Journal of Nematology 43(1):49–60. 2011.

Abstracts of NEMASYM: The Second Nematode-Bacteria Symbioses Research Coordination Network Meeting

S. Patricia Stock,[1] David McK. Bird,[2] Elodie Ghedin,[3] Heidi Godrich-Blair[4]

Preface

Intimate associations between microbes and eukaryotes are widespread in nature, occurring in every type of ecological niche. The spectrum of such interactions ranges from highly integrated obligatory symbioses to 'loose' associations. Microbial symbioses are integral to the function of every ecosystem on Earth. Therefore to gain insights into fundamental processes underlying symbiosis and the role of symbiosis in earth/human ecosystems, researchers in this project have focused on one of the most common eukaryote-prokaryote interactions: that between nematodes and bacteria. Associations between nematodes and bacteria range from fortuitous to obligate and from beneficial to pathogenic. The ubiquity and diversity of nematode-bacterium symbioses make them an excellent model to understand the key questions in symbiosis. However, while numerous researchers worldwide are currently studying associations between these two groups of organisms, these scientists rarely interact because their field of research is often defined by the organism studied rather than by the process (in this case, symbiosis). To reddress this critical need for crossing disciplinary lines, a Research Coordination Network on 'Nematode-Bacteria Symbioses' (NEMASYM) was established in 2008 to promote the intellectual discourse among scientists studying bacteria-nematode associations. This network is funded by the National Science Foundation and its specific goals of are: 1) Foster interdisciplinary collaborations between scientists; 2) Encourage scientists engaged in basic and applied research to explore how cross-talk and networking can enhance and advance science in this field; and 3) Develop and distribute educational materials to scientists and educators to promote the study of nematode-bacteria symbioses as model systems in science and education.

There is no other group in the United States or elsewhere that is similar to this research work group in its broad scope of nematode-bacteria interactions. A focal point of the network is the organization of annual meetings for core group members and their post-doctoral associates and graduate students. The inaugural NEMASYM meeting was held in Madison, Wisconsin on August 9-16, 2009 in conjunction with the 6th International Symbiosis Society meeting. The theme focus of that meeting was "Contributions of bacteria to nematode development, nutrition, and behavior". A total of 45 participants representing nine countries from five continents attended this event. There were 42 oral and 25 poster presentations.

The second NEMASYM meeting took place in Tucson, Arizona on November 11-14, 2010. The theme of this meeting was "Contributions of bacterial symbionts to nematode pathogenesis". There were 46 participants representing eight countries from four continents. Keynote speakers were: 1) John (Jack) Werren from University of Rochester, New York, who talked about the evolutionary consequences of *Wolbachia* symbionts in insects; 2) Raffi Aroian from University of California, San Diego who discussed the use of bacteria to cure human nematode diseases; and 3) David Clarke From University Cork College, Ireland, who presented results of his research program on the regulation of pathogenicity and mutualism in *Photorhabdus* symbionts. In addition to these keynote presentations there were 30 oral presentations that are herein presented.

Wolbachia's Evolutionary Consequences

John (Jack) H. Werren

Department of Biology, University of Rochester, Rochester, NY 14627 USA

Wolbachia are among the most abundant intracellular parasites on the planet, infecting upwards of 65% of terrestrial arthropods and many filarial nematodes. The implications of these bacteria to invertebrate cell biology, ecology and evolution are potentially profound, but still remain controversial. A key question is whether *Wolbachia* accelerate adaptive evolution and speciation in their hosts. Here I present data relating to the diversity of *Wolbachia*, their co-evolutionary interactions with hosts, and their role in accelerating host adaptive evolution. Lateral gene transfers from *Wolbachia* to invertebrate genomes are common and widespread. A major question is whether

[1]Department of Entomology, University of Arizona, Tucson Arizona 85721.
[2]Bioinformatics Research Center, NC State University, Raleigh, NC 27695.
[3]Department of Computational and Systems Biology, Center for Vaccine Research, University of Pittsburgh School of Medicine, 3501 Fifth Avenue, Pittsburgh, PA 15261.
[4]Department of Bacteriology, University of Wisconsin-Madison.1550 Linden Dr. Madison, WI 52706.

these can lead to acquisition of novel gene functions or simply degenerate by mutational degradation following transfer. Recent data relating to this question will be discussed.

