THIRD NEMASYM MEETING

"PROTEOMES AND GENOMES FOR THE STUDY OF EUKARYOTE-PROKARYOTE SYMBIOSES"

Oregon State University
LaSells Stewart Center

Corvallis, Oregon
July 16-17, 2011

National Science Foundation
WHERE DISCOVERIES BEGIN

NemaSym
A Research Coordination Network for the Study of Nematode-Bacterium Symbioses

PROGRAM AT GLANCE

Friday July 15th

Evening

Gather in the lobby of the Hilton Garden Inn at 6.30PM for evening dinning at a local restaurant (TBA).

Saturday July 16

LaSells Stewart Center, Agriculture Production Room
Oregon State University

Morning

8.00–8.45	Registration
9.00–9.15	Opening Remarks
9.15-10.00	Invited Keynote Speaker- ***Silvia Bulgheresi, University of Vienna, Austria***
10.00-10.15	**Coffee break**
10-15-12:00	Oral Presentations I
12:00-12.30	Round Table Discussion
12.30-1.50	Lunch (on your own) at local restaurants

Afternoon

2.00-3.00	Invited Keynote Speaker- ***Paul Sternberg, California Institute of Technology, USA***
3.00-3.30	**Coffee break**
3.30-5.30	Oral Presentations II
5.30-6.30	Round Table Discussion

Sunday July 17

LaSells Stewart Center, Agriculture Production Room
Oregon State University

Morning

8.00-10.00	Oral Presentations III
10:00-10.30	**Coffee break**
10.30-11.00	Round Table Discussion
11.00-12.00	Closing remarks. Plans for 2012 activities
12.00	Meeting adjourned

Monday July 18

Location TBA

Evening

6.00-7.00 **SON Poster Session I**

Tuesday July 19

Location TBA

Afternoon

1:00-2:45 **SON NEMASYM SYMPOSIUM.** "Synergistic and antagonistic interactions between nematodes and bacteria"

DETAILED PROGRAM

Saturday July 16 - Morning

9:00–9:15		**Opening Remarks**
9:15-10.00	Silvia Bulgheresi	**Invited Keynote Speaker**
10:00-10:20		**Coffee break**
10:20-10:40	Peter Di Gennaro	Host biology places constraints on parasitic strategies
10:40-11:00	Kristen Murfin	*Steinernema feltiae – Xenorhabdus bovienii* specificity
11:00-11:20	Barton Slatko	A laterally transferred ferrochelatase gene is functional and essential in filarial nematode parasites
11:20-11:40	Elena Melnikow	The involvement of *Brugia malayi* cathepsin-like cysteine proteases in the endosymbiotic interaction with *Wolbachia*
11:40-12:00	Elodie Ghedin	*B. malayi* spliced variants of an ORFan protein that interacts with a *Wolbachia* surface protein
12:00-12:30		**Round Table Discussion**
12:30-2:00		**Lunch Break**

Saturday July 16 – Afternoon

2:00-3.00	Paul Sternberg	**Invited Keynote Speaker**
3:00-3:30		**Coffee break**
3:30-3:50	Sujai Kumar	Novel ways of extracting complete *Wolbachia* genomes from second-gen nematode genome sequencing projects
3:50-4;10	Sammy Sedky	Evidence of *Wolbachia* endosymbionts present in new nematode genomes
4:10:4:30	David Clarke	Genomic analysis of mutualism in *Photorhabdus luminescens*
4:30-4:50	Sheldon Hurst	Elucidation of the *Photorhabdus temperata* genome and comparative genomics
4:50-5:10	Swati Singh	Genomic and biological analysis of the role of antibiotics in the life cycle of the entomopathogen *Xenorhabdus nematophila*
5:10-5:40		**Round Table Discussion**

Sunday July 17- Morning

8:00-8:20	Hillel Schwartz	Establishing Heterorhabditis nematodes as a system for molecular genetics
8:20-8:40	Brian Darby	Bacterial-responsive transcriptomes of rhabditid soil nematodes
8:40-9:00	Todd Ciche	Global change in *Photorhabdus luminescens* gene expression in symbiotic vs. pathogenic forms of the bacteria
9:00-9:20	Jason Crawford	Rhabduscin is an immunosuppressant localized to the periphery of bacterial cells to evade the phenoloxidase-mediated immune response of its animal host
9:20-9:40	Lou Tisa	Identification of *Photorhabdus temperata* Mutants Altered in Nematode Symbiosis and/or Insect Pathogenesis
9:40-10:00	Heidi Goodrich-Blair	Phenotypic variation and host interactions of the *Steinernema jollieti* symbiont *Xenorhabdus bovienii*
10:00-10.30		**Coffee break**
10.30-11.00		**Round Table Discussion**
11.00-12.00		**Closing remarks. Plans for 2012 activities**
12.00		**Meeting adjourned**

ABSTRACTS

1. Host biology places constraints on parasitic strategies

Peter DiGennaro[1,2], Elizabeth H. Scholl[1]and David McK Bird[1,2,3]

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It is well established that during their evolutionary history, plant parasitic nematodes (PPN) acquired prokaryotic functions *via* horizontal gene transfer. These inter-kingdom exchanges, which imbued PPN with a range of novel (for animals) enzymatic functions, most likely reflect the legacy of ancient nematode-bacterial symbioses, and presumably were pivotal in permitting PPN to form symbioses with plants. Like all symbionts, PPN must function within the constraints defined by the biology of the host, identification of these constraints is pre-requisite to fully understand the interaction. This is particularly true for those PPN (such as root-knot nematode: RKN: *Meloidogyne* spp.) where the symbiotic interaction culminates in a fundamental re-direction of host cell development. The classical view of plant development emphasizes the role of secondary-metabolite hormones (auxins, cytokinins, ethylene, etc.), but it recently has been discovered that small peptides also have a central role in plant development. These peptides permit long- and short-range cell-to-cell communication and may function to integrate the concerted action of the classical hormones. We hypothesize that the biology regulated by plant peptide hormones may represent a constraint on the mechanism(s) available to PPN for them to form an intimate symbiotic relationship with their host. Further, we postulate that the PPN with more intricate plant interactions might directly exploit peptide hormones to establish the parasitic symbiosis. Consistent with this, bioinformatic analysis of the RKN genomes identified multiple families of genes encoding plant peptide hormones. Close examination of plant and nematode peptide hormone revealed unique structural motifs. An algorithm based on these defining characteristics was used to screen available PPN genomes revealing correlation between symbiotic complexity and peptide hormone gene complement. This information is guiding functional analyses.

2. *Steinernema feltiae - Xenorhabdus bovienii* specificity

Kristen E. Murfin[1], Steven Forst[2], S. Patricia Stock[3] and H. Goodrich-Blair[1]

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Gram-negative bacteria of the *Xenorhabdus* genus are beneficial symbionts of entomopathogenic *Steinernema* spp. nematodes. The genetics and processes of the mutualism between *X. nematophila* and *S. carpocapsae* have been extensively studied. However, much remains to be learned about trends of co-adaption of *Steinernema* nematodes with their *Xenorhabdus* partners, particularly with *Xenorhabdus* that have broader nematode host ranges. To begin to characterize the specificity of the nematode *Steinernema feltiae* with its bacterial symbiont, *Xenrorhabdus bovienii*, I have performed *in vitro* cultivations of three strains of *S. feltiae* with nine strains of *X. bovienii* symbionts. These assays indicate that eight of nine bacterial strains are able to support the growth and development and colonize the infective

stage of all three nematode strains. Further testing indicates that the strain that is unable to support the *S. feltiae* nematode life cycle may be toxic to *S. feltiae* nematodes. Our current research is aimed to characterize the bacterial factor that is contributing to this phenomenon.

3. A Laterally Transferred Ferrochelatase Gene is Functional and Essential in Filarial Nematode Parasites

Barton E. Slatko[1], Bo Wu[1], Jeremy M. Foster[1] Jacopo Novelli[1, 2], Daojun Jiang[3], Harry A. Daily[4] and Frederic Landmann[5]

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Species in the phylum Nematoda lack a heme biosynthetic pathway and require exogenous heme. Many filarial nematodes contain an obligate endosymbiont, *Wolbachia,* which has a functional heme biosynthesis pathway. Sequencing of the human filarial nematode *Brugia malayi* revealed a genomic ferrochelatase (BmFc) gene, the terminal step in heme biosynthesis. The BmFc gene contains 9 exons spanning ~ 4.5 kb and includes a mitochondrial-targeting domain. The ferrochelatase is functional based upon genetic studies (complementation to an *E. coli* hemH$^-$ mutant and as a transgene in *C. elegans),* biochemical analysis (*in vitro* enzyme assays) and inhibitor studies (using a ferrochelatase–specific inhibitor and RNAi inhibition experiments in *B. malayi*). While the mitochondrial targeting domain is required for mitochondrial location, it is not required for enzyme activity. FISH reveals the BmFc gene is almost universally expressed in both male and females tissues, except for female late-stage embryos and male late stage sperm cells. Orthologues have been identified in several other filarial nematodes, as well as from non-*Wolbachia* containing species and a non-filarial nematode. Phylogenetics suggests a non-*Wolbachial*, but α-proteobacterial, origin with the lateral transfer acquisition predating the split of the Rhabditida into the Spirurina and Rhabtinia clades. This is the first reported functional LGT gene in animal or human filarial nematodes. Its requirement for worm viability suggests it could play a role in the symbiotic relationship between the filarial nematode host and its symbiont and may be a potential drug target to combat filariasis.

4. The involvement of *Brugia malayi* cathepsin-like cysteine proteases in the endosymbiotic interaction with *Wolbachia*

Elena Melnikow[1*], Tiruneh Hailemariam[1], Thomas R Unnasch[3], Elodie Ghedin[2] and Sara Lustigman[1]

[1]Lindsley F. Kimball Research Institute, New York Blood Center, New York, NY 10065 USA [2]University of Pittsburgh School of Medicine, Center for Vaccine Research, 3501 5th Avenue Pittsburgh, PA 15261 USA [3]Department of Global Health, University of South Florida, Tampa, FL 33612 USA

Wolbachia is essential for the development and reproduction of *B. malayi*, the causative agent of Lymphatic Filariasis. Our study was aimed at identifying proteins that potentially have an essential function in this endosymbiotic relationship. When the effects of tetracycline treatment on the regulation of *B. malayi* transcripts 7 and 14 days post treatment were characterized using

a whole genome microarray studies, we found that transcripts corresponding to *B. malayi* cysteine proteases were regulated. Filarial cysteine proteases are known to be involved in molting and embryogenesis, processes shown to be dependant on *Wolbachia*. To elucidate the role cysteine proteases play during this symbiosis, we studied their expression pattern after anti-*Wolbachia* treatment. A bimodal regulation pattern of transcripts encoding cysteine proteases was observed when *in vitro* tetracycline treated worms were tested. Transcripts corresponding to *Bm-cpl-3* and *Bmcpl- 6* were found to be up-regulated 1 day post treatment, unchanged or down-regulated by day 3, but then up-regulated at day 6. Using tetracycline treated infertile female worms and purified embryos we established that this bimodal pattern represents the worms' response to *Wolbachia* death in different tissues; earlier effect on embryogenesis and a later effect within the hypodermis of the adult worms. To further determine the association between cysteine protease expression and *Wolbachia*, we analyzed *Bm-cpl-5* RNAi treated worms, in which embryogenesis is affected, and found a specific reduction of *Wolbachia* in the hypodermis and microfilariae but not in the oocytes and embryos. Moreover, the transcript levels of a *Wolbachia*-specific ankyrin gene (wBm0287) were down-regulated by 10.2 fold in these RNAi treated worms. Treatment of adult female worms with a cysteine protease inhibitor, which affect embryogenesis, was shown to alter both the expression levels of *B. malayi* cysteine protease transcripts and *Wolbachia* genes. The possible role cysteine proteases play in the endosymbiosis will be further discussed.

