

Antibacterial properties of medical leech (*Hirudo medicinalis*) intestinal tract contents

Elizabeth Pierson
Advisor: Dr. David Brown
Marietta College

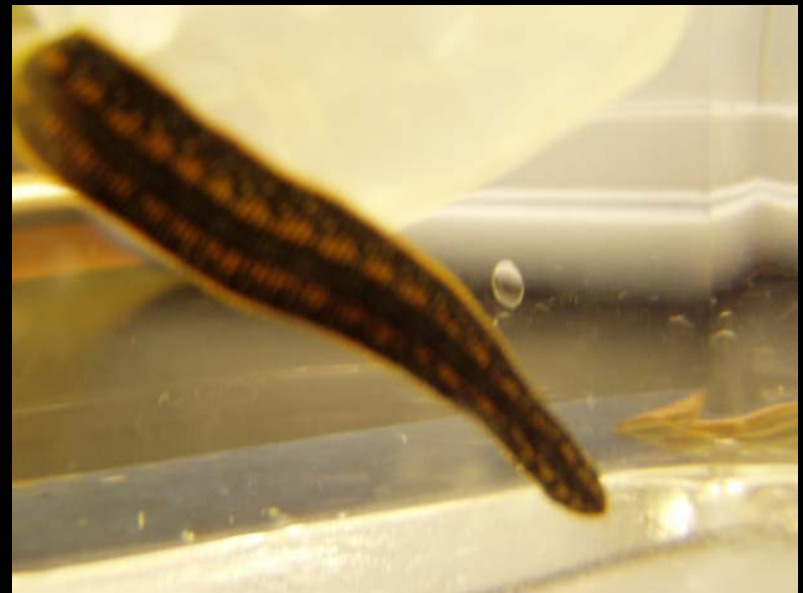
Senior Research Presentation
Spring 2004

Overview

- Background
- Rationale and Hypotheses
- Experimental Methods and Procedures
- Data and Results
- Discussion and Conclusions
- Acknowledgements and Questions

Background: Why leeches?

- History of leech “medicine”
 - “Humors”
 - Decreased use
- Microsurgery
 - Increased use
 - Research concerning native flora and infection
- Useful compounds from saliva:
 - Anticoagulant
 - Vasodilator



Rationale: Again, why leeches?

- Further research
 - Bacterial colonization
 - Prompted investigation of gut contents
 - Antibacterial properties

- Purpose

Hypotheses

- *Hirudo medicinalis* gut contents will exhibit antibacterial activity against selected bacteria

and

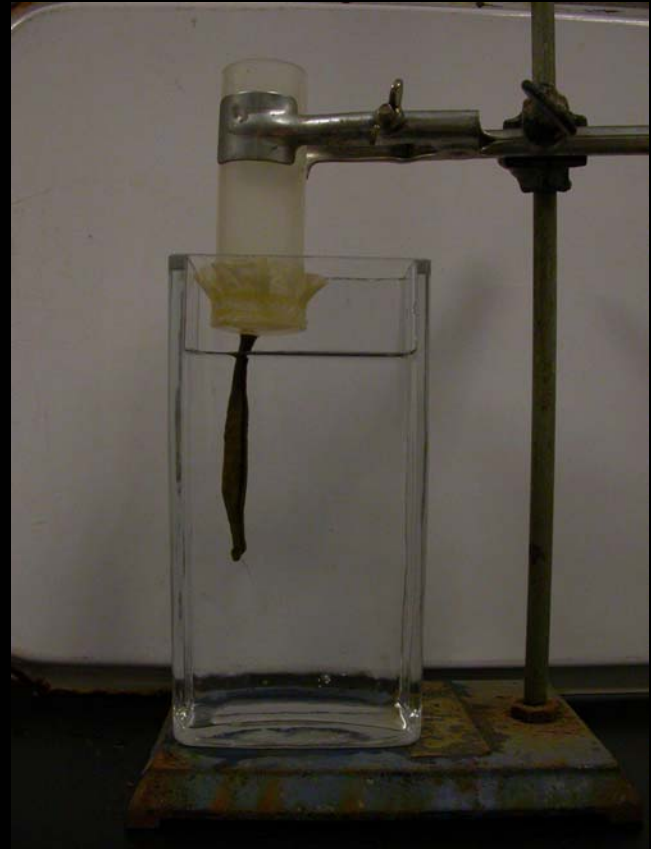
- Leech gut contents contain a large molecular weight compound responsible for antibacterial activity.