Serratia proteamaculans strains associated with novel insect pathogenic nematodes in UK soils

Fada'a Al Own[1], Ed Feil[1], Patricia Stock[2], and Nick R. Waterfield[1]
[1]Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY, UK.
[2]Department of Molecular and Cellular Biology,
[2]Department of Entomology, University of Arizona, Tucson AZ 85721, USA.

Galleria larvae were used to bait UK soil samples collected in February 2009 from different habitats collected along two transect-lines, east-west (along the south coast of England) and north-south (from England into Wales). To do this we pioneered the use of a new smart phone technology (Epicollect) to manage sample collection data. Insects which became infected were placed on White traps to isolate any emerging nematodes. Hemolymph was also sampled from all insects, even if no nematodes emerged, and plated onto rich medium to assess the dominant bacteria. Samples of emergent nematodes were also washed; surface sterilised and then crushed into rich growth medium, and again plated out to assess the dominant bacterial content. Genomic DNA was prepared from all bacterial isolates and the genus characterized by sequencing PCR products of the bacterial 16s rDNA. We were surprised to find that only one sample gave a *Steinernema/Xenorhabdus* entomopathogenic nematode (EPN) complex. The majority of infected insects contained either free living *Serratia proteamaculans* (Sp) like strains or produced nematodes which also contained similar strains. We further characterized these Sp-like strains by sequencing two housekeeping genes, *recA* and *dnaJ*. A phylogeny based on this analysis suggests that the nematode associated strains belong to specific clades, distinct from the free living infective strains. We selected three nematode/Sp-like complexes for a more detailed analysis. The nematode hosts were characterized, confirming two Rhabditid nematodes and one *Pristionchus* sp. Sand trap infection studies with Galleria, plate feeding assays and further nematode crushing confirmed that these nematodes could feed and reproduce on the Sp like strains and that unlike *E. coli*, could always be recovered from surface sterilized and crushed worms. Two of these bacterial strains have now been chromosomally labeled with GFP to further assess their association with the nematode hosts.

Establishment of new host relationship with an invasive? Or hitchhiker across the Atlantic?

Eleanor Groden[1] and S. Patricia Stock[2]
[1]School of Biology and Ecology, University of Maine, Orono ME 04469, USA.
[2]Department of Entomology, University of Arizona, Tucson AZ 85721, USA.

The European red ant, *Myrmica rubra*, is a northern temperate species common in damp pastures, riverbanks and woodland edges in its native range in Europe and western Asia. It was first reported in the U.S. in 1908, and confirmed in Maine in 1952. After a seeming long period of establishment, populations of this ant have rapidly spread over the past 10 to 15 years in many coastal communities in Maine and neighboring states and Canadian provinces, where it has become locally dense and pestiferous. A nematode was first observed emerging from these ants collected at two sites in Maine in 2003 and 2004, and a similar diplogasterid-like nematode was retrieved at various sites in its native range in England in 2003. Unfortunately, the nematodes were not maintained alive and further identification was not possible at that time. In July and August 2008, *M. rubra* colonies collected from seven different sites in Maine were parasitized with nematodes, and affected colonies collapsed. Nematodes from each site were maintained separately, reared on baby-food agar and *Galleria mellonella* larvae, and used to challenge *M. rubra* colonies. Subsequent ant mortality varied significantly with the site of origin of the nematode, ranging from 2.5% (not different from control) to 27.3% 18 days post exposure. Nematode populations were identified with a combination of molecular and morphological methods. Sequences were generated from the PCR amplicons derived with nematode 18S (barcode) and 28SrDNA primers for molecular characterization identification of nematodes, and DIC microscopy was used to depict key morphological traits and to confirm nematode molecular identification. Data indicates nematodes are members of the family Diplogasteridae and belong to the genus *Pristionchus*, specifically the species *P. entomophagus*, which has previously been found in association with scarab beetles. Implications of this host-parasite relationship in an introduced species will be considered.

The evolution of virulence and resistance mechanisms in the interaction of Pristionchus nematodes with Bacillus bacteria

Robbie Rae, Igor Iatsenko, Amit Sinha & Ralf J. Sommer
Max-Planck Institute for Developmental Biology, Tuebingen, Germany.

Bacillus bacteria are highly prevalent in the soil ecosystem and some species have evolved the ability to kill mammals and invertebrates, including insects and nematodes. Understanding how these bacteria evolved an array of mechanisms to target a range of hosts and how the nematodes evolved specific resistance mechanisms against a diversity of *Bacilli* is of prime importance. We are using two model nematodes *Prisitonchus pacificus* and *Caenorhabditis elegans*

and nematocidal *Bacillus* species to answer these questions. These nematodes have been developed as genetic model organisms with an array of forward and reverse genetic tools, transgenic techniques as well as full sequenced genomes. In the natural environment *Pristionchus* nematodes can be isolated from scarab beetles: Dauer larvae infect beetles, wait for the host to die and then resume development and grow prolifically on the decaying carcass feeding on microorganisms. It has been shown that *Pristionchus* associates with a wealth of plant and animal pathogenic bacteria and is generally more resistant to these bacteria than *C. elegans*. Interestingly, *P. pacificus* has been shown to have an array of genes responsible for detoxification of xenobiotic compounds. To understand the virulence/resistance mechanisms involving nematodes and bacteria we have developed a *Pristionchus/Bacillus* model. We are interested in how novel *Bacillus* strains manage to cause rapid mortality to nematodes, why this differs between nematode hosts and how these strains evolved virulence factors. In order to gain more insight in difference in host susceptibility and pathogenicity of naturally isolated bacteria we screened through a collection of 1400 *Bacillus* strains to identify those that specifically kill either *C. elegans* or *P. pacificus*. Of these 1400 grown to vegetative cell stage, 20 were identified that kill either *C. elegans* or *P. pacificus*. The most pathogenic of which cause mortality to *C. elegans* in under 12 hours but *P. pacificus* remains resistant. When grown on standard Nematode Growing Media (NGM) these strains are the most nematode pathogenic bacteria reported in the literature so far. Using 16SrRNA and multi locus sequencing (in cooperation with Dr. Alex Hoffmaster, CDC; Athens, USA) these 3 toxic strains are thought to be *B. thuringiensis*. Pathogenicity of this bacterium to nematodes is mostly reported when bacteria are grown to spore stage and express crystal proteins. Hence our strains are novel in their ability to kill nematodes at vegetative cell stage and in their virulence strength. We will report on our current studies, which aim to understand what genes are responsible for extreme virulence of *B. thuringiensis* when fed to nematodes and what are the specific susceptibility mechanisms in *C. elegans* and the resistance mechanisms in *P. pacificus*? We are tackling the nematode - bacterial interaction from both sites of the equation and will report the results of our genetic and microarray analyses.

Effects of pathogenic bacteria associated with Rhabditis blumi on some insect pests

Hae Woong Park[1], Yong Ook Kim[1], Anwar L. Bilgrami[4], Jae-Seok Ha[2], Jong Hoon Kim[1], Sung Hun Young[1], Hyeong Hwan Kim[3], Chul Soo Shin[1]

[1]Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University, Shinchon-dong, Seodaemun-gu, Seoul 120-749, South Korea;

[2]Industrial Biotechnology & Bioenergy Research Center, KRIBB, Daejeon, Chungnam 305-806, South Korea;

[3]Horticultural & Herbal Crop Environment Division, National Institute of Horticultural & Herbal Science, RDA, Suwon, Gyeonggi 441-440, South Korea

[4]Department of Mosquito Control, Cape May Court House, New Jersey 08210, U.S.A.

Effects of pathogenic bacteria associated with a rhabditid nematode *Rhabditis blumi* were studied on *Galleria mellonella* larvae and reproduction of *Rhabditis blumi*. Three species of bacteria i.e, *Alcaligenes faecalis*, *Flavobacterium* sp., and *Providencia vermicola* were isolated from infective juveniles of *R. blumi*. *Providencia vermicola* and *Flavobacterium* sp. showed 100% mortality of *G. mellonella* larvae 48 h after hemocoelic injection, whereas *A. faecalis* showed only 30% mortality. Infective juveniles of *R. blumi,* showed 100% mortality of *G. mellonella* larvae. On the other hand sterile first and second stages of *R. blumi* exhibited insignificant pathogenic effects on *G. mellonella*. Nematodes fed and grew well on associated bacteria resulting in significant increase in nematode reproduction and fecundity. The highest nematode yield was recorded on *P. vermicola*. Fluorescent microscopic observation of bacterial cells labeled with an Alexa Fluor dye inside the nematodes revealed that bacteria were digested, indicating that associated bacteria are used as a food source for nematodes. In mixed cultures using two of the three associated bacteria, one kind stimulated the other. The highest total bacterial yield of 12.6 g/l was obtained when the inoculum ratio of *P. vermicola* to *A. faecalis* was 10:1. In air-lift bioreactors, the nematode growth rate increased with an increasing level of dissolved oxygen. The maximum nematode yield of 1.75 x 105 per ml was obtained at 192 h with an aeration rate of 6 vvm.

Polymorphisms in generation times and growth curves of* Xenorhabdus bovienii *isolated from Steinernema nematode hosts

William Soto[1], Diana A. Stan[1], and S. Patricia Stock[1]

[1]Department of Entomology, University of Arizona, Tucson, AZ 85721, USA.

A bacterial growth curve consists of a lag, exponential, stationary, and death phase. Shaking flasks with high-nutrient liquid media is as homogenous an environment as a growing bacterial population can possibly experience, since microhabitat complexity and niche heterogeneity is minimized with extensive orbital agitation. Additionally, bacterial cells in exponential phase, growing under optimal liquid culture conditions (e.g., aeration, temperature, pH, salinity, etc.), are physiologically the most uniform relative to one another than any other part of a growth curve. Therefore, one should generally expect the variation of generation times within a bacterial species

to be the least under such conditions. This is what has been observed in the marine bioluminescent bacteria *Vibrio fischeri*, light organ symbionts of sepiolid squids and monocentrid fishes. Genetically distinguishable *V. fischeri* strains display almost no dissimilarity in generation times and growth curve characteristics at optimal liquid culture conditions under orbital agitation, despite being isolated from diverse fish and squid host species from oceans located on opposite sides of the globe. Enteric *Xenorhabdus* bacteria are symbionts of entomopathogenic nematodes in the genus *Steinernema*, with *X. bovienii* having the widest nematode host range. Contrary to *V. fischeri*, *X. bovienii* isolates demonstrate substantial variation in generation times and growth curves, even when coming from the same nematode host individual. Although genetic differentiation among *X. bovienii* isolates could potentially explain this intriguing result, phase variation (which has not been reported in *V. fischeri*) is another phenomenon that could account for these *X. bovienii* polymorphisms in population growth kinetics.

Induction of Heat Shock Protein (Hsp) response in Steinernema websteri A10.

Andrea M. Binnebose[1,2] and Susan Bornstein-Forst[2]
[1]Department of Biology, [2]Marian University, Fond du Lac, WI 54935, USA.

Post-application persistence for entomopathogenic nematodes (EPN) is impacted by environmental conditions of fluctuating temperature and desiccation stress. This study investigates the effect of slow desiccation during development of *Steinernema websteri* inside insect hosts and examines levels of heat shock proteins (Hsp) synthesis in emergent EPN populations. *Galleria mellonella* were infected with *Steinernema websteri* ssp. A-10 at a dose of 20 infective juveniles (IJ)/host. Productive infections were subjected to slow air-desiccation in an environmental chamber at 23°C for up to 31 days post-infection. Desiccated hosts were rehydrated in White traps using sterile reverse osmosis (RO) water and IJ were collected over a three-day time period beginning at T= 10, 17, 24, and 31 days post-infection, respectively. For each day of collection 100 randomly selected IJ were measured for length and width using a stage micrometer and population numbers were determined using a dilution estimation method. IJ were pelleted, sonified and aqueous supernatants were applied to 10% Laemmli SDS-gels for protein separation and Western blotting with antibodies directed to Hsp 27, 40, 60, 70, 90. Results suggest that the day of emergence combined with desiccation stress positively impacts population numbers and sizes and that Hsp90 may be important for survival under desiccation stress during development.

Longitudinal binary fission in a stilbonematid symbiont

Niels R. Heindl[1], Jörg A. Ott [2] and Silvia Bulgheresi[1]
[1]Department for Genetics in Ecology, University of Vienna, Austria,
[2]Department for Marine Biology, University of Vienna, Austria

Rod-shaped sulfur-oxidizing bacteria cover the cuticle of the free-living marine nematode *Robbea sp.3*. They are aligned perpendicularly to the host surface and set their division plane parallel to their long axis (longitudinal division). This is an extremely rare cytoengineering feat exclusively observed in few other bacterial symbionts. It enables both symbiont daughter cells to reproduce without loosing contact with the worm. Accordingly, the contractile Z-ring formed by the tubulin homolog FtsZ is also positioned longitudinally. Another conspicuity of the symbiont fission is that the furrow ingresses asynchronously: it starts at the pole in contact with the worm's cuticle (proximal pole). In *E. coli* the MinCDE proteins position the division plane. MinC inhibits Z-ring formation and in time average it is maximally concentrated at the poles and minimally at midcell. We wanted to know if this system is also positioning the fission plane in the symbionts. In order to analyze the symbiont MinCDE and FtsZ proteins distribution, we immunostained them with antibodies against the corresponding *E. coli* proteins. It appears that, prior to cell constriction, MinC and MinD are maximally concentrated laterally and not at the poles. As for MinE, preliminary data suggest that, surprisingly, it accumulates at the proximal pole. By using degenerate primers, we cloned and sequenced a 1,174 nt-long fragment of the symbiont *minCDE*-operon. Symbiont MinD amino acid sequence is 66% identical to its *E. coli* homolog. Although the full-length sequences of *minC* and *minE* are still lacking, the symbiont Min proteins might function very differently from their *E. coli* homologs. Alternatively or additionally they could be differently regulated. Having sequenced the full-length symbiont *minCDE*-operon, we will ectopically express the symbiont operon in *minCDE*-knocked out *E. coli* to shed light on the function of the symbiont MinCDE system.

A bacterial symbiont protects its insect host against nematode parasites

Steve Perlman
Department of Biology, University of Victoria, Victoria BC, Canada

The mushroom-feeding fly *Drosophila neotestacea* is commonly infected with a virulent parasitic nematode, *Howardula aoronymphium (Tylenchida: Allantonematidae)*. In addition to reduced survival and male mating success, until recently, virtually all females were rendered completely sterile upon infection. We have found that *D. neotestacea* harbors a *Spiroplasma* bacterial symbiont that restores fertility to nematode-infected flies. *Spiroplasma* infection is dynamic and appears to have dramatically increased in frequency in less than 20 years, and also appears to be

spreading across N. America. This study demonstrates the importance of defensive symbionts, which are increasingly being recognized as major players in the ecology of species interactions. This is also the first example of natural symbiont-mediated defense against nematodes.

New insights on the role of R-type bacteriocins of Xenorhabdus nematophila in interspecies competition

Nydia Morales Soto[1] and Steven Forst[1]
[1]Department of Biological Sciences, University of Wisconsin, Milwaukee WI, 53211, USA.

Xenorhabdus and *Photorhabdus* are sister taxa that engage in mutualistic associations with distinct entomopathogenic nematodes (EPNs) and also function as pathogens against an array of insects. Competition occurs when two EPNs co-invade an insect and release their respective bacterial symbionts into the hemocoel. Of the 20 *Xenorhabdus* species identified, *in vitro* production of R-type bacteriocins (phage tail-like particles) had only been characterized in *X. nematophila* isolated from the *Steinernema carpocapsae*. To test the hypothesis that R-type bacteriocins are an important component of the antimicrobial repertoire produced to suppress natural competitors, we first demonstrated that *X. nematophila* indeed produced bacteriocins *in vivo*. The P2 phage gene cluster of *X. nematophila* (*xnp*) encoding tail synthesis proteins involved in bacteriocin production was identified. Deletion of *xnpS* that encodes the tail sheath protein eliminated bacteriocin activity. Supernatant preparations from the wild type strain strongly inhibited growth of *Photorhabdus luminescens* while preparations from the *xnpS* strain were inactive suggesting that R-type bacteriocins are involved in suppressing *Photorhabdus* during interspecies competition. Sensitivity of different *Xenorhabdus* species to R-type bacteriocins was found to vary considerably and was not related to phylogenetic distance. In addition, R-type bacteriocin sensitivity of different *X. bovienii* strains ranged from highly sensitive to low or undetectable sensitivity. Production of R-type bacteriocins by other *Xenorhabdus* species was also shown to be variable. Finally, intra-species R-type bacteriocin activity was demonstrated for the first time. Bacteriocins of *X. nematophila* (*S. carpocapsae*) were shown to be active against *X. nematophila* isolated from *S. anatoliense*. These findings suggest that the *in vivo* contribution of R-type bacteriocins to suppression of inter- and intra-species competitors and reproductive success of the nematode partner may vary considerably.

***Genotypic, phenotypic, and lypolytic variation in* Xenorhabdus bovienii**

Murfin, K.[1], Forst, S.[2], Stock, P.[3], and Goodrich-Blair, H.[1]
[1]Department of Bacteriology, University of Wisconsin Madison, Madison WI 53706, USA,
[2]Department of Biological Sciences, University of Wisconsin Milwaukee, Milwaukee WI 53201, USA,
[3]Department of Entomology, University of Arizona, Tucson AZ 85721, USA

Steinernema nematodes mutualistically associate with gram-negative bacteria from the genus *Xenorhabdus*. Within this genus, *X. bovienii* appears to be the most promiscuous symbiont, associating with more than eight *Steinernema* species. Previous studies have shown that *S. felitae* shows specificity for certain strains of *X. bovienii*, but the differences among *X. bovienii* strains that underlie this specificity have not been examined. To date, the factors required for *X. bovienii* symbiosis with its hosts have not been identified. To determine genetic strain variation among *X. bovienii* strains, the CRISPR (clustered regularly interspaced short palindromic repeats) regions of eight *X. bovienii* strains were examined. These data support the idea that *X. bovienii* has considerable intra-specific variation. Previous studies of the *S. carpocapsae-X. nematophila* system have shown that lipolytic activity impacts the ability of the symbiont to support nematode development, where *Δ xlpA* strains lacking lipase activity are less able to support nematode reproduction. *X. bovienii* has an *xlpA* homolog that likely encodes a lipase, and we have shown that *X. bovienii* strains have lipolytic activity similar to *X. nematophila* towards Tween substrates. Ongoing research is being done to identify if *X. bovienii* lipolytic activity is encoded by *xlpA* and how it affects the nematode life cycle. We found no obvious variation in the lipase activities expressed by *X. bovienii* strains, but did observe differences in the phenotypic regulation. Lipolytic activity in many *Xenorhabdus* species is subject to phenotypic variation. We have shown that the form in which lipase is expressed varies among *X. bovienii* strains, and that the presence of lipids results in populations enriched for the lipase-expressing form. These differences in regulation may affect the ability of the bacterial strain to support the nematode life cycles of different *Steinernema* species, and current research is aimed at determining how these differences impact host specificity.

Fitness of mutualistic partners in relation to host suitability as inferred by insect diet and life history stage

Victoria A. Miranda and S. