Chemically synthesized antimicrobial peptides inhibit *in vitro* growth of *Campylobacter* spp.

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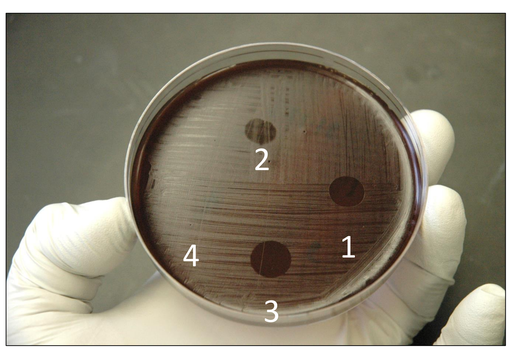
Introduction and Hypothesis

Novel alternatives to traditional antibiotics are needed for food-animal production. One goal of our laboratories is to discover and evaluate antimicrobial peptides (AMP) to reduce foodborne bacterial pathogens during poultry production. AMPs permeabilize membranes and are found in most every class of living organism where they have evolved as a defense mechanism against invading microorganisms. Our working hypothesis is that AMPs can be identified that inhibit growth of *Campylobacter jejuni* then subsequently utilized to reduce gastrointestinal *Campylobacter* among commercially produced chickens.

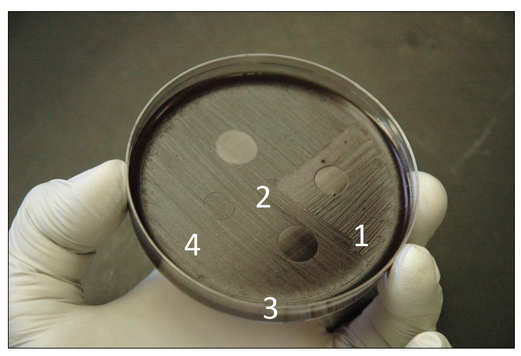
METHODS AND SUMMARY OF RESULTS

A set of 11 unique AMPs chemically synthesized commercially and evaluated for ability to inhibit growth of two *C. jejuni* strains. Six of the AMPs assayed produced zones of inhibition on lawns of *C. jejuni*. These included: NRC-13, a variant of pleurocidin isolated from the American plaice-flounder; RL-37, a 37-residue AMP of the cathelicidin family which is expressed in bone marrow of the rhesus monkey; temporin, from the frog, *Rana temporaria*; a potent hybrid AMP (Cec-Mag) composed of residues 1-8 of cecropin A (from the *Cecropia* moth) fused to residues 1-12 of magainin 2 (from the African clawed frog, *Xenopus laevis*); dermaseptin from the skin of *Phyllomedusa* frogs; and the synthetic OAK, C12K-2b12. Three AMPs were chosen for further investigation on the basis of reported reduced cytotoxicity to mammalian cells: Cec-Mag, RL-37 and C12K-2b12. These AMPs produced zones of inhibition on lawn assays against 19 different bacteria, including *C. jejuni*, *C. coli* and *C. lari* as well as two strains of *Salmonella* and *Lactobacillus*. Modifications of the NCCLS M26A and Hancock assays were utilized to determine minimum inhibitory concentrations (MIC) in microtiter plates for these AMPs against three strains of *C. jejuni*. MICs were approximately 3.1 ug/ml for the AMP RL-37 and C12K-2b12, while the MIC for Cec-Mag was in the range of 12.5 to 50 ug/ml.

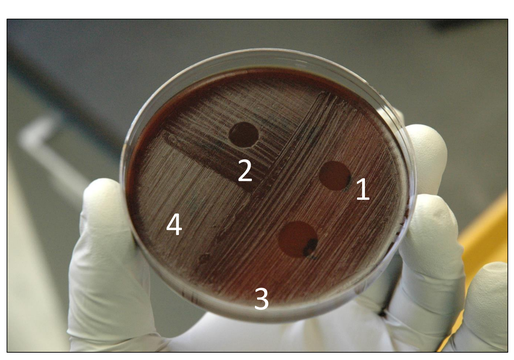
RESULTS



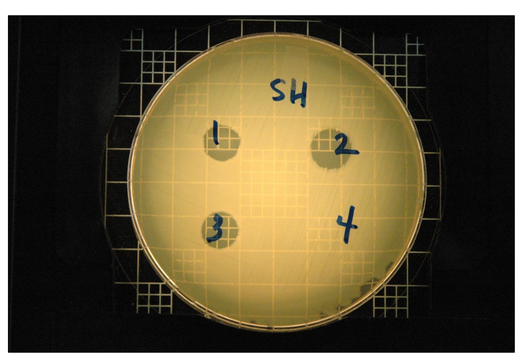
Campylobacter jejuni A74C



Campylobacter coli 49941



Campylobacter lari A35221



Salmonella enterica serotype Heidelberg

Figure 1. Examples of AMP zones of inhibition in a spot-on-lawn assay of 1) C12K-2β12; 2) Cecropin A-Maganin 2; 3) RL-37 and 4) sterile water.

