

## Effects of Synthetic Cecropin Analogs on in Vitro Growth of *Acholeplasma laidlawii*†

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**Four synthetic peptides (Peptidyl MIMs; Demeter Biotechnologies, Inc.) were evaluated for their in vitro activity against *Acholeplasma laidlawii*. Fifty percent effective concentration values ranged from 1 to 15  $\mu\text{M}$ . Three of these compounds are more lethal than cecropin B against *A. laidlawii*.**

Lytic polypeptides with antimicrobial activity have been isolated from insect hemolymph, amphibians, and mammals (3). Encoded by single genes, these short peptides have potent antimicrobial activity against a wide spectrum of microorganisms, including bacteria and fungi, yet have very low toxicities to higher organisms. Biologically active synthetic peptides have been produced, and gene constructs coding for lytic polypeptides have been introduced into a number of higher plants for the control of bacterial and fungal phytopathogens (1, 2, 7, 9, 10, 11, 12, 14, 17, 18, 19). We have evaluated the activity of four synthetic cecropin analogs for their antimicrobial activities against the mollicute *Acholeplasma laidlawii*. These analogs were designed for enhanced membrane lysis activity, and each possesses features characteristic of cecropin-like lytic polypeptides (6). Specifically, these peptides each are strongly basic polar molecules which are predicted to form hydrophilic amphipathic  $\alpha$ -helices in their N-terminal regions and more hydrophobic  $\alpha$ -helices in their C-terminal regions.

*A. laidlawii* strain PG-8 (ATCC 23206) was grown in liquid SP-4 medium (22) at 37°C. To assess the lytic activity of each peptide, early-log-phase cultures were exposed to six concentrations of four synthetic Peptidyl Membrane Interactive Molecules (Peptidyl MIMs; Demeter Biotechnologies Inc., Triangle Park, N.C.). The amino acid sequences of these peptides and cecropin B are as follows: 6M1, FKLRAKIKVRLRAKIKL; 2M5, KRKRAVKRVGRRLKLLARKIARLGVAKLAGLR AVKLF; 2M2, KRKRAVKRVGRRLKLLARKIARLGVAF; 2L2, FAKKFAKKFKKFAKKFAKFAFAF; cecropin B, KWKVFKKIEKMGRNIRNGIVKAGPAIAVLGEAKAL.

To each of seven 270- $\mu\text{l}$  aliquots of early-log-phase *A. laidlawii* ( $10^7$  to  $10^8$  CFU/ml) was added 30  $\mu\text{l}$  of dilutions of each Peptidyl MIM. Solutions and cells were incubated at 37°C for 60 min, and three 20- $\mu\text{l}$  aliquots of each dilution were plated and allowed to dry, without spreading, onto SP-4 medium solidified with 12% Noble agar. Plates were incubated at 37°C for 2 to 3 days in a humidified chamber, and CFUs were

counted after staining in Diene's stain. All treatments with each Peptidyl MIM were replicated three times. Mean viable CFU/ml values were calculated from each replicated dilution plating. Percent survival for each Peptidyl MIM at each peptide concentration was calculated based upon the CFU/ml from each dilution plating divided by the CFU/ml of the water control.  $\text{EC}_{50}$ s (estimated concentrations resulting in 50% survival) were calculated for each compound by regressing the percent survival against the natural logarithm of the compound concentration. The resulting regression equation was used to calculate the concentration of compound that would result in 50% survival. Paired *t* tests were used to compare mean  $\text{EC}_{50}$ s for significant differences.

Of the four Peptidyl MIMs tested, peptides 6M1 (mean  $\text{EC}_{50}$  for three replicates, 1.1  $\mu\text{M}$ ) and 2M5 (mean  $\text{EC}_{50}$  for three replicates, 0.8  $\mu\text{M}$ ) were the most lethal to *A. laidlawii*, with  $\text{EC}_{50}$ s of about 1  $\mu\text{M}$ . Peptide 2L2 (mean  $\text{EC}_{50}$  for three replicates, 1.5  $\mu\text{M}$ ) was moderately effective, while peptide 2M2 (mean  $\text{EC}_{50}$  for two replicates, 16.3  $\mu\text{M}$ ) was the least toxic of those tested (Fig. 1). The  $\text{EC}_{50}$  values, with the exception of those for peptide 2M2, were 5 to 15 times lower than that of purified cecropin B, which had  $\text{EC}_{50}$  values of about 15  $\mu\text{M}$  (data not shown) (mean values for 2M5 and 6M1 were not significantly different from each other but were significantly different from mean values for 2L2 and 2M2; mean values for 2M2 and 2L2 were significantly different from each other [ $P < 0.05$ ]). These effective concentrations are of the same magnitude as those reported for other synthetic lytic peptides derived from cecropin B against a variety of gram-positive and gram-negative bacteria (4, 5, 6, 11, 13, 14, 15, 17, 18, 20, 21).