Methods and Procedures

- Feeding procedure
 - Gut contents
- Kirby-Bauer
 - Determine if antibacterial activity
- Minimum Inhibitory Concentration Assay
- *Aeromonas sobria* tests

Methods and Procedures

- *Hirudo medicinalis*
 - Leeches given feeding solution (0.15M sodium chloride and 1mM arginine in dH₂O)
 - Squeezed to obtain gut contents
 - Gut contents filter-sterilized

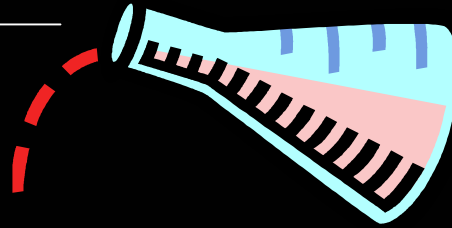


What is the Purpose of Kirby-Bauer?

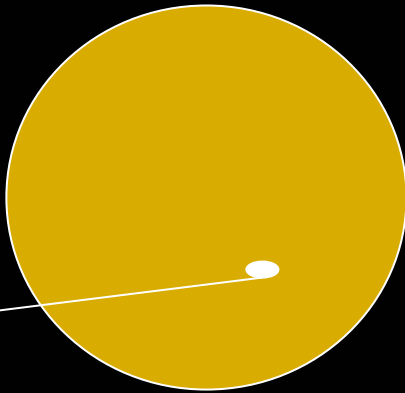
- Bacteria and antibiotic susceptibility
- Gut contents and antibacterial activity
- “Zones of inhibition”
 - a distinct lack of bacterial growth
- Screening for activity from gut contents
 - 1st batch of leeches
 - 1st and 2nd feeding
 - 2nd batch of leeches

