Screening *Photorhabdus luminescens* for Antibacterial Properties Using a Modified Version of the Kirby-Bauer Method

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Introduction

Photorhabdus luminescens is a Gram negative rod-shaped bacterium that belongs to the family *Enterobacteriaceae* (Figure 1). The family of *Enterobacteriaceae* is a large one, in which this family consists of all known organisms that inhabit the gut of humans and animals. Common genus members of this family include *Escherichia, Salmonella*, *Shigella*, *Serratia*, *Citrobacter, Klebsiella*, *Proteus*, and *Yersinia*, just to name a few.

P. luminescens is a symbiotic bacterium that lives within the gut of the entomopathogenic nematode, *Heterorhabditis bacteriophora*, and has very interesting properties. When this nematode infects the host insect larvae, they regurgitate *P. luminescens* into the haemocele of the larvae killing it within 48 hours. *P. luminescens* invades by producing agents that depresses the immune system of the larvae. This bacterium also produces degradative enzymes that digest the carcass of the larvae and induces the non-feeding juvenile nematodes to become reproductively active. While the degradation of the carcass is crucial to the survival of the two organisms, it is known that *P. luminescens* produces antimicrobial agents to ward off other organisms from existing within the decaying carcass, but these agents are yet to be identified.

In this experiment, these antimicrobial properties are being investigated using a modified Kirby-Bauer Method. Using this method, a collection of 28 different bacteria (Table 1) are used to observe these properties of *P. luminescens*.

Abstract

The aim of this work was to observe and determine the bacteriocidal properties of *Photorhabdus luminescens*. A modified version of the Kirby-Bauer method was used to screen this bacterium for bacteriocidal properties against a collection of 28 different bacteria. The collection of bacteria used contained bacterial species with different cellular morphologies and Gram stain reactions. The measurements of the zones of sensitivity were used to observe and determine these bacteriocidal properties. The Kirby-Bauer method was slightly modified to achieve the aim of this work. The modification consisted of using blank, sterile disks that had been inoculated with a liquid culture of mid-log phased *P. luminescens*. From these 28 different species of bacteria, 11 bacterial species was determined to be sensitive to *P. luminescens*.

Purpose

The purpose and goal of this experiment is to screen *P. luminescens* against a collection of bacteria to observe for bacteriocidal properties, in hope that this experiment will aid in the identification of some of these agents for potential use as new antibiotics for humans and animals.

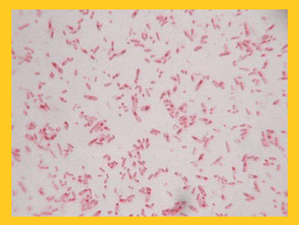


Figure 1 – Gram stain of *P. luminescens*

Methodology

1. Mueller-Hinton (MH) plates were prepared according to manufacturer's instructions and were poured to a depth of 4 millimeters and stored at 4°C.
2. A nutrient broth culture of the phase 1 variant of *P. luminescens* was started to obtain cells that were in mid-log phase and will be used to infuse sterile, blank antibiotic discs.
3. Broth cultures of the bacterial collection were prepared and grown overnight at their respective temperatures and compared to a 0.5 McFarland turbidity standard.
4. The bacterial cultures were then streaked onto pre-labeled MH plates using a special streaking technique, creating a lawn of bacterial growth.
5. Infusion of the sterile, blank discs was performed aseptically in a laminar flow hood. To infuse these discs, 2 drops of *P. luminescens* broth culture was added to each disc and allowed to soak for 10 minutes.
6. One blank disc and one P.lum disc was placed aseptically onto the streaked MH plates. All discs were placed approximately 29 mm from the edge of the plate and approximately 22 mm apart from each other to avoid any possible zone overlaps.
7. The streaked MH plates were then incubated at 30°C overnight in a dry incubator. 30°C was the temperature used due to the fact that it is the optimum temperature for *P. luminescens*.
8. Observations of plates and any zones of sensitivity were recorded and the zones of sensitivity were measured in millimeters.

Data Analysis/Results

Observations were made to identify those plates that showed a circular clearing around the P.lum disc. Also, the plates that did not show a circular clearing were observed and recorded as well. These circular clearings are known as the zones of sensitivity, the diameter of the zones were measured and reported in millimeters. The plates without a circular clearing were deemed to show tolerance and this is known as resistance. Out of the 28 different bacterial species that was tested, 11 of them showed sensitivity to *P. luminescens* while the other 17 exhibited resistance to *P. luminescens*. Figure 2 depicts a photo collage of 10 of the tested organisms that showed sensitivity to *P. luminescens*. Graph 1 depicts all of the sensitive organisms with their respective zones of sensitivity. Graph 2 exhibits the organisms that belong to the same family as *P. luminescens*, in which no zones of sensitivity was observed. Graph 3 reveals the organisms that were tested in the genus *Bacillus*, in which four out of the six tested, showed sensitivity. Graph 4 depicts organisms of the genera *Moraxella* and *Neisseria*, in which the three organisms tested, showed a great deal of sensitivity. Graph 5 exhibits the families of *Streptococcaceae*, *Planococcaceae* and *Micrococcaceae*, in which the later 2 families were observed to be sensitive while the *Streptococcaceae* family showed resistance.

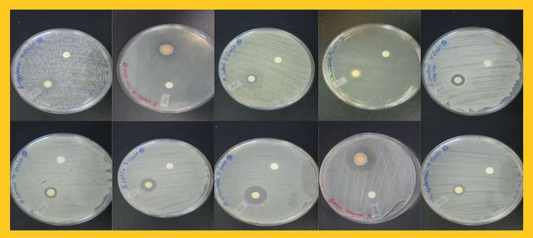
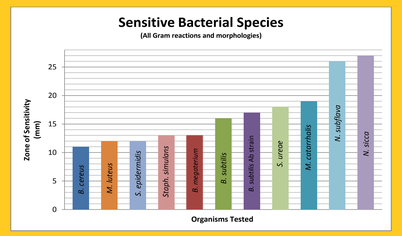


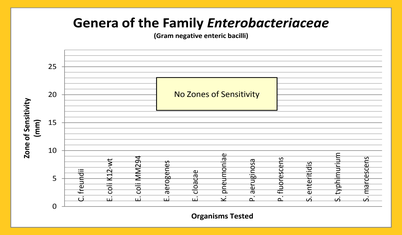
Figure 2 – *P. luminescens* susceptible organisms

Bacillus cereus	*Lactococcus lactis*
Bacillus licheniformis	*Micrococcus luteus*
Bacillus megaterium	*Neisseria sicca*
Bacillus subtilis	*Neisseria subflava*
Bacillus subtilis Ab producing strain	*Pseudomonas aeruginosa*
Bacillus thuringensis	*Pseudomonas fluorescens*
Branhamella (Moraxella) catarrhalis	*Salmonella enteritidis*
Citrobacter freundii	*Salmonella typhimurium*
Enterobacter aerogenes	*Serratia marcescens*
Enterobacter cloacae	*Sporosarcina ureae*
Enterococcus faecalis	*Staphylococcus epidermidis*
Escherichia coli MM294	*Staphylococcus simulans*
Escherichia coli K12-wt	*Streptococcus durans*
Klebsiella pneumoniae	*Streptococcus mutans*

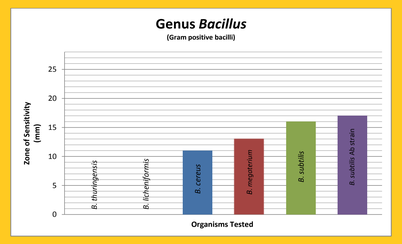
Table 1 – Bacterial Collection Panel



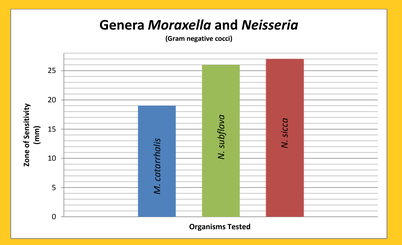
Graph 1 – All sensitive bacterial species



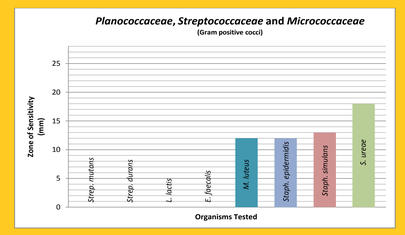
Graph 2 – Sensitivity of the family *Enterobacteriaceae*



Graph 3 – Sensitivity of the Genus *Bacillus*



Graph 4 – Sensitivity of *Moraxella* and *Neisseria*



Graph 5 – Sensitivity of *Planococcaceae*, *Streptococcaceae* and *Micrococcaceae*

Discussion/Conclusion

Sensitivity is a term that is used to describe the susceptibility of bacteria to antimicrobial substances while resistance is a term that is used to describe the tolerance of bacteria to withstand the effects of antimicrobial substances. Antibiotic sensitivity testing is often performed using the Kirby-Bauer method where small discs that contain different antibiotics are placed onto a bacterial lawn and if the bacteria of the lawn are sensitive to an antibiotic then they will not grow thus creating a clear halo around the disc. This project used a modification of this method, in which the discs were infused with a broth culture of *P. luminescens* instead of antibiotics.

P. luminescens is a Gram-negative bacterium that belongs to the family, *Enterobacteriaceae*. In Graph 2, all of the tested members of this family showed no sensitivity and therefore an assumption can be made. The assumption is that *P. luminescens* will not inhibit the growth of other members of the *Enterobacteriaceae* family and that these antimicrobial properties may not be useful in creating a drug that is effective for Gram-negative enteric infections.

On the other hand, Graph 4 depicts the sensitivity of some Gram-negative cocci. *Branhamella* (*Moraxella*) *catarrhalis* is a notorious bug that can cause an upper respiratory infection or conjunctivitis and is very sensitive to *P. luminescens* as well as the two *Neisseria* species, *sicca* and *subflava*. Further experimentation will be needed to determine if *Neisseria meningitidis* and *Neisseria gonorrhoeae* are sensitive as well. According to these results, *P. luminescens* is very effective against Gram-negative cocci and the antimicrobial properties may be exploited to create new drugs for these infections, especially for treating gonorrhea, meningitis, sinus infections and otitis media in children.

Graph 3 shows six different *Bacillus* species which four of the six tested was indeed sensitive to *P. luminescens*. It was found that *Bacillus thuringensis* and *Bacillus licheniformis* were not sensitive to *P. luminescens*.

Graph 5 depicts results from three different families of Gram-positive cocci, *Planococcaceae*, *Streptococcaceae* and *Micrococcaceae*. In this graph, the genera *Micrococcus*, *Staphylococcus* and *Sporosarcina* did show sensitivity, but the genera of *Enterococcus*, *Streptococcus* and *Lactococcus* did not.

The purpose of this presentation was to demonstrate the antimicrobial properties of *P. luminescens* and to determine which species of bacteria were sensitive or resistant to these antimicrobial properties.

Acknowledgements

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