
- Determine if the γ -core motif activity can predict full-length defensin activity.
- Evaluate the defensins for potential transgenic expression in alfalfa to develop lines with increased crown rot resistance.

Methods

Spread-plate Assay

A spread-plate assay was used to monitor bacterial growth inhibition as previously described (1).

- Bacteria were grown out to an OD_{600} value of 0.1
- 200 μ L of bacteria was incubated with shaking for 3 hours with concentrations of defensin peptide up to 30 μ g/mL
- Bacteria were serially diluted, and 100 μ L was plated onto NBY plates.
- After 48 hours of incubation, the bacterial colonies were counted.
- From these values, the amount of defensin needed to inhibit growth of the pathogens strains by 50% (IC_{50}) was calculated.

Results

Table 1. Amino acid sequences of γ -core region (bold) and C-terminal region (italics) of defensins tested *in vitro*.

Defensin	Amino Acid Sequence
MsDef1	GRCRDDFRC <i>WCTKRC</i>
So-D2	GDCKGIRRC <i>MCSKPL</i>
MtDef4	GRCRGFRRC <i>FCTTHC</i>
MtDef5	GACHRQGFG <i>FACFCYKCC</i>

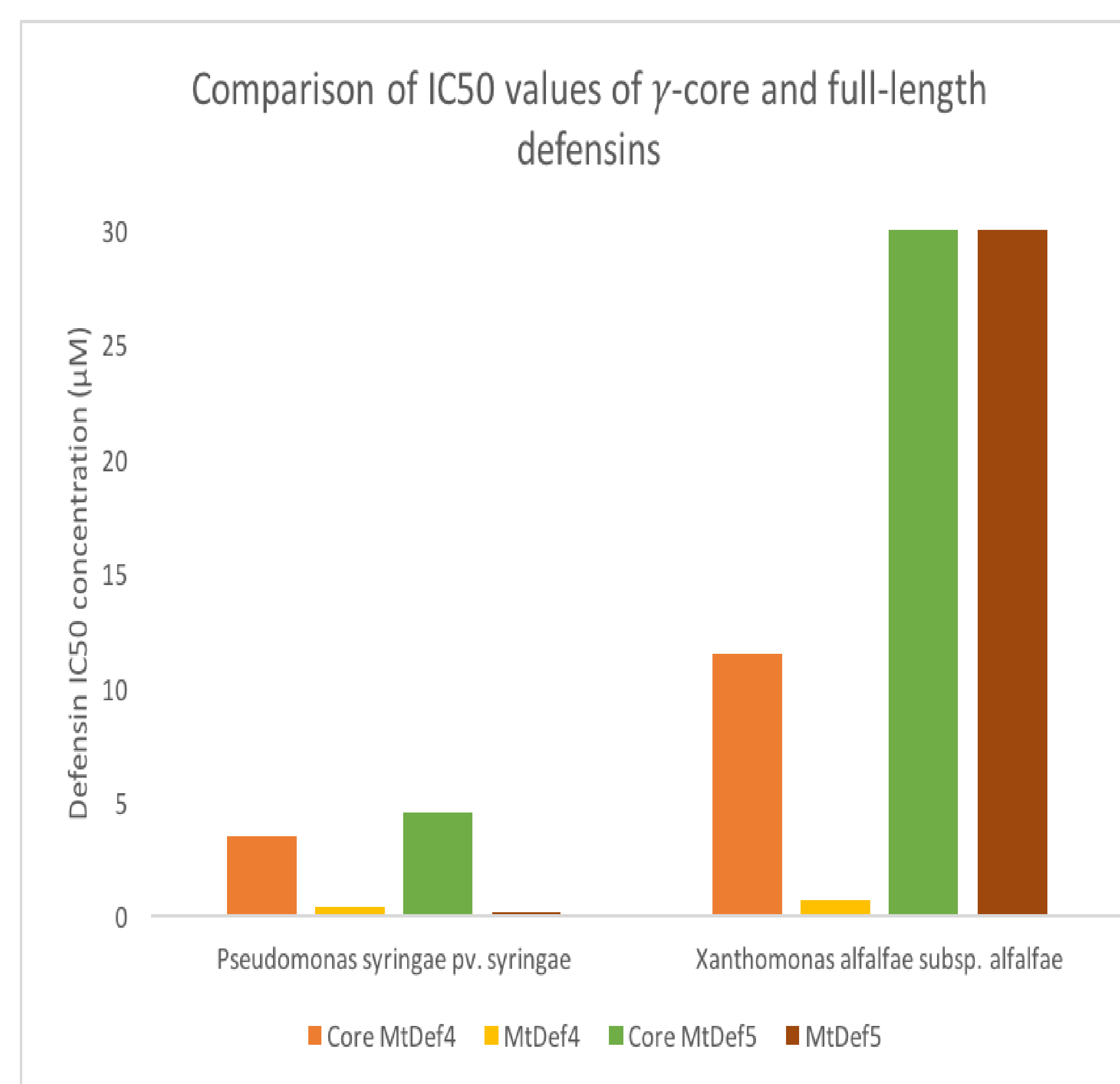


Figure 1. MtDef4, MtDef5, and their corresponding γ -core motifs displayed high biological activity against *P. syringae* pv. *syringae*. MtDef5 and its associated γ -core motif failed to suppress the growth of *X. alfalfae* subsp. *alfalfae*.

Table 2. Activity of γ -core motif defensin peptides and full-length defensin peptides against bacterial alfalfa crown rot pathogens. The IC_{50} (μ M) is reported.

Defensin	<i>Xanthomonas alfalfae</i> subsp. <i>alfalfae</i>	<i>Pseudomonas syringae</i> pv. <i>syringae</i>
Core MtDef4	11.48	3.47
Core MtDef5	> 30	4.52
Core So-D2	19.39	25.93
Core MsDef1	7.99	8.80
MtDef4	0.684	0.430
MtDef5	> 30	0.198

Synthetic γ -core motifs of four defensins (Table 1) along with two full-length defensins were tested *in vitro* against bacterial pathogens associated with the alfalfa crown rot disease complex. Full length defensins had greater biological activity than the corresponding γ -core motif (Table 2). Overall, the crown rotting pathogens were highly susceptible to defensin and γ -core motif peptides. Both the γ -core of MtDef5 and full-length MtDef5 displayed no antimicrobial activity against *X. alfalfae* subsp. *alfalfae*, which reveals that the γ -core motif is an indicator of the biological activity of the full-length defensin (Figure 1).

Conclusions

- Plant defensins have high biological activity of against bacterial pathogens, which was previously overlooked. Well-characterized plant defensins should be retested for antibacterial activity.
- The γ -core motif of plant defensins can be used to predict the biological activity of the full-length defensin. This could greatly simplify and expedite defensin bioassays.
- Transgenic expression of plant defensins in alfalfa has the potential to lead to improved crown rot resistance and reciprocal gains in alfalfa yield.

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.