Kirby-Bauer Procedure

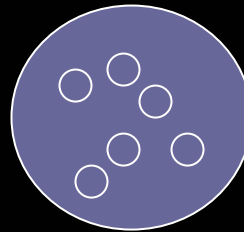
1. Liquid culture of bacteria



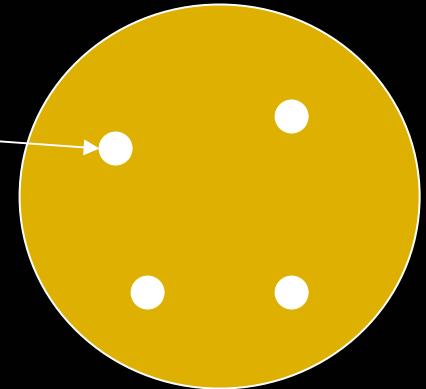
2. Nutrient agar



Sterile disks saturated with compound



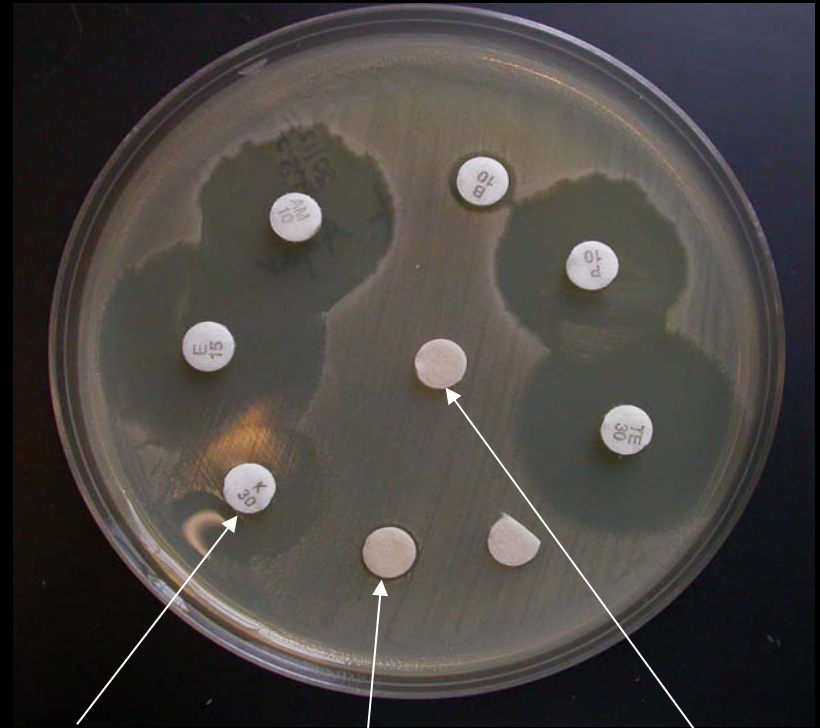
3. Compound diffuses out into surrounding medium



Kirby-Bauer, cont'd

■ Kirby-Bauer

- Bacteria
 - *Bacillus subtilis*
 - *Micrococcus luteus*
- Gut contents
- Antibiotic disks
 - (positive control)
- Feeding solution
 - (negative control)



Pos. control

Gut contents

Neg. control

Kirby-Bauer Data

<i>Bacillus subtilis</i>					
	Gut 1	Gut 2	Lowly 1	George 2	Pooled
Zones of Inhibition (mm)	8.00	8.00	8.00	11.60	7.00
<i>Micrococcus luteus</i>					
	Gut 1	Gut 2		George 2	
Zones of Inhibition (mm)	10.60	10.60		8.0	

George's 2nd feeding (without heme) still shows inhibition

What is the Purpose of Minimum Inhibitory Concentration (MIC)?

- To determine the minimum amount of antimicrobial agent that will inhibit growth
- Measure the absorbance of samples
 - Higher numbers signify more bacterial growth
 - Lower numbers show inhibited growth
- To demonstrate a difference between the growth of bacteria with gut contents, and the growth without

MIC Procedure



Bacillus subtilis

Broth culture



With gut contents

Broth culture

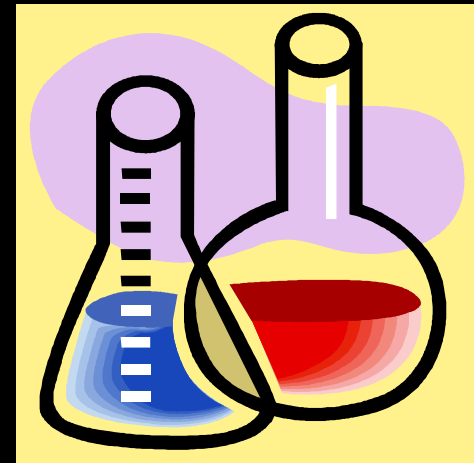


With feeding solution

Without gut contents

MIC, cont'd

- Minimum Inhibitory Concentration Assay
 - Broth cultures
 - Bacteria
 - *Bacillus subtilis*
 - With gut contents
 - With feeding solution (control)
 - Without gut contents
- Absorbance at 660 nm



