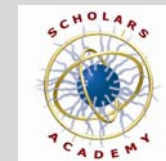


Empirical Tests of the Antibiotic Properties of Snake Venom: A New Twist on an Old Tale



Brendon Bingwa*, Anish Chavda*, Amanda Walker*
Aaron Krochmal, and Lisa Morano



Department of Natural Sciences, University of Houston-Downtown
*Undergraduate Research Students

Introduction

Snake venoms are complex mixtures of proteins, including small peptides, enzymatic and non-enzymatic proteins produced by specialized oral glands of certain snake lineages. Venoms have been empirically shown to aid in the acquisition and digestion of prey, but could have additional utilities, a concept best evidenced by the lack of a venom delivery system among several lineages of venom-producing snakes. Typical foraging behavior and prey types expose snakes to an array of pathogenic bacteria; selection would favor snakes capable of killing these bacteria, an ability which could be performed by venoms. Using a variety of microbiological assays, we investigated the antibiotic activity of Western diamond-backed rattlesnake (*Crotalus atrox*) venom against bacteria most commonly found on the snake's prey.

Methods

Radial Diffusion Assay

Bacterial cultures of *Pantoea agglomerans*, *Staphylococcus epidermidis* and *Escheria coli* were used to create bacterial lawns for experimentation.

- Cells were cultured in Luria-Bertani (LB) broth and incubated overnight at 30°C.
- LB agar plates were made and divided into four quadrants for disk placement.

Bacteria were subjected to the following treatment levels using filter-paper disks:

- Snake venom mixed with water in concentrations of 250mg/ml, 125mg/ml, and 62.5mg/ml.
- Bovine Serum Albumin (BSA) mixed at the above concentrations was used as a positive control
- Water and blank disks were also used as controls.

P. agglomerans plates were incubated over a 24-hr period at 30°C. *S. epidermidis* and *E. coli* were both incubated 37°C.

Zones of inhibition (Figure 2) were measured in triplicate and averaged for each disk. A grand mean was then taken for each treatment. Only *E. coli* values were analyzed for simplicity.

Optical Density Analysis of Bacterial Growth

E. coli was cultured in LB broth and incubated overnight.

A 96-well ELISA plate was loaded with 75µl of bacterial cells (10⁶/ml) and 75µl of one of the following treatments:

- Snake venom at concentrations of either 250mg/ml, 125mg/ml, or 62.5mg/ml.

- Control
 - LB
 - Water

Optical density readings (650nm) were taken at 30 minute intervals over a 6 hour period and then again after 24 hours.



Figure 1: Performing Radial Diffusion Assay

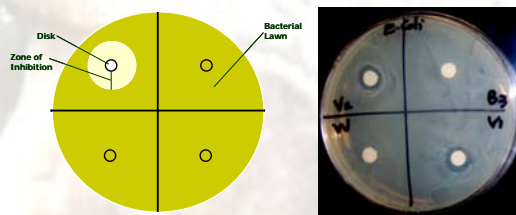


Figure 2. *C. atrox* Venom Inhibits Bacterial Growth. (Left) Schematic of zones of inhibition. (Right) Significant zones of inhibition surround the quadrants labeled V₁ and V₂, venom concentrations of 250 mg/ml and 125 mg/ml respectively. Quadrants W (water) and B₃ (BSA concentration of 62.5 mg/ml) do not show any zones of inhibition.

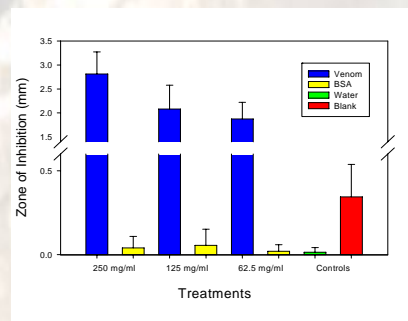


Figure 3: Mean Zones of Inhibition. Significant mean zones of inhibition show that *C. atrox* venom is antibacterial. Blank, water, and BSA disks did not appreciably inhibit bacterial growth.

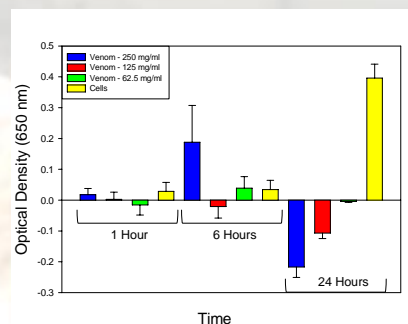


Figure 4: Optical Density Analysis of Bacterial Growth. Optical density readings after 1, 6, and 24 hours show that *C. atrox* venom significantly inhibits bacterial growth.

Results

Radial Diffusion Assay

- At all concentrations, snake venom strongly inhibited *E. coli* growth.
- The size of inhibition zone positively correlated with venom concentration. The 250mg/ml, 125mg/ml, and 62.5mg/ml concentrations had mean inhibition zones of 2.814mm, 2.082mm, and 1.874mm respectively (Figure 3).
- The strongest venom concentration, 250mg/ml, inhibited growth significantly more than the other two concentrations. There was no significant difference between the 125mg/ml and 62.5mg/ml concentrations.
- BSA, water, and blank disks did not inhibit bacterial growth.
- The lack of inhibition by the BSA control indicates that protein and osmotic lysis were not solely responsible for bacterial cell death.

Optical Density Analysis of Bacterial Growth

- Bacterial growth was inhibited in all three venom concentrations, while the untreated control (cells only) exhibited substantial growth (Figure 4).
- After 24 hours, the cell/venom mixtures had optical density below that of the initial starting point.
- The untreated *E. coli* exhibited an approximate ten fold increase in optical density after the 24 hour period.

Discussion

- The results show that *C. atrox* venom has inherent antimicrobial properties.
- Though the mechanism by which the antimicrobial properties are expressed is currently unknown, it could be rooted in enzymatic lysis of cells (*i.e.* membrane disruption) or inhibition of chemical signaling pathways by small peptides in the venom.
- Our results support the previously reported antimicrobial activity in the venoms of other vipers (Krochmal & Gardner 2004) and the inland taipan (Nair *et al.* 2007).
- Future work will focus on investigating this phenomenon with a phylogenetic context to elucidate the evolutionary origins of venoms and determining the functional mechanisms by which snake venoms inhibit bacterial growth.

Acknowledgements

- Financial support through the UHD Scholars Academy with funding from the U.S. Army Research Office (Award No. W911NF-04-1-0024)
- Dr. Stephen Mackessy for technical support

References

- Krochmal A, Gardner C. Potent Poison or Liquid Lysol: On the evolutionary origins of snake venom. *Integrative and Comparative Biology* (2004) 44:6-586.
- Nair D, Fry B, Alewood P, Kumar P, Kini R. Antimicrobial Activity of Omwaprin, a New Member of Waprin Family of Snake Venom Proteins. *The Biochemical Journal* (2007) 402:93-104.