5. *B. malayi* spliced variants of an ORFan protein that interacts with a *Wolbachia* surface protein

Alan Twaddle[1], Elena Melnikow[2], Benjamin Policicchio[1], Shulin Xu[3], Jing Liu[2], Lin Li[2], Yelena Oksov[2], Thomas Unnasch[3], Sara Lustigman[2], Elodie Ghedin[1*]

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The parasitic nematode *Brugia malayi* is a lymphatic dwelling filarial worm that infects close to 150 million people worldwide. *B. malayi* harbors an endosymbiotic intracellular bacterium *Wolbachia* that is required for the development and reproduction of the worm. Relatively little is known about *Wolbachia* proteins that interact with the filarial host and which might be important in maintaining the obligate symbiotic relationship. The *Wolbachia* surface proteins (WSPs) are members of the outer membrane protein family and we hypothesize that they are involved in the *Wolbachia-Brugia* symbiotic relationship. We have observed that some of the WSPs bind specifically to *B. malayi* crude protein extracts and have found a specific *B. malayi* protein involved in one of these interactions. We provide a detailed analysis on the variant structures of the gene encoding this hypothetical protein and on its expression profile. Tetracycline treatment of female adult worms *in vitro*, which kills the *Wolbachia*, induced upregulation of the corresponding transcript. We are targeting this gene for RNAi to determine whether any effect can be observed on the worm and the bacteria. Characterizing proteins involved in specific interactions between *B. malayi* and *Wolbachia* should lead to a better understanding of the molecular basis of the symbiosis.

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6.

Sujai Kumar and Mark Blaxter.

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Second generation sequencing has made it possible to obtain a high quality draft nematode genome for less than the cost of 4 round trip tickets from the UK to the NemaSym meeting. At the Blaxter Lab, we are currently sequencing half a dozen nematodes, three of which are filarial Onchocercidae with wolbachia endosymbionts: *Litomosoides sigmodontis, Onchocerca ochengi,* and *Dirofilaria immitis*. It is very difficult to separate the genomes of the *Wolbachia* from the nematodes at the library preparation or the raw-read stage. After much experimentation, we have developed a robust pipeline that allows us to separate *Wolbachi*a genomic data from nematode genomic data using preliminary assemblies and a combination of several factors: coverage, GC content, and differential matches to known bacterial and nematode sequences. With only one library of short-read small-insert Illumina 100bp paired-end sequencing, we can assemble the approximately 1 Mb genomes in as few as 60 contigs, with an average contig size as high as 15 kb and N50 as high as 25 kb. The addition of long-insert mate-paired libraries allows the contigs to be scaffolded into even fewer pieces. We have compared these three genome sequences with previously sequenced *Wolbachia* endosymbionts of other filarial nematodes (*Brugia malayi, Onchocerca volvulus,* and *Wuchereria bancrofti*) as well as more distantly related wolbachia of arthropods (*Drosophila* and *Culex*). These comparisons highlight the evolutionary dynamics of key proteins and other genome elements in this group of prokaryotes. The worms are being sequenced in collaboration with: David Taylor, Judith Allen, and Simon Babayan (*L. sigmodontis*); David Taylor, Judith Allen, Ben Makepeace, and Alistair Derby (*O. ochengi*); Pascal Maeser, Daniel Nilsson, and Adrian Wolstenholme (*D. immitis*).

7. Evidence of *Wolbachia* endosymbionts present in new nematode genomes

Sammy Sedky and Paul De Ley

Department of Nematology, University of California Riverside, Riverside CA, USA

Wolbachia are alpha-proteobacteria that are endosymbiotic partners of many animal species—most notably arthropods and the filarial clade of animal-parasitic nematodes. The relationship between nematode *Wolbachia* and their hosts has features of mutualism, in particular that of intracellular symbiosis. Why certain nematodes carry *Wolbachia* and how these bacteria are affecting nematode biology is poorly understood, although it is becoming apparent that these bacteria play an essential role in the lifestyle of certain nematodes. The plant-parasitic nematode, *Radopholus similis* is the first plant-parasitic nematode in which the presence of a *Wolbachia* endosymbiont was found (Haegeman, et al). We have used highly conserved *Wolbachia* genes (ftsZ, CTP synthase, groEL, GroES) to search for homologs of *Wolbachia* endosymbionts in five new nematode genomes (an unidentified oncholaimid sp., *Aphelenchus avenae*, *Pseudaphelenchus yukiae*, *Plectus aquaticus*, *Panagrolaimus* sp. PS1159). Preliminary results indicate the presence of homologs of *Wolbachia* endosymbionts in *Pseudaphelenchus yukiae* and *Plectus aquaticus* genomes.

8. Genomic analysis of mutualism in *Photorhabdus luminescens*

David J. Clarke

Department of Microbiology, University College Cork, Ireland

Photorhabdus is a genus of bioluminescent, Gram-negative bacterium belonging to the family *Enterobacteriaceae*. *Photorhabdus* is highly virulent to insect larvae whilst, at the same time, maintaining a mutualistic relationship with entomophagous nematodes of the family Heterorhabditiae. During this mutualistic interaction the bacteria must be competent to a) support nematode growth and development and b) successfully colonize the infective juvenile (IJ) stage of the nematode, in a process called transmission. We have recently shown that the TCA cycle is required in order for *P. luminescens* to be able to support nematode growth and development. Moreover the TCA cycle also controls the transition of *P. luminescens* from pathogen to mutualist suggesting that a metabolic switch plays a key role in regulating mutualism. Using transcriptomics we have characterized the changes that occur in bacterial gene expression during this metabolic switch. In addition to observing an increase in the expression of genes known to be involved in mutualism we also see a shift in the method of energy generation from aerobic respiration to fermentation. In other studies we have also identified genes that are required for transmission, including a LysR-type transcriptional regulator called HdfR. Using confocal laser scanning microscopy we have shown that the *DhdfR* mutant can colonize, and grow within, adult hermaphrodites as well as wild-type bacteria. However subsequent steps in transmission appear to be delayed with the *DhdfR* mutant suggesting a possible explanation for the defect in colonization. HdfR controls the expression of more than 100 genes in *P. luminescens*, including genes involved in arginine metabolism, the *ant* operon (required for the production of the anthraquinone pigment) and the *hpa* operon (involved in hyxroxyphenylacetate catabolism). The role(s) of these genes in transmission will be discussed.

9. Elucidation of the *Photorhabdus temperata* genome and comparative genomics

Sheldon Hurst IV, Holli Rowedder, Fesha Abebe-Akele , Hannah Bullock, and Louis S. Tisa.

Department of Molecular, Cellular and Biomedical Sciences, University of New Hampshire, Durham, NH 03824, USA.

Entomopathogenic nematodes in the family Heterorhabditidae form an obligate association with its bacterial partner members of *Photorhabdus sp.* The growth and development of this nematode has an obligate requirement for the microbial symbiont. Joined with the bacteria, the nematode actively seeks out insects to infect. After penetrating the insect, the bacteria are released within the hemocoel, where *Photorhabdus* rapidly replicates producing a multitude of virulence factors and other natural products. For three *Photorhabdus* species, genome sequences for only two species (*P. luminescens* and *P. asymbiotica*) have been established. We have determined a draft genome sequence of the third species, *P. temperata* strain NC19. The 5.2 Mb genome was organized into 17 scaffolds and has an average G + C content of 43.3% The genome encoded for 3 rRNA operons, 69 tRNAs, and 4,808 CDS. Analysis of the genome identified genes involved in important predicted functions including nonribosomal peptide synthases (NRPS), toxins, proteases, lipases, and adhesins. These data were compared to genomes of the other sequenced *Photorhabdus* genomes to provide greater insight into the symbiosis and host-pathogen associations and will be discussed. Analysis by the use of IslandViewer program predicted 42 genomic islands within the *P.temperata* genome compared to 24 and 25 for *P. luminescens* and *P. asymbiotica,* respectively.

10. Genomic and biological analysis of the role of antibiotics in the life cycle of the entomopathogen *Xenorhabdus nematophila*

Swati Singh *, Jordan Reese and Steven Forst. University of Wisconsin, Milwaukee, USA. Email: swati@uwm.edu

Xenorhabdus nematophila is an enteric bacterium that is a mutualist of the nematode *Steinernema carpocapsae* and a pathogen for numerous insect larvae. *S. carpocapsae* vectors *X. nematophila* into the insect gut and invades the body cavity (hemocoel) where both the nematode and bacteria reproduce. While *X. nematophila* produces diverse antimicrobial compounds little is known about the microbial competitors that presumably exist in the hemocoel during early infection and the role that antibiotics may play in interspecies competition. Using culture-dependent and culture-independent analyses we show that insect gut microbiota enter the hemocoel and proliferate during early stages (5-10 h) of invasion and that *X. nematophila* appears at 10 h and becomes the dominant species by 18 h. The gene cluster responsible for synthesis of xenocoumacin (Xcn), the major antibiotic of *X. nematophila,* has been identified. During infection with a Xcn-deficient strain *X. nematophila* does not become dominant until 24 h post-invasion. This delay was correlated with delay in insect mortality. In addition, the Xcn-deficient strain produced translucent colonies similar to that of virulence modulation (vmo) strain. These results suggest, for the first time, that an antibiotic of *X. nematophila* is important for optimally infecting the host and suppressing competitors, an effect that can also be attributed to its possible role in immunomodulation. We have also identified six other gene clusters in the genome of *X. nematophila* that potentially encode antimicrobial compounds. Their role in interspecies competition and host infection is presently being investigated.

11. Establishing *Heterorhabdiitis* nematodes as a system for molecular genetics

Hillel Schwartz and Paul Sternberg

Division of Biology and HHMI, California Institute of Technology, Pasadena, CA

Nematodes of the genus *Heterorhabditis* are insect-parasitic nematodes that live in mutually beneficial symbiosis with pathogenic *Photorhabdus* bacteria. *Photorhabdus* bacteria are lethal to insects and to other nematodes but are required for *Heterorhabditis* growth. The symbiosis between *Heterorhabditis* nematodes and *Photorhabdus* bacteria therefore offers the potential to study the molecular genetic basis of their cooperative relationship. We are interested in developing tools to make such studies more feasible; in particular, we have obtained and inbred independent isolates of *H. bacteriophora* and identified polymorphisms using high-throughput sequencing. We are in the process of creating a SNP map; comparison of genomic sequences of the reference *H. bacteriophora* strain M31e and an inbred derivative of a Moldovan isolate identified approximately one SNP for every 3.5 kbp; these polymorphisms are being used to score recombinant inbred lines to place supercontigs in a genetic map. We are conducting screens for mutant *H. bacteriophora* with altered properties that could make them more useful in

a laboratory environment and with altered interactions with *Photorhabdu*s bacteria. In addition to our work with *H. bacteriophora*, we are collaborating with Rousel Orozco and Patricia Stock to determine the genomes and transcriptomes of the additional species *H. indica, H. megidis*, and *H. sonorensis* and the genomes of their endogenous *Photorhabdus symbionts*. We anticipate that these resources will enable us and the wider insect-parasitic nematode community to identify induced mutations and natural variations affecting the interactions involved in the symbiosis between *Heterorhabditis* nematodes and pathogenic *Photorhabdus* bacteria.

12. Bacterial-responsive transcriptomes of rhabditid soil nematodes

Brian J. Darby and Michael A. Herman.

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Soil bacteria are an important part of the biotic environment for bacterial feeding soil nematodes, serving as both prey and potential pathogens. Interactions between nematodes and bacteria are thought to be of significant importance to the ecological dynamics of the soil community, but teasing out the underlying biology of these interactions remains a challenge. We addressed this question by isolating four rhabditid nematodes (*Oscheius tipulae*, *Oscheius sp*. 2, *Rhabditis sp*., and *Mesorhabditis monhystera*) from soils collected at Konza Prairie Biological Station to investigate their genomic responses to diverse soil bacteria. First, we co-assembled sequences from two RNA-seq libraries, one that was normalized and sequenced using the Roche GS-FLX Genome Sequencer and another that was not normalized and sequenced using the Illumina Genome Analyzer IIx. Although most contigs were identifiable in the *C. elegans* transcriptome, some contigs that could not be attributed to bacteria or rRNA were common to both species of *Oscheius* and were possibly lineage specific genes. Second, we used digital gene expression analysis to test the genomic response of nematodes to feeding on certain bacteria. Sequences from the Illumina GAIIx run were from libraries that had been prepared from nematodes that were grown on one of six bacteria (three gram-negative: *E. coli* OP50, *Pseudomonas* sp., *Stenotrophomonas maltophilia*, and three gram-positive: *Bacillus subtilis, B. megaterium*, and *B. thuringiensis*), in duplicate. Reads from each library were tagged with a unique barcode sequence to identify their treatment and replicate, and they were mapped against the hybrid assemblies as a digital estimate of transcript abundance (i.e. "gene expression"). These transcriptome sequences will complement genomic information available within the Rhabditidae family and be useful in comparative genome studies. Ultimately, our studies will help us understand the gene functions involved in the formation and maintenance of dynamic soil nematode communities in changing environments.

13. Rhabduscin is an immunosuppressant localized to the periphery of bacterial cells to evade the phenoloxidase-mediated immune response of its animal host

Jason M. Crawford,* Cyril Portmann,* and Jon Clardy.

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* Equal contributions

Photorhabdus luminescens and *Xenorhabdus nematophila* are entomopathogenic Gammaproteobacteria that produce the glycoside rhabduscin, an aminoglycosyl- and vinyl-isocyanide-functionalized tyrosine derivative. The bacteria are in a mutualistic partnership with nematode hosts and together infect a broad range of insect larvae, making the duo an effective biocontrol agent for agricultural applications. We found that rhabduscin is a potent nanomolar-level inhibitor of phenoloxidase, an important component of the insect's innate immune system. The producing bacterial cells themselves effectively inhibit phenoloxidase. If left uninhibited, phenoloxidase's natural function is to synthesize a defensive melanin barrier on and around the invading cells, which entraps the bacteria in nodules and exhibits bactericidal activity. The vinylisocyanide biosynthetic genes, *isnA* and *isnB*, and the glycosyltransferase gene were deleted in *X. nematophila* to investigate the cell's inhibitory activity. A scar-less deletion mutant excising *isnA* and *isnB* destroyed rhabduscin production and cellular inhibition. However, deletion of the glycosyltransferase led to aglycone intermediate accumulation, which inhibited phenoloxidase to near wildtype levels but increased cellular toxicity to the host bacterium. Using a precursor-directed biosynthetic strategy, a synthetic vinyl-azide or a vinyl-nitrile mimic enabled bypass of the native *isnA-isnB* product, which was accepted by the downstream glycosyltransferase. These products showed nearly a one million-fold drop in inhibitory activity, indicating that the isocyanide functional group was critical for phenoloxidase inhibition. Furthermore, the azide mimic was used as a chemical handle to investigate the localization of the small molecule on or in the bacterial cell. Fluorophores were linked to the azide mimic *in vivo* using click chemistry, revealing that the small molecule mimic was localized to the periphery of the bacterial cell. It is likely that this peripheral localization increases the effective concentration of the immunosuppressant directly at the site most needed to provide the bacteria with a competitive advantage for evading the insect's innate immune system.

14. Global change in *Photorhabdus luminescens* gene expression in symbiotic vs. pathogenic forms of the bacteria.

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Heterorhabditis bacteriophora is associated with *Photorhabdus luminescens* bacteria in an entomopathogenic symbiosis and also is closely related to *Caenorhabditis elegans*. Infective juvenile stage nematodes harbor symbiotic bacteria in their intestines, sometimes for many months while searching for an insect meal, before regurgitating the bacteria into insect blood. Genome sequences along with forward and reverse genetics are available for host and symbiont. Maternal nematodes acquire the symbionts as a persistent biofilm attached to the posterior intestine. Persistent biofilm cells are transmitted to infective juveniles developing inside of and ultimately consuming the maternal nematode. Screening for symbiont genes required for transmission revealed that Mad fimbriae are essential. A minority of symbiont cells express Mad fimbriae essential for initiating symbiosis as a persistent biofilm. Expression requires inversion of the *madswitch* promoter from the off orientation of most wild type cells to the on orientation of cells initiating symbiosis. Cells initiating symbiosis (S-form) are small (ca. 1/8 vol) and develop small colonies compared to the wild type (P-form). Locking the *madswitch* on locked the S-form that otherwise was severely outcompeted by P-form revertants in culture. Repressing many

phenotypes associated with *Photorhabdus* such as insect virulence, bioluminescence and antibiotic production, the S-form differentially expresses more than 10% genes. The S-form has decreased expression of crystalline inclusion proteins, luciferase, protease, stilbene antibiotic biosynthesis and several Tc toxin genes. The S-form has increased expression of CRISPR associated sequences (CAS), Mad fimbriae, putative Type 6 secretion system and *hexA* genes. In summary, drastic changes in global gene expression are caused by a single 257 bp inversion of the *madswitch* promoter.

15. Identification of *Photorhabdus temperata* mutants altered in nematode symbiosis and/or Insect pathogenesis

Holli Rowedder, Hannah Bullock, Christine Chapman, Cintia Felix, Jon Gately, Sheldon Hurst IV, and Louis S. Tisa.

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The entomopathogenic nematode *Heterorhabditis bacteriophora* forms a specific mutualistic association with its bacterial partner *Photorhabdus temperata*. For the nematode, growth and development have an obligate requirement for the microbial symbiont, and recognition is strain-specific. We are interested in identifying genes involved in insect pathogenesis and nematode symbiosis and have used a genetic approach to address this question. Previously we generated a bank of 10,000 transposon mutants and identified 86 motility mutants that were isolated for further study. Besides motility defects, the mutant library has been screened for three other properties: (1) hemolysin activity, (2) antibiotic production, and (3) altered surface properties via a calcoflour dye binding assay. The motility mutants were screened for insect pathogenesis by the insect injection assay with host model, *Galleria mellonella* and for mutualism, qualitatively by an *in vitro* nematode development assay. Preliminary screening identified 14 and 8 mutants with altered insect pathogenesis and symbiosis, respectively. From insect pathogenesis assays using different MOI values, two mutants (UNH5832 and UNH8309) showed an enhanced pathogenesis response compared to the parental wild-type, while two other mutants (UNH1307 and UNH6441) exhibited a delayed response that was not related to growth rate differences. Further symbiosis assays yielded two mutants with alternative symbiosis properties: UNH6427 showed delayed nematode growth, and UNH5832 accelerated nematode development. All of these verified mutants have been classified as defective motility mutants, except for one which is a hyperswarmer mutant (UNH8309). The transposon insertion site in Mutant UNH1307 causing delayed pathogenesis was identified as an RNaseII defect. Genetic complementation of the mutant UNH1307 restored wild type activity including motility, virulence and antibiotic production.

16. Phenotypic variation and host interactions of the *Steinernema jollieti* symbiont *Xenorhabdus bovienii*

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Xenorhabdus bovienii bacteria reside in the intestine of the infective-juvenile (IJ) stage of the entomopathogenic nematode, *Steinernema jollieti.* The recent sequencing of the *X. bovienii* genome facilitates its use as a model to understand host-symbiont interactions. To provide a biological foundation for such studies, we characterized *X. bovienii* in vitro and host-interaction phenotypes. Within the nematode host *X. bovienii* was contained within a membrane bound envelope that also enclosed the nematode-derived intravesicular structure. *S. jollieti* nematodes cultivated on mixed lawns of *X. bovienii* expressing green or DsRed fluorescent proteins were predominantly colonized by one or the other strain, suggesting the colonizing population is founded by a few cells. *X. bovienii* exhibits phenotypic variation between orange-pigmented primary form and cream-pigmented secondary form. Each form can colonize IJ nematodes when cultured *in vitro* on agar. However, IJs did not develop or emerge from *Galleria mellonella* insects infected with secondary form. Unlike primary-form infected insects that were soft and flexible, secondary-form infected insects retained a rigid exoskeleton structure. *X. bovienii* primary and secondary form isolates are virulent toward *Manduca sexta* and several other insects. However, primary form stocks present attenuated virulence, suggesting that *X. bovienii,* like *X. nematophila* may undergo virulence modulation.

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17. NEMASYM: Nematode-bacterium Symbioses Research Coordination Network

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Numerous laboratories worldwide are currently dedicated to the study of nematode-bacteria associations. The themes for studying such associations have been dictated mostly by nematode trophic groups or parasitic associations (including plant-parasitic nematodes and bacteria associations, filarid nematodes and *Wolbachia*, entomopathogenic nematodes and symbiotic bacteria, free-living bacterivore nematodes, etc). The diversity of interactions between nematodes and bacteria provides an unprecedented opportunity to use a comparative approach to understand fundamental processes underlying the inter-dependency of nematodes and bacteria as a model system for other eukaryote-prokaryote symbioses. Furthermore, many nematode-bacteria symbiotic partnerships offer excellent opportunities for their development as model systems that can be studied mechanistically and from both sides of the partnerships. However, to achieve these worthy goals we must remove historical divisions separating scientists, promote collaborations and communication between them, and facilitate application of the tools and perspectives of multiple disciplines. Given the multiplicity and diversity of orientations in the field of 'Nematode-Bacteria Symbioses', there is a need for increased communication among researchers favoring exchange of data, sharing of methodology, and proactive identification and pursuit of the major questions in the field. In this presentation I will summarize current goals and activities being developed by NEMASYM, a research coordination research network that fosters growth and advancement of the field of "nematode-bacterium symbioses".

18. Do mucin-like molecules have a role in the attachment of *Pasteuria penetrans* to the cuticle of plant-parasitic nematodes?

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Mucin-like molecules belong to a family of glycosylated proteins that are high in serine and threonine in which up to 85 percent of their molecular weight is made up of carbohydrate. They are produced by epithelial tissues and can be in secretions or membrane bound, functioning as lubricants and forming barriers. They also are involved in cell signalling and in immunity. In animal-parasitic nematodes they are present in the cuticle surface coat and play an important role in evasion of the host animals' immune system but very little is known about them in plant-parasitic nematodes, although mucins identified in *Toxocara canis* have putative orthologs in plant-parasitic nematodes. Mucin-like proteins have also been identified in *Caenorhabditis elegans* and RNAi knockdown of these genes has been associated with

changes of lectin staining of the nematode cuticle surface. Orthologs to mucin-like genes from *C. elegans* were identified in *M. incognita* amplified by PCR, cloned, sequenced and dsRNA synthesised by *in vitro* transcription. RNAi knockdown was performed by oral feeding, and *Pasteuria* endospore attachment assays performed at 4, 12 and 24 hours post feeding to test the hypothesis that mucin-like genes of the cuticle surface of infective juveniles have a role in endospore attachment. Endospore attachment tests revealed the orthologus mucin-like gene H43E16.1 increased endospore attachment, whereas C26G2.2 decreased endospore attachment and the effects were time dependent. Orthologus genes to K11D12 and F35E12 had no effect. The results will be discussed from the hypothetical view that a velcro-like mechanism is involved in endospore attachment and that mucin-like molecules play a role in endospore attachment and specificity.

19. Do bacterial ectoparasites modulate the predation rate of entomopathogenic nematodes in nature?

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Independent lineages of *Paenibacillus* spp. appear to have converged as ectoparasites of entomopathogenic nematodes (EPNs) in the genera *Steinernema* and *Heterorhabditis*. In Florida, a non-entomopathgenic *Paenibacillus* sp., closely related to the entomopathogens *P. lentimorbus* and *P. popilliae,* is commonly observed adhering to the cuticle of *S. diaprepesi* infective juveniles that emerge from insect prey. The bacterium is phoretic on and specific to *S. diaprepesi.* It completes its life cycle within insects killed by *Xenorhabdus doucetiae,* the entomopathogenic bacterial symbiont of *S. diaprepesi.* The presence of *Paenibacillus* sp. does not affect the development rate or population size of *S. diaprepesi* in its insect host. However, steinernematid and heterorhabditid nematodes encumbered by *Paenibacillus* endospores migrated less and killed fewer insects in controlled experiments. Whether these bacteria regulate entomopathogenic nematode populations in nature is unknown. We employ real-time quantitative PCR (qPCR) in ongoing field experiments and surveys to study relationships between EPNs and potential competitors and antagonists, including *Paenibacillus* sp., in citrus orchard soils. Positive relationships between the abundance of *Paenibacillus* sp. and *S. diaprepesi* in nematode samples from geospatial ($r=0.38$, $n=54$, $P<0.01$) and temporal ($r=0.36$, $n=432$, $P<0.0001$) surveys support the likelihood of species specificity, both for the primer/probe designs and the bacterium-nematode relationship. Moreover, the temporal patterns of *Paenibacillus* sp. in two orchards on Florida's central ridge were consistent with the possibility that the bacteria may regulate numbers of *S. diaprepesi*. We detected fewer *Paenibacillus* sp. ($P<0.05$) and more *S. diaprepesi* ($P<0.05$) in plots of citrus trees managed conventionally (weekly, microjet irrigation; dry fertilizer) compared to those of trees under intensive management (daily, drip fertigation). Major differences in the soil environments of the two treatments (plant nutrients, pH, EC, soil moisture) provide factors that potentially regulate the bacterium and might be used to infer the role of *Paenibacillus* sp. in EPN population dynamics and perhaps modify soils in ways that favor biological control by EPNs.

20. Genomics of *Heterorhabditis* and *Steinernema*.

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We are analyzing genome sequences for a set of entomopathenogenic nematodes in order to facilitate molecular genetic studies, define transcript and protein sets, and assess the phylogeny of these nematodes. We have obtained genomic and cDNA sequence for *Steinernema carpocapsae*, *moticolom*, *scapterisci*, *feltiae* and *glaseri*, assembled their genomes using RNA-mediated scaffolding (Mortazavi et al. Genome Research 2009), and inferred gene sets. As an outgroup, we have assembled *Panagrellus redivivus*. We are in the process of sequencing *Heterorhabditis sonorensis* and *indica*. As part of these projects we have also sequenced associated bacteria. The state of genome annotation and biologically relevant results so far will be discussed.

21. How to be a Laxus

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The marine nematode *Laxus oneistus* (Stilbonematinae, Desmodoridae, Chromadoria) establishes a binary association with a sulfur-oxidizing gammaproteobacterium. Hypodermal glandular sensory organs (GSOs) produce the mucus the symbionts are embedded in. The molecular composition of this mucus and how it changes during worm development must play a pivotal role in partners' recognition. In order to molecularly dissect this mucus, we performed second generation sequencing of the *L. oneistus* transcriptome. This lead to the identification of putative GSO-secreted proteins that may either involved in symbiont-binding or in the killing of non symbiotic bacteria (e.g antimicrobial peptides). In order to understand their function, we are in the process of analyzing their spatial and temporal expression pattern and of expressing them in heterologous systems.

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22. Characterization and phylogenetic relationships of a new *Photorhabdus luminescens* subspecies (Gamma-Proteobacteria: Enterobacteriaceae), the symbiont of *Heterorhabditis sonorensis* (Nematoda: Heterorhabditidae)

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Photorhabdus are motile Gram-negative bacteria that have a mutualistic association with *Heterorhabditis* nematodes (Heterorhabditidae). These bacteria possess peculiar biochemical characteristics such as inability to reduce nitrates, and the capacity to ferment only a limited number of carbohydrates. *Heterorhabditis* nematodes vector the bacteria from one insect host to another and also provide shelter to the bacteria from soil stressors and antagonists. Once inside the insect host, the bacterial symbionts are released and produce toxins and secondary metabolites and broad spectrum antibiotics, that kill that the host by septicemia within 48 hrs. At present, three *Photorhabdus* spp. have been identified: *P. luminescens, P. temperata* and *P.*

asymbiotica, and many subspecies for each taxon have also been described. Characterization of new species and subspecies has been based on sequence data, mostly 16SrDNA gene, and also on a selection of protein coding genes. In addition to this, phenotypic traits including temperature growth, colony morphology, color, light production, carbohydrate response and assimilation have been considered. In this study, we characterize the bacterial symbiont of *Heterorhabditis sonorensis,* a recently discovered entomopathogenic nematode species form the Sonoran desert in Arizona. We considered a selection of classic biochemical and molecular methods including sequence data from six genes: 16s rDNA, and five protein coding genes: *ser*C, *gyr*B, recA, *glt*X, *dna*N. Evolutionary relationships of this new *Photorhabdus* subsp. was inferred considering maximum parsimony and Bayesian analyses. Results from this study indicate this bacterium has a maximum temperature growth in nutrient broth at 37 °C. Cells are Gram-negative, motile, oxidase negative, catalase, arginine dihydrolase and gelatinase positive. This subspecies reacts negatively to m- inositol, formic, propionic, galacturonic and citric acid . But it reacts positively to D-alanine. Phase I colonies are bioluminescent, granulated, convex and opaque and have a sticky consistency. Phylogenetic analyses indicate this subspecies belongs to the *luminescens* group and has *P. luminescens luminescens* as their sister taxa.

23. Diversity and distribution of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) in Egypt.

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At present, there is scatter information on the diversity and natural occurrence of entomopathogenic nematodes in Egypt. Therefore, this study reports results from a survey conducted in Egypt which considered this country's four natural geographic regions: a) northern (Mediterranean Sea coast and Nile Delta), b) middle (Nile valley), c) southern and d) Sinai Peninsula regions. These regions have different climate, soil types and crops. Overall a total of 1,000 soil samples were collected. Of them, more than 180 samples (18%) were EPN-positive. Currently, 100 isolates have been molecularly characterized to species level. ITS rDNA sequence analysis was considered as an initial screening/diagnostic approach. Molecular analysis revealed that 58% of the EPN positive samples contained nematodesin the family Heterorhabditidae and the remaining 42% of the samples yielded nematodes in the Steinernematidae. All recovered *Heterorhabditis* isolates were identified as *Heterorhabditis indica* Poinar, (Karunakar and David, 1994). Among steinernematids, isolates were placed mainly into two species: *S. abbasi* Elawad et al., 2002 and *S. carpocapsae* (Weiser, 1954). In addition to these results, one sample from Aswan governorate (southern region) yielded a *Steinernema* species that is currently under study. This nematode is a member of the *glaseri*-group and is closely related to *S. arenarium*. Species in the *glaseri*-group have been reported from Mediterranean countries including Italy, Morocco and Israel. Apparently, this isolate is a novel species, however further studies are warrant to confirm its complete identification. Surprisingly, EPN fauna in Egypt does not seem to be very diverse with respect to number of species. We speculate factors such as climate and soil types limit species diversity. However, those EPN species such as *S. abbasi* and *H. indica* which are adapted to hot and dry climate conditions prevail and are widely distributed.

24. Ultrastructure of the Intestine and Rectal Glands in *Heterorhabditis* Hermaphrodites (Rhabditida: Heterorhabditidae)

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In this study, we examined the ultrastructure of the posterior intestine and rectal glands of *Heterorhabditis* hermaphrodites. Three *Heterorhabditis* spp.: *H. bacteriophora* Poinar (NC1 strain), *H. indica* Poinar, Karunakar & David (India-H01 strain) and *H. sonorensis* Stock, Rivera-Orduño & Flores Lara (Caborca strain) were considered. TEM observations revealed the presence of microvilli in the apical portion of the intestinal cells. The density and arrangement of microvilli are more profuse in the posterior intestinal cells. At this level, intestinal cells of *Photorhabdus*-fed gravid and non-gravid hermopahrodites, may contain vacuoles of various sizes harboring bacterial cells. We speculate these vacuoles are involved in the digestion of bacteria. *Photorhabdus* cells were also observed in the intestinal lumen, especially in terminal portion. Three unicellular rectal glands (two sub-ventral and one dorsal) were observed in all examined specimens. The terminal portion of these glands opens into the rectum by means of a duct. Microvilli were not present in either the apical or terminal portion of these glands. The cytoplasmic nature of these glands suggests a secretion role, with various lysosome-like bodies present. No vacuoles or other similar structures containing *Photorhabdus* bacteria were observed in these glands.

25. Effects of *Fusarium oxysporum* f. sp. asparagi (Ascomycota:Hypocreales) on the *Photorhabdus luminescens sonorensis* (Enterobacteriales: (Enterobacteriaceae), the bacterial symbiont of Heterorhabditis sonorensis (Nematoda: Heterorhabditidae)

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Entomopathogenic nematodes (EPNs) have only one free living stage which occurs in the soil. In this stage the nematode is associated with the bacterial symbiont *Photorhabdus luminescens* which growth and multiplies and plays a vital role in this nematode's successful reproduction and overall fitness. Many natural enemies live in the soil environment where this EPN lives including fungi, protozoa and scavengers with which the EPN-bacteria complex has to interact. The genus *Fusarium* is one of the most ubiquitous and cosmopolitan soilborne fungi. Strains and formae speciales of this fungus vary widely in their effects on hosts, ranging from virulent to avirulent, and from pathogenic to saprophyte. *F. oxysporum* has been reported as both pathogenic (Hasan and Vago, 1972; Roberts, 1981) and non-pathogenic in its relationship with insects. The effects of *Fusarium oxysporum* f. sp asparagi were tested on the symbiotic bacterium *Photorhabdus luminescens* under laboratory conditions including three different media, NBTA (NeutralBormothymol Blue Agar), LB (Luria-Berthani Agar), and PDA (Potato Dextrose Agar), and three fungus ages (7, 10, and 15-day old) were considered. Bacteria were extracted from IJs, and maintained in glycerol stocks. Treatments considered will be: NBTA (fungus 7, 10, 15 days old), LB (fungus 7, 10, 15 days old), and PDA (fungus 7, 10, 15 days old). In each case half of the plate was seeded with the bacteria and the other with each respective treatment. Not antagonistic interactions were observed between the fungus and the symbiotic bacteria; however there were significant differences in the bacterial growth in relation to the different medium used. No significant differences were observed among the different

ages of the fungus studied. Data was analyzed using ANOVA, and means were compared using Tukey's test (JMP, 2008).

26. Molecular characterization of entomopathogenic nematodes' biodiversity occurring in different agro-climatic regions of India

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Entomopathogenic nematodes were isolated from the rhizosphere soil of different cropping systems in 11 states of India, representing 11 agro-climatic regions varying from hot arid, semi-arid, temperate hilly region to tropical hilly region with high annual rainfall (>400 cm). Altogether 20 isolates of *Steinernema* and 5 of *Heterorhabditis* were collected. These were characterized based on the sequence as well as composition of ITS region of ribosomal DNA of their infective juveniles. Genomic DNA was isolated and the PCR amplification was carried out using the specific primers by Vrain et al followed by DNA sequencing of the ITS region of rDNA. Sequences obtained were subjected to BLAST to know their close relatives. All the sequences were submitted in the NCBI GenBank. The isolates and their respective GenBank accession numbers are: *S. thermophilum* (DQ66565), *Steinernema* species 19 Isolates viz., SGdl1(EF216870), SGdl2 (EF216871), SGgj1 (GQ373382), SGchh1 (GQ438788), SGmg1 (EF219458), SGas1 (FJ715946), SGwb2 (FJ418045), SGrj16 (GU354214), SGrj4 (HQ003711), SGrj5(HQ003712), SG rj20 (HQ148711), SGmtr10 (GU354213), SGmtr11 (GU35421), SGmtr12 (GU354216), SGkr (FJ715947), SGjk (FJ418046), SGut1(GQ353372), SGup1(GQ373381), SGor1(GQ353373); and *Heterorhabditis* species 5 isolates viz., SGmtr15 (GU354219), SGmtr14(GU354218), SGmtr13(GU354217), SGmg3 (FJ751864) and SGgj (FJ744544). A dendrogram was constructed by hierarchical cluster analysis based on these sequences and the 18 known species of *Steinernema*, exhibited trichotomy. Three isolates of Steinernema, one isolate each from Kerala (SGkr) and Rajasthan (SGrj20) and Assam (SGas1) states having paired horn-like structures on their lip region formed a clade along with other 5 species of the *bicornutum* group (*S. bicornutum, S. riobrave, S. ceratophorum, S. thermophilum, S. abbasi*), which showed the robustness of this molecular tool in taxonomy. Most of the native isolates of *Steinernema* formed clusters either within the *carpocapsae* group or *siamkayai* group. All the *Heterorhabditis* isolates were grouped in distinctive one clade. It is indicated that unlike *feltiae* and *glaseri* groups, the species with small sized infective juveniles (*carpocapsae* and *siamkayai* groups) are highly adaptive to tropical and subtropical climatic conditions prevalent in the Indian subcontinent.

27. Ecological characterization of native entomopathogenic nematodes and their bacterial symbionts in Steinernema-Xenorhabdus complex

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The 10 isolates of *Steinernema* from different agro-climatic zones of India and their respective bacterial symbionts were characterized ecologically. Among the all isolates *S. thermophilum* was found to be highly virulent by having least LC 50 values (< 5 & 9 IJs /insect) for *Galleria mellonella* and *Helicoverpa armigera* respectively. Its bacterial symbionts *Xenorhabdus indica* alone could induce 100 % mortality when injected in the hemolymph of insect at the rate of 20 µl comprising 528 CFU (Colony Forming Units) per insect. The *Xenorhabdus* isolate from Orissa state was found to be least effective. The optimum temperature requirement for infectivity and development was found to be dependent on the thermal niche range of the respective *Steinernema* isolates. The optimum temperature was restricted to 25^0 C for *Steinernema* isolate from temperate hilly regions of northern India. While it varied from 25 to 35^0 C for most of the other isolates from tropical and subtropical climatic conditions. Unlike *Steinernema* isolates, the optimum temperature for the *Xenorhabdus* was constant (28^o C) for all the isolates irrespective of their origin. Secondary metabolites of *X. indica* were extracted in different solvents, of which ethyl acetate fraction exhibited high fungicidal activity even at the lowest concentration (31.2 ppm) inducing 50 % inhibition in growth. HPLC analysis of this fraction revealed two groups of compounds resembling xenorhabdins and xenocoumacins.

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