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Attractant Role of Bacterial Bioluminescence of *Photorhabdusluminescens*on a *Galleria mellonella* Model

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Abstract: Though the intricate relationship between the entomopathogenic nematode *Heterorhabditis bacteriophora* and its symbiotic bacterial counterpart *Photorhabdus luminescens*is generally known; the role of bioluminescence produced by the bacterial symbiont is yet to be identified. The objective of this study was to determine if bacterial luminosity plays a crucial role in attraction of larval insect hosts. This study focused on bacterial bioluminescence produced from both *in vitro* and *in vivo* culturing of the bacterial symbiont. The obtained results portrays that the average distance between *Galleria mellonella*larvae and the bacterial light source (*P. luminescens*)decreased in a linear fashion as a function of increasing intensities of luminosity; thereby supporting the hypothesis that bioluminescence offers a symbiotic role to attract insect host larvae.

Keywords: *Photorhabdus Luminescens*, *Heterorhabditis Bacteriophora*, Symbiosis, Bioluminescence, Biological Control Agent

1. Introduction

Heterorhabditis bacteriophora is an entomopathogenic nematode (EPN)that is currently being explored as a biological control agent for particular crop insect pests. *H. bacteriophora* is a cruiser species of nematode, meaning that infective juveniles “seek” out their insect host. In other words, populations of new infected juveniles that are generated from an infected host will exit the infected carcass and disseminate into the surrounding environment in search of anotherinsect host. This predation strategy is rather different than the ambush method which is employed by the sister genus *Steinernema*[1,2]. During the ambush method, infective juveniles of *Steinernema* remain sessile in a single location until an appropriate host is detected.

EPNs arelethal to the insect host due to the transmission of symbiotic entomopathogenic bacteria [3]. *H. bacteriophora* is associated with its bacterial symbiont *Photorhabdusluminescens* [4]. The bacterial symbiont is carried within the gut of *H. bacteriophora*until the nematode reaches the hemolymph of an insect larva. Upon signaling, *H. bacteriophora*expels the bacterial symbiont, resulting in bacterial proliferation that quickly consumes available nutrients within the insect. During this time, the bacterial symbiontbegins to secrete digestive enzymes and insecticidal toxinsthat result in insect death within 24-48 hours [5, 6]. Additionally,*P. luminescens* and other sister species produce bioluminescence; which to date, are the only knownterrestrialbacterialcapable of doing so [1].A possible symbiotic role for bioluminescence is that nematodes exploit this mechanismas a way to attract host insects to the infected carcass. As a result, this mechanism would greatly increase survival of the nematode-bacterium complex.

Galleriamellonella is typically used as a model host

organism to study nematode efficiency and bacterial toxicity. *G. mellonella* is also employed for *in vivo* culturing of EPNs and bacterial symbiontsfor laboratory use[7]. In relevance to this study, larvae of *G.mellonella* use 4 small organs (i.e. stemmata) on each side of its head in order to perceive low-quality vision. Stemmata contain light sensitive pigments (i.e. rhodopsins) that enable insect larvae to detectlight when excited [8]. *G. mellonella* rhodopsins havea lambda max (510 nm), proximal to the peak wavelength (490 nm) ofthe bioluminescence produced by of *P. luminescens* [8, 9]. Many other Lepidopteran species have rhodopsins whose lambda max falls within the same spectrum of bioluminescenceof*P. luminescens*such as *Manducasexta* and Spodopteraexigua(520nm and 515nm, respectively)[9,10]. The relatively small distances between emission peaks and insect rhodopsins may not have a significant impact on light detection within insect larvae.

In this study, larvae of G. mellonella were used to observe the effects of luminosity produced by P. luminescens on insect attraction. This study was performed utilizing two different bacterial culturing conditions to producein vitro and in vivo light sources. These light sources, along with live, uninfected insect larvae, were placed into a gridded chamber and left in low external light conditions. Distances between light sources and live insects were determined from digital images and plotted against the corresponding luminosity reading. The results of this study support the idea that bacterial luminosity does attract insect larvae. Furthermore, this research suggests that bacterial luminosity is a symbiotic trait that infective juveniles of H. bacteriophora exploit to attract insect hosts.

2. Materials and Methods

2.1. Preparation of Materials and Supplies

Infective juveniles of *H. bacteriophora* were obtained through in-house mass production protocols. *P. luminescens* cultures were isolated from infected *G. mellonella* using the method of Inman III and Holmes [11]. *G. mellonella* were obtained from Carolina Biological Supply Company (Burlington, North Carolina, USA). Using an inoculation loop, samples of infected hemolymph were aseptically obtained and streaked for bacterial isolation onto nutrient agar(g/L: beef extract, 6; digested gelatin, 10; and agar, 15).

P. luminescens was cultured overnight at 28°C and isolated colonies werethen subcultured onto a second plate. Isolated colonies from the second plateweretransferred to tubes containing3 mL ofnutrient broth (g/L: beef extract 6; digested gelatin, 10) and incubated at 150 rpm and 28°C overnight. Following incubation, the liquid culture was scaled up to 50ml nutrient broth in a 250 mL Erlenmeyer flask. Cultures were incubated at 28°Cand agitated on an orbital shaker at 150 rpm overnight. Unused infected *Galleria*larvae from this step were used for *in vivo* luminosity studies.

Behavior chambers used in this experimentwere made from 100 mm x 20mm cell culture dishes (Corning, Inc.) and to them PetriStickers™containing32 square gridswere affixed to the top for determining insect positions.Inside chamberswere lined with pre-moistened 9 cm Whatman filter paper and assembled. Chamber tops were drilled with four equidistant holes (~ 2 mm in diameters)to allow gas exchange.

2.2. Methods

2.2.1. Bacterial and Larvae Sampling

*G. mellonella*were screened for signs of infection based on melanization (darkening of pigment) and motor defects based on lack of response to physical stimulation. Larvae who did not display any of the traits where separated from the population received.

P. luminescens isolates where screened for contamination and phase variation. Contamination was screened for by gram-staining at transferring to new media. Phase variation was screened by only selecting cultures with relatively high bioluminescence.

2.2.2. Exposure to Invitro Bioluminescence

For*in vitro* trials, 1 mL aliquots of the 50 mL overnight culture of*P. luminescens* culture were obtained and dispensed into 1.5 mLmicrocentrifuge tubes. Luminosity of sample aliquots was measured with a Modulus™single tube luminometer (Turner Biosystems) and reported in terms of relative luminosity units (RLU). Afterwards, each culture tube was placed at theperipheryof each chamber. Healthy*G.mellonella*larvae were added carefully to the center of each chamber and placed in a lowlight environment for 10 minutes. After the 10 minute period, each chamber was briefly exposed to laboratory light and a digital photograph was taken at a fixed 90º angle to the plate surface. Controlsconsisting of sterile culture broth,were also performed in the same manner.

2.2.3. Exposure to in Vivo Bioluminescence

H. bacteriophora – P. luminescens infected larvae of *G.mellonella* were measured for luminosity. The infected and highly luminescent carcasses of *G. mellonella* were placed at the peripheral of each chamber. Healthy, non-infected*G. mellonella*were added to the center of each chamber. Methodology of *in vivo* testing was conducted in the same manner as *in vitro* testing, with dead, suffocated larvae acting as a control, which were discarded upon melanization.

2.2.4. Analyses of Images

Utilizing the known distances ofthe grid and the number of pixelsalong the grid from the digital photograph were used to determine ratios of pixels todistance. From the pixel grid,the location of the culture and the head of the *G. mellonella*were found, thenumber of pixels separating those locations was then counted.The pixels to millimeter ratio of the individual photographs were used to calculate the distance of the *Galleria* larvae to the light source based on the number of pixels separating them.

3. Results

3.1. Effects of Bioluminescence from in Vitro Cultures on Galleria Mellonella

*Galleria*larvae exposed to *in vitro* cultures of *P. luminescens*tend to follow a set pattern of behavior towards luminosity. Results indicate that distancesbetween luminescent cultures and larval heads decrease as luminosity increases. When the RLU of the culture increase, larvaetend to move closer to the tube in aninversely linear fashion. This observation suggests that at higher intensities of bioluminescence attraction of the insect is greater; thereby reducing the distance between the insect and the luminescent culture. The slope produced from the *in vitro* data indicates an attraction rate of -7.54 mm/10^5 RLU as shown in Figure 1.

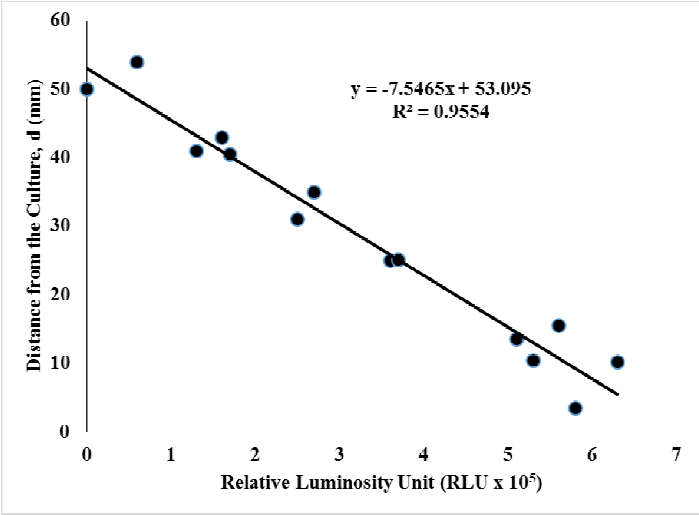

Figure 1. *Effects of in vitrobioluminosity on Galleria mellonella position relative to P. luminescens culture. The overall trend ofin vitro culturing depicts an inversely linear relationship. The linear portion depicts an attractionrate of -7.54 mm/10^5 RLU.*

3.2. Effects of Bioluminescenceproduced from in Vivo Cultures on Galleria Mellonella

G.mellonella exposed to *in vivo* culturing of *P. luminescens* and *H. bacteriophora* within carcasses of otherlarvae were foundto display a similar inversely linear correlation between RLU and distances to carcasses. With the *in vivo* testing there is a linear trend that exhibits a decreasing distance to the carcass as RLU of *in vivo*cultures increase (Figure 2). The rate of this linear trend was at -0.75 mm/10^5RLU.During the period of*in vivo*experimentation (~ 5 days), no healthy *Galleria*subjects were infected by *H. bacteriophora*. Thissuggests that more incubation time is needed to force the emergence of infective juvenile nematodes from insect carcasses.

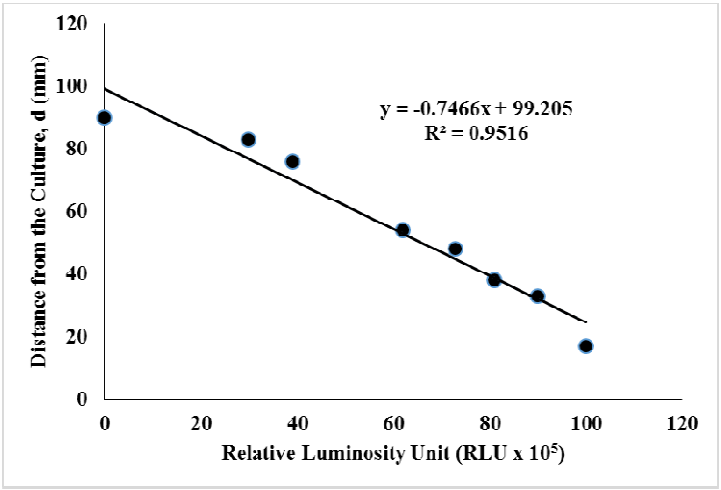

Figure 2. *Experimental correlation between distance and RLU determined during in vivo trials. A linear correlation is observed that depicts a decreasing rate of -0.75 mm/10^5RLU which is highly similar to the rate obtained through in vitro trials.*

4. Discussion

H. bacteriophora is classified as a cruiser species and appears to conflict with the role of bioluminescence from *P. luminescens* as a tool for host attraction[2,12].The differentiation between cruiser and ambush EPNs is based on

the observation of species' microscopic movements, rather than their macroscopic distribution in an ecosystem.This conflict can be resolved by considering the relative effectiveness of *P. luminescens* on *G.mellonella,*and its ability to attract larvae from portions of the environment in which *H. bacteriophora*does not inhabit.From this study, onlyhigh luminosityreadings, both *in vitro* and *in vivo*culturing systems,show effective attraction of *G.mellonella* larvae. The possible explanation of the role of bacterial bioluminescence is that at high RLU, host insects are attracted to a certain distance from the infected insect carcass. Once infective juveniles (IJs) of *H. bacteriophora*emergefrom the carcass, they wouldmobilize within the environment to detect and attack baited hosts. Ultimately, luminosity draws insect hosts closer to the host cadaver to reduce the distance nematode would have to travel before attacking a new host and as a consequence, survival of both *P. luminescens* and *H. bacteriophora* is greatly increased.

Both culturing methods resulted in linear curvesthat depicted decreasing distancesbetween insect and bacterial cultures as luminosity increased. These trends indicate that high*in vivo* luminosity would be expected in a natural setting that issufficient to attract larval insect hosts.The differences in slopes could be due to secondary metabolites or other compounds produced by *P. luminescens.*Researchwith ant models has shown, that *P. luminescens*secretes a secondary metabolite that repeals insects from the host cadaver. This offers protection to their nematode counterparts from being consumed by other organisms [13]. Controls from both *in vivo* and *in vitro* tests may provide some evidence in support of this. When*G. mellonella*larvae were exposed to non-luminescent *in vitro* cultures of *P. luminescens* (Phase II), larvaewere found to have an average distance (~50 mm) from the non-luminescent culture.On the other hand,*in vivo* control larvae exposed todead non-luminescent larvae were found at the furthest distance away from the cadaver (~100 mm).

5. Conclusion and Recommendations

The results of this experiment demonstrate that bioluminescence produced by *P. luminescens* can attract healthy insect larvae towards an infected host. This is evidenced by the employment of *G. mellonella* larvae and measuring the distancebetween luminescent cultures and larval insect heads as a relation to increasing luminosity. The results of bioluminescence enable a greater probability that healthy larvae will become infected by attraction to the infectious host carcass. This experiment concludes that bioluminescence produced by *P. luminescens*may be used to attract new insect hosts and as a consequence, *H. bacteriophora* nematodes benefit by the reduction of distance to infect another insect host.Further studies are required to determine the nature of the relationship between the attraction and repulsion of *G. mellonella*to bioluminescence and secreted bacterial compounds, respectively, produced by *P. luminescens.*

There are two mechanisms by which *Galleria* can be infected. Cruisingnematodesmay utilizebioluminescence to expand its range.This study certainly demonstrates that *P. luminescens* will attract and infect *G. mellonella.* However, the study does not eliminate the possibility that *Galleria*may also become infected through contact with other infected larvae (dead or alive).Further study may provide evidence that bioluminescent surface-sanitized *G. mellonella*canalso infect insect larvae.

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References

[1] Georgis R, Gaugler R (1991) Predictability in biological control using entomopathogenic nematodes.J Econ Entomol 84:713-720

[2] Ehlers RU (1992)Environmental and biotic factors influencing the control potential of entomopathogenic nematodes of the genus *Steinernema* and *Heterorhabditis*. In:Gommers F J, Mass PWT (eds) Nematology from Molecule to Ecosystem. European Society of Nematologists,UK, pp201-212

[3] Ciche TA, Darby C, Ehlers RU,ForstS, Goodrich-Blair H (2006)Dangerous liaisons: The symbiosis of entomopathogenic nematodes and bacteria. Biol Control 38:22–46

[4] Fischer-Le SauxM, Viallard V, Brunel B, Normand P, Boemare NE(1999)Polyphasic classification of the genus Photorhabdus and proposal of new taxa: P. luminescens subsp.luminescens subsp. nov.,P. luminescens subsp. akhurstii subsp. nov., P. luminescens subsp.laumondii subsp. nov., P. temperata sp. nov., P. temperata subsp. temperata subsp. nov.and P. asymbiotica sp. nov. Int J SystEvolMicr49:1645–1656

[5] Hinchliffe S, Hares M, Dowling A, ffrench-Constant RH (2010)Insecticidal Toxins from the *Photorhabdus* and *Xenorhabdus*bacteria. Open Toxicol J 3:101-118

[6] DabornPJ, Waterfield N, Silva CP, Au CPY, Sharma S, ffrench-ConstantR H(2002)A single *Photorhabdus* gene, makes caterpillarsfloppy (*mcf*), allows *Escherichia coli* topersist within and kill insects. P NatlAcadSci USA 99:10742-10747

[7] Hu K and Webster J (2000) Antibiotic production in relation to bacterial growth and nematode development in Photorhabdus Heterorhabditis infected Galleria mellonella larvae. FEMS Microbiology Letters 189:219-223

[8] Pande C, Deng H, Rath P, Callender RH, Schwemer J(1989) Resonance raman spectroscopy of an ultraviolet-sensitive insect rhodopsin.Biochemistry-US 26:7426-7430

[9] Welham P, Stekel D(2009)Mathematical model of the lux luminescence system in the terrestrial bacterium *Photorhabdusluminescens*. Mol Biosyst5:68-76

[10] Briscoe A, Bernard G (2009) Eye shine and spectral tuning of long wavelength-sensitive rhodopsins: no evidence for red-sensitive photoreceptors among five *Nymphalini* butterfly species. J ExpBiol208:687-696

[11] Inman III FL, Holmes LD (2012)The effects of trehalose on the bioluminescence and pigmentation of the phase I variant of *Photorhabdus luminescens.* J Life Sci6:119-129

[12] Inman III FL, Singh S, Holmes LD (2012) Mass production of the beneficial nematode *Heterorhabditis bacteriophora* and its bacterial symbiont *Photorhabdus luminescens*. Indian J Microbiol 52:316-324

[13] Zhou X, Kaya HK, Heungens K, Goodrich-Blair H (2002) Response of ants to a deterrent factor(s) produced by the symbiotic bacteria of entomopathogenic nematodes. Appl Environ Microbiol 68:6202-6209