International Journal of Medical Science and Clinical Research Studies

ISSN(print): 2767-8326, ISSN(online): 2767-8342

Volume 03 Issue 03 March 2023

Page No: 302-305

DOI: https://doi.org/10.47191/ijmscrs/v3-i3-04, Impact Factor: 6.597

Phosphorescence and Potential Antibiosis Secondary to *Photorhabdus Luminescens* Wound Contaminations at the Battle of Shiloh, Tennessee 1862

E. Scott Sills[1,2]

[1]Center for Advanced Genetics/FertiGen; San Clemente, California 92673 USA

[2]Fertility Reserve Bank, Regenerative Biology Group; Harriman, Tennessee 37748 USA

ABSTRACT

During the American Civil War, contemporary accounts describe a peculiar luminescence phenomenon associated with untreated injuries sustained by fallen troops after the Battle of Shiloh (6-7 April 1862). While not experienced by all soldiers, field surgeons observed that the glow was somehow linked to reduced rates of wound infection and better outcomes. Given the wet springtime weather and unsheltered overnight conditions of the injured, it is plausible that some lacerations or punctures were incidentally infected with microscopic contaminants, including common soil nematodes. One endemic group likely present on riverbank groundcover is fam. *Heterorhabditidae*, a symbiotic host to *Photorhabdus luminescens.* Classified within the *Morganellaceae*, this Gram-negative pathogen is characterized by its unusual life cycle. With particular relevance to Shiloh, this sequence includes a blue-green phosphorescence phase accompanied by release of protein toxins having local antibiotic properties. Here, natural progression of this microbe from nematode symbiont to lethal secondary insect parasite lodged in an unclean wound is considered. *Photorhabdus* life-cycle is discussed as a component of the Martin-Curtis Hypothesis (2001), with new information on fluorophore isolation and recent genomic sequencing data. The Shiloh campaign is also placed within the context of the larger Western Theatre, noting that the engagement marked the heaviest Civil War casualty toll up to that point.

ARTICLE DETAILS

Published On:
03 March 2023

KEYWORDS: bioluminescence; U.S. Civil War; wound infection; nematode; *Photorhabdus luminescens*

Available on:
https://ijmscr.org/

INTRODUCTION

Over two days in early April 1862, the U.S. Civil War Battle of Shiloh was fought near a steamboat dock in rural west Tennessee (see Figure 1). As springtime rains covered the South, Union troops sought to dominate the Tennessee and Cumberland riverways. Kentucky was already firmly under Union control and most of Tennessee was also occupied, including Nashville. A battle was inevitable, since control of the region would provide a major strategic attainment to the victor. Indeed, Shiloh was the scene where some 24,000 were killed or wounded, delivering a shocking prelude to both sides regarding what would come later in the war.

Figure 1. Major riverways and Battle of Shiloh (red) located on the west Tennessee River bank, north of the Mississippi—Tennessee state line.

Before sunrise on April 6th the Confederates made a surprise attack on a nearby Union camp, pushing them back to a makeshift trench. Although CSA troops assailed the Federal line, the charge stalled and was unable to break Union defenses. Importantly, Senior Confederate General Albert Sydney Johnston—a key figure and chief organizer of the entire campaign—was fatally wounded during the ensuing mayhem, resulting in overall command devolving to Gen. P.G.T. Beauregard.

Under new generalship, the vast extent of Confederate losses was surveyed and the original plan was put under caution. Gen. Beauregard recalibrated, and called off the attack. Because Gen. Grant soon received fresh Ohio reinforcements to strengthen his position, the South's decision proved fateful. The next morning, Union troops massed their own surprise action with overwhelming force, securing victory. The defeat at Shiloh permanently ended the Confederates' influence in the Western Theatre [1].