MIC Data

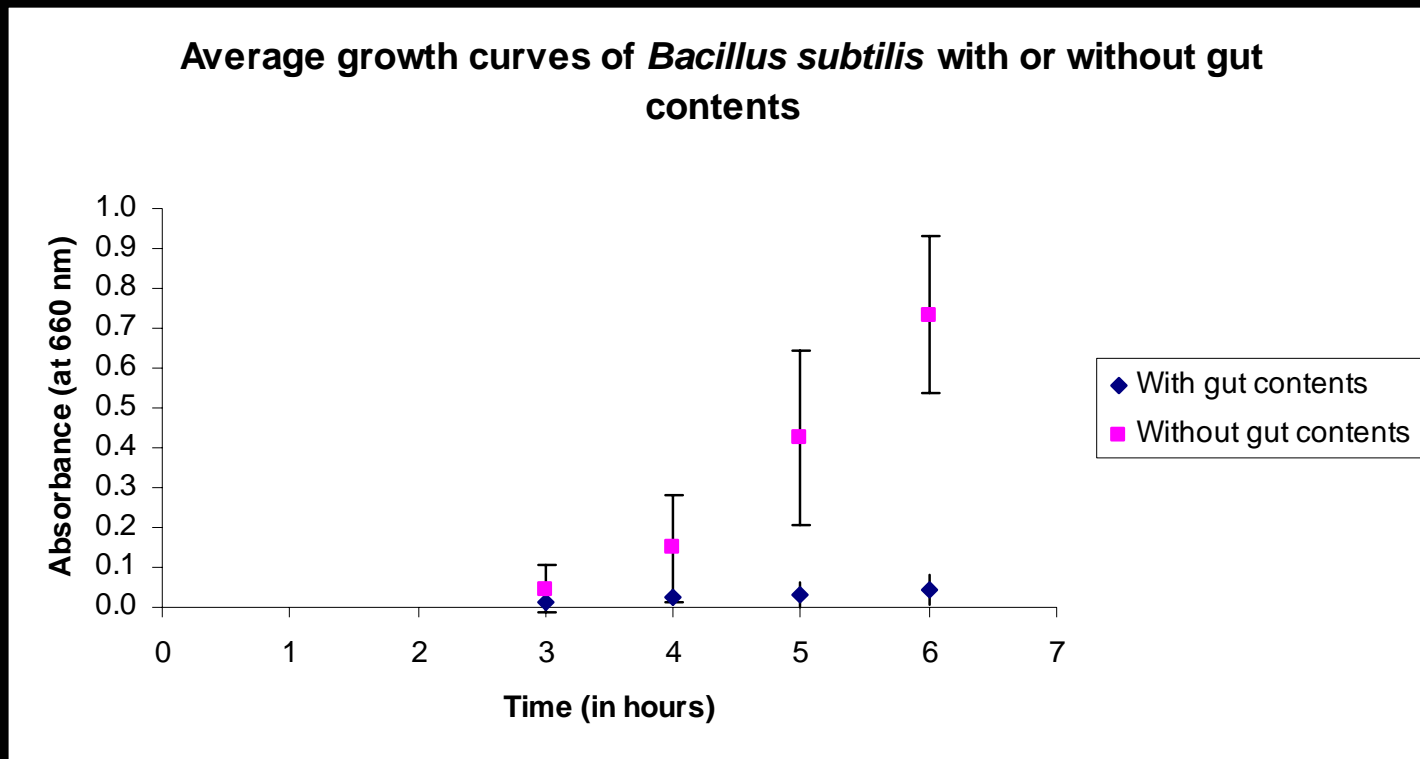
L1 gut cont.	Herb gut cont.	George 2	Pooled gut con.	Average	Std dev		Arg. 1ml	Arg. .5 ml	Arg. #2	Arg. #3	Average	Std dev
0.018	0.026	-0.015	0.021	0.013	0.019		0.039	0.131	0.001	0.013	0.046	0.059
0.023	0.049	-0.008	0.033	0.024	0.021		0.201	0.315	0.035	0.044	0.149	0.135
0.034	0.077	-0.011	0.032	0.033	0.031		0.535	0.681	0.23	0.251	0.424	0.221
0.04	0.097	-0.011	0.041	0.042	0.038		0.85	0.948	0.615	0.521	0.734	0.199
						TTEST						
						0.045468						

Significant at <0.05

Results of ttest= 0.045

Therefore, there is a significant difference between bacteria grown with gut contents and without gut contents (with feeding solution)

MIC (First batch of leeches)



Addition of gut contents inhibited growth rate of *Bacillus subtilis*

Aeromonas sobria experimentation

- Evidence for antibacterial compounds
 - Symbiotic bacteria
 - Paper proposed that inhibition of bacteria was due to membrane-attack complex (MAC) of immune system
- Zones of inhibition
 - Broth culture: bacteria
 - Blood culture w/o bacteria: MAC
 - Blood culture w/ bacteria: MAC or bacteria

Medium	Bacteria	Inhibition
Broth	No	None
Broth	<i>Aeromonas sobria</i>	None: from bacteria?
Blood	No	None: from MAC?
Blood	<i>Aeromonas sobria</i>	None: from bacteria?

Results

- Kirby-Bauer
 - First batch of leeches: Distinct zones of inhibition
 - Without heme (George)
 - Second batch of leeches: No zones of inhibition
- MIC
 - First batch: Different rate of growth b/n flasks w/gut contents and w/o gut contents
 - Second batch: no MIC due to paucity of gut contents
- *Aeromonas sobria* KB
 - No inhibition from broth or blood noted
 - No inhibition from controls

Discussion and Conclusions

- First hypothesis was supported
 - 1st batch of leeches
 - Some inhibition noted
- Membrane-attack complex
 - Results from George/first batch of leeches suggest inhibitory activity was not due to ingested blood



Discussion and Conclusions, cont'd

- *Aeromonas sobria* experimentation
 - Results
 - Culture conditions

- Second hypothesis: Did not conclusively determine if large molecular weight compound was responsible
 - Small zones of inhibition indicate that compound may be large
 - Membrane filtration
 - Centrifugation results

Technical Problems

- Maintaining them
- Unsure of conditions before arrival
- Finding information about the system
- Getting leeches to feed

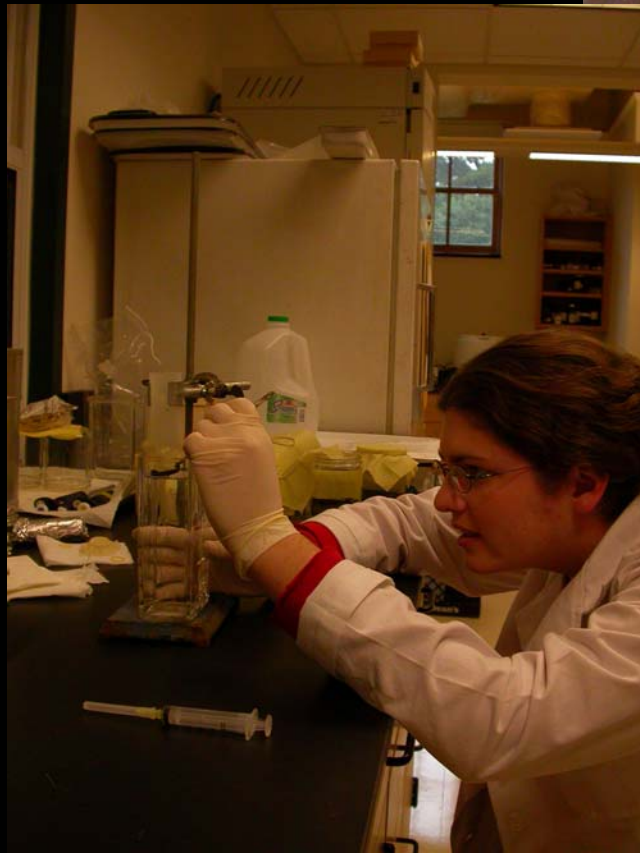
Majority of time was spent learning about the system and attempting to obtain gut contents.

Future experiments



- Replicating results!
- Experimentation with *Aeromonas sobria*
- Size-exclusion chromatography
- Temperature stability trials
- Leech dissection

Pictures



When leeches bite and release, their three jaws leave a triangular mark similar to the Mercedes-Benz symbol.

Acknowledgements



- ❖ Dr. David Brown
- ❖ Dr. Steven Spilatro
- ❖ Capstone Class
- ❖ MC Biology Department
- ❖ Investigative Studies Program
- ❖ My parents
- ❖ Timothy Hayes, D.V.M.

Questions