Interestingly, the activity of peptide 2M5 is much greater than that of peptide 2M2, even though peptide 2M2 is a truncated form of peptide 2M5. Peptide 2M5 contains 10 additional C-terminal amino acid residues that are not present in peptide 2M2 and which extend the hydrophobic  $\alpha$ -helix at the C terminus of the molecule. This extended hydrophobic tail may facilitate the embedding of the molecule in the cell membrane of mollicutes, which may lead to the destabilization or dissolution of the membrane.

Peptide 6M1 was also very effective against *A. laidlawii*; however, this compound does not share the same overall structural similarities as the other peptides tested. Although it is composed of mostly basic peptide residues that form amphi-

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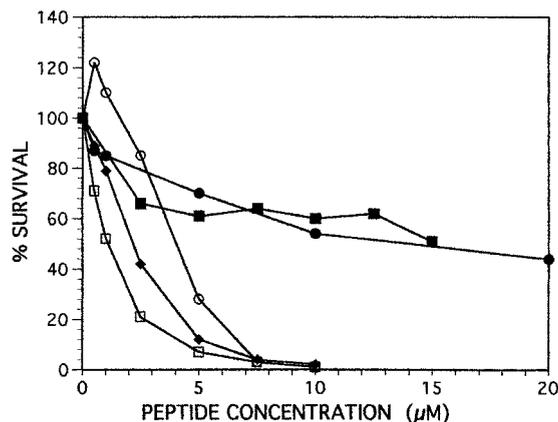


FIG. 1. Survival of *A. laidlawii* after 1-h exposures to dilutions of four synthetic cecropins. Data points are mean values. Symbols: ■, 2M2; □, 2M5; ◆, 6M1; ○, 2L2; ●, cecropin B.

pathic  $\alpha$ -helices, it does not have the strongly hydrophilic N terminus and hydrophobic C terminus that the other peptides have in common with cecropin B. Its antimicrobial activity, however, is comparable to that of peptide 2M5, the most effective compound used in this study. Peptide 6M1 is predicted to form amphipathic  $\alpha$ -helices at its N and C termini that are connected by a short, rather flexible, intervening segment. A similar conformation has been shown for the cecropins, and it has been proposed that this general structure is integral to their mode of action (6). However, the other peptides used in this study are predicted to exist along their entire lengths as amphipathic  $\alpha$ -helices without any intervening flexible region. Therefore, although peptide 6M1 contains a linker segment and has relatively high lytic activity, the presence of such a segment linking the N- and C-terminal  $\alpha$ -helices seems to not be required for lytic activity of these compounds against *A. laidlawii*.

An interesting effect of Peptidyl MIM 2L2 was the 20 to 30% increase in cell titers of *A. laidlawii* at concentrations of less than 1  $\mu$ M. Such stimulation of cell growth by low concentrations of lytic peptides has also been reported in other systems (12). This effect was reproducible and may reflect alterations to the membrane structure that result in increased cell metabolism or reproduction of mollicutes.

The results presented here demonstrate that these cecropin-like analogs have powerful antimicrobial effects on *A. laidlawii* growth in vitro. *A. laidlawii* is closely related to unculturable phytoplasmas based on 16S ribosomal DNA sequences (8, 16). Although such sequence homology provides only taxonomic placement and does not necessarily reflect similar physiological characteristics, it is possible that these cecropin analogs may have similar effects on phytoplasmas, which also are prokaryotes devoid of cell walls. Therefore, these analogs may prove useful in the development of transgenic plants with high resistance to phytoplasmas.

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