All AMPs examined produced zones of inhibition on lawns of *Campylobacter* spp. and *Salmonella enterica* type Heidelberg. Sterile water and PBS did not cause zones of inhibition.

RESULTS CONTINUED

Table 1. Formation of zones of inhibition by AMP against various target bacteria.

Target Bacteria	1. C12K-2β12	2. Cecropin A-Magainin 2	3. RL-37	4. Water-Control
Cj 14118	Pos	Pos	Pos	Neg
Cj 81-116	Pos	Pos	Pos	Neg
Cj 81-176[a]	Pos	Pos	Pos	Neg
Cj 11168^[a]	Pos	Wk	Pos	Neg
Cj RM1221[a]	Pos	Pos	Pos	Neg
Cj A74C	Pos	Pos	Pos	Neg
Cj A49943*	Pos	Pos	Pos	Neg
Cj A33250*	Pos	Pos	Pos	Neg
Cj A29428*	Pos	Pos	Pos	Neg
Cc Epi 33-WT	Pos	Pos	Pos	Neg
Cc A49941*	Pos	Pos	Pos	Neg
Cc A33559*	Pos	Pos	Pos	Neg
Cl RM2100	Pos	Pos	Pos	Neg
Cl A35221*	Pos	Pos	Pos	Neg
Cl "slaughter beach"	Pos	Pos	Pos	Neg
S. Typhimurium Epi 3	Pos	Pos	Pos	Neg
S. Heidelberg Epi 42	Pos	Pos	Pos	Neg
L. acidophilus-WT	Pos	Pos	Pos	Neg
L. helveticus-WT	Pos	Neg	Neg	Neg

Pos = inhibition zone acquired
Neg = no zone of inhibition
Wk = weak activity without full zone of inhibition
[a] Alternate ATCC designations
^National Collection of Type Cultures (NCTC) isolate
*American Type Culture Collection (ATCC) isolate
Cj = *Campylobacter jejuni*
Cc = *Campylobacter coli*
Cc Epi 33 (3309-61099A hog)
Cl = *Campylobacter lari*
L = *Lactobacillus*
Salmonella enterica serotype Typhimurium
Salmonella enterica serotype Heidelberg
WT = wild type
Epi = Acquired from epidemiological surveillance

Figure 2. Typical MIC response for AMP RL-37 in microtiter well assay.

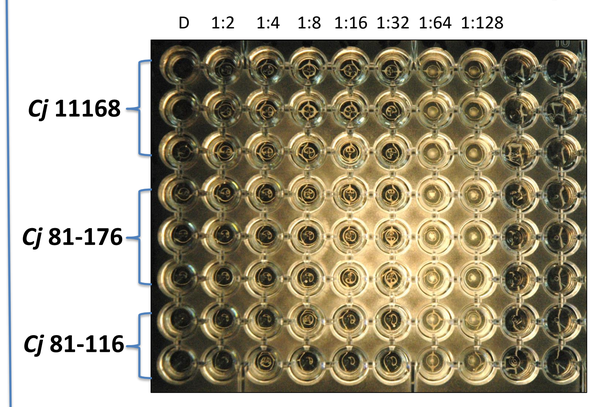
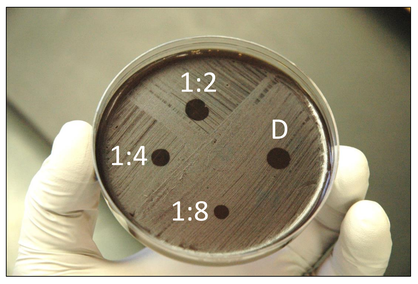


Figure 3. Typical response for diluted AMP C12K-2β12 against *Cj* 11168.



CONCLUSIONS

- The selected AMPs produced obvious zones of inhibition against growth of *C. jejuni*, *C. coli* and *C. lari* isolates in the classic spot-on lawn plating assay.
- MICs were approximately 3.1 ug/ml for the AMP RL-37 and C12K-2b12.
- MICs were slightly higher for the Cec-Mag AMP in the range of 12.5 to 50 ug/ml.
- The selected AMPs also inhibited growth of a pathogenic *Salmonella* isolate.
- Research must be done to determine the effect of AMP on the non-pathogenic natural flora of broiler chickens.
- Our next approaches are to express AMPs in yeast and explore encapsulation technologies to stabilize the AMP for *in vivo* trials in broiler chickens.

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