By April 7th when all the fighting was done, temperatures were dropping in the damp field where countless wounded soldiers were scattered. As darkness came, several troops noticed some open wounds began to emit a faint blue-green light. Army doctors were puzzled how this peculiar glow seemed to presage better outcomes; wounds with this feature had fewer complications & lower infection rates. Although widespread acceptance of germ theory or the ability to assess would cultures was still years away, it may be that fallen soldiers nevertheless gained from contamination by endemic luminescent bacteria with antibiotic action. Martin & Curtis (2001) were the first to propose *Photorhabdus luminescens* as the biological mediator for what was initially unexplained folklore [2].

At Shiloh, the most notable battlefield casualty was Confederate Maj. Gen. Albert Sidney Johnston (1803-1862), as Rebel forces were deprived of a talented strategist and revered leader. As one of the original five senior Generals directly commissioned by Confederate President Jefferson Davis, Johnston was the highest-ranking officer to die in battle during the entire war, on either side. Despite the Martin-Curtis Hypothesis of useful microbiology at work after Shiloh, far more than antibiotics would have been needed to help Johnston survive his catastrophic gunshot wound and likely hemorrhagic shock [3].

LIFE-CYCLE STRATEGY & MOLECULAR TACTICS

Perhaps *P. luminescens* most notable characteristic is its obligate, symbiotic relationship with the soil nematode family *Heterorhabditidae*. When the nematode is eaten by an insect, *P. luminescens* releases toxins which are fatal to the ultimate host, but not the nematode. In addition, lytic enzymes are produced which convert insect tissue into nutrients which can be utilized both by nematode and bacteria to facilitate reproduction & growth. These substances block invasion of the host insect by competitors, becoming visible in dark environments by bioluminescence, a display which might lure other insects to consume the nematodes in which it lives.

In this sequence, *P. luminescens* must both continue within the gut of its (nematode) carrier but eventually neutralize the secondary insect host. This two-stage lifestyle presumably involves a switch between a nematode-symbiotic then insect-pathogenic state, and virulence factors have been deduced to enable these functions [4]. Of note, no other organism has been found to exhibit this dual, nested host phenotype—symbiotic with one while pathogenic for another [5,6] (see Figure 2).

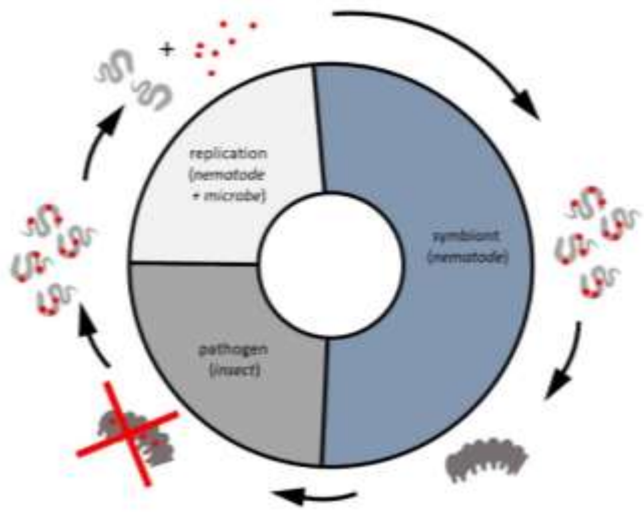

Figure 2. *Photorhabdus luminescens* (red) begins in symbiont mode within the gut of entomopathogenic nematodes fam. *Heterorhabditidae*. When larvae/insects later consume these worms, the microbe quickly kills and converts this (X) to nutrients to benefit itself and the nematode host.

The *P. luminescens* genome encodes a set of high molecular weight toxins which appear evolutionarily favored to supply a natural insecticide [7]. Unlike any other known sequence, the protein triggers diffuse and rapid apoptosis throughout the insect gut and its hemocytes [8]. Subsequent research has isolated multiple toxins consistent with destructive operations via multiple insecticidal actions with functional redundancy [9]. Given that consumption of the insect host is essential for successful release of new infective juvenile nematodes, these possibilities seem reasonable [8]. While usually intolerant of normal human body temperature or other warm environments, *P. luminescens* may have opportunistically encountered a damp April night after the Battle of Shiloh, providing permissive conditions for superficial infection as well as the reported glow.

This unusual light emission associated with *P. luminescens* has been directly observed under controlled culture conditions [10]. While the organism does produces proteins toxic to insects [11], *Photorhabdus* extracts also strongly inhibit growth of Methicillin-resistant *Staphylococcus aureus* [12]. Regarding the molecular mechanism responsible for the glow, expression of *lux* operon is directed by a promoter expressed constitutively to activate housekeeping factors inside the bacterium [13]. The distinctive blue-green glow derives from lumazine activation, a protein with a non-covalently bound fluorophore, 6,7-dimethyl-8-ribityllumazine which reacts with the α-subunit of luciferase [14]. Recently this protein was cloned upstream of the *lux* operon in *P. luminescens* [13]. Bioluminescent signaling is seen in other species as warning in the larvae stage, to convey information on unpalatability to potential predators [15]. Pulsed light flashes are also employed by adult insects in seasonal courtship [16,17]. Earlier this year, a previously unreported *Photorhabdus* species was discovered from *Heterorhabditis* using comparative genomic analysis [18].

CONCLUSION

The glow effect noted after this battle appears to stand as a unique event—there are no reports of anything comparable to Shiloh at other Civil War engagements. Because innumerable nematodes and microbes would be present at many battlefields, the convergence of other variables to give the result at Shiloh invites speculation. Climate factors may have favored robust overgrowth of soil nematodes, increasing infection risk for unbandaged wounds in prolonged contact with wet ground. The role of chance occurrence is always impossible to measure, especially in the frenzy of war. Likewise, nature can prove consequential in the painful calmness following fierce fighting—as at Shiloh.

FUNDING

This project received no grant support from public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

None.

ETHICAL APPROVAL

Not applicable.

REFERENCES

I. Groom W. Why Shiloh matters. The New York Times [*newspaper*] April 6, 2012 doi:https://archive.nytimes.com/opinionator.blogs.nytimes.com/2012/04/06/why-shiloh-matters/

II. Durham S. Students may have answer for faster-healing Civil War wounds that glowed. U.S. Department of Agriculture Research Service; May 29, 2001 [*newsletter*] doi:https://web.archive.org/web/20010617013104/http://www.ars.usda.gov/is/pr/2001/010529.htm

III. Anderson J, Peace D, Okun MS. Albert Sidney Johnston's sciatic dueling injury did not contribute to his death at the Battle of Shiloh. Neurosurgery 2008;63(6):1192-7. doi:http://dx.doi.org/10.1227/01.NEU.0000327687.45271.45

IV. Liu J, Berry RE, Blouin MS. Identification of symbiotic bacteria (*Photorhabdus* and *Xenorhabdus*) from the entomopathogenic nematodes *Heterorhabditis marelatus* and *Steinernema oregonense* based on 16S rDNA sequence.

V. J Invertebr Pathol 2001;77(2):87-91. doi: http://dx.doi.org/10.1006/jipa.2001.5007

VI. Forst S, Dowds B, Boemare N, Stackebrandt E. Xenorhabdus and Photorhabdus spp.: Bugs that kill bugs. Annu Rev Microbiol 1997;51:47-72.

VII. doi:http://dx.doi.org/10.1146/annurev.micro.51.1.47

VIII. Chaston JM, Suen G, Tucker SL, Andersen AW, Bhasin A, Bode E, et al. The entomopathogenic bacterial endosymbionts *Xenorhabdus* and *Photorhabdus*: Convergent lifestyles from divergent genomes. PLoS One 2011;6(11):e27909. doi:http://dx.doi.org/10.1371/journal.pone.0027909

IX. Waterfield NR, Bowen DJ, Fetherston JD, Perry RD, ffrench-Constant RH. The tc genes of *Photorhabdus*: A growing family. Trends Microbiol 2001;9(4):185-91. doi:http://dx.doi.org/10.1016/s0966842x(01)01978-3

X. Daborn PJ, Waterfield N, Silva CP, Au CP, Sharma S, Ffrench-Constant RH. A single Photorhabdus gene, makes caterpillars floppy (mcf), allows Escherichia coli to persist within and kill insects. Proc Natl Acad Sci USA 2002;99(16):10742-7. doi: http://dx.doi.org/10.1073/pnas.102068099

XI. ffrench-Constant RH, Dowling A, Waterfield NR. Insecticidal toxins from *Photorhabdus* bacteria and their potential use in agriculture. Toxicon 2007;49(4):436-51. doi:http://dx.doi.org/10.1016/j.toxicon.2006.11.019

XII. Gerrard JG, McNevin S, Alfredson D, Forgan-Smith R, Fraser N. Photorhabdus species: Bioluminescent bacteria as emerging human pathogens? Emerg Infect Dis 2003;9(2):251-4. doi: http://dx.doi.org/10.3201/eid0902.020222

XIII. Ensign JC, Bowen DJ, Petell J, Fatig R, Schoonover S, ffrench-Constant RH et al. Insecticidal protein toxin from *Photorhabdus*. Japan Patent Office 2005: JP3657593B2.doi:https://patents.google.com/patent/JP2004089189A/en?inventor=Jerald+C+Ensign

XIV. Muangpat P, Meesil W, Ngoenkam J, Teethaisong Y, Thummeepak R, Sitthisak S, et al. Genome analysis of secondary metabolite-biosynthetic gene clusters of *Photorhabdus akhurstii* subsp. *akhurstii* and its antibacterial activity against antibiotic-resistant bacteria. PLoS One 2022;17(9):e0274956. doi:http://dx.doi.org/10.1371/journal.pone.0274956

XV. Wellawa DH, Lam PS, White AP, Allan B, Köster W. Characterization of colonization kinetics and virulence potential of *Salmonella enteritidis* in chickens by photonic detection. Front Vet Sci 2022;9:948448. doi: http://dx.doi.org/10.3389/fvets.2022.948448

XVI. Sato Y, Shimizu S, Ohtaki A, Noguchi K, Miyatake H, Dohmae N, et al. Crystal structures of the lumazine protein from *Photobacterium kishitanii* in complexes with the authentic chromophore, 6,7-dimethyl- 8-(1'-D-ribityl) lumazine, and its analogues, riboflavin and flavin mononucleotide at high resolution. J Bacteriol 2010;192(1):127-33. doi: http://dx.doi.org/10.1128/JB.01015-09

XVII. Kusy D, He J, Bybee SM, Motyka M, Bi W, Podsiadlowski L, et al. Phylogenomic relationships of bioluminescent elateroids define the 'lampyroid' clade with clicking *Sinopyrophoridae* as its earliest member. Syst Entomol 2021;46:111-23. doi:https://resjournals.onlinelibrary.wiley.com/doi/10.1111/syen.12451

XVIII. Owens ACS, Lewis SM. Artificial light impacts the mate success of female fireflies.R Soc Open Sci 2022;9(8):220468. doi: http://dx.doi.org/10.1098/rsos.220468

XIX. Yang L, Zhao Z, Luo D, Liang M, Zhang Q. Global metabolomics of fireflies (*Coleoptera: Lampyridae*) explore metabolic adaptation to fresh water in insects. Insects 2022;13(9):823. doi: http://dx.doi.org/10.3390/insects13090823

XX. Castaneda-Alvarez C, Machado RAR, Morales-Montero P, Boss A, Muller A, Prodan S, et al. *Photorhabdus antumapuensis* sp. nov., a novel symbiotic bacterial species associated with *Heterorhabditis atacamensis* entomopathogenic nematodes. Int J Syst Evol Microbiol 2022;72(10). doi: http://dx.doi.org/10.1099/ijsem.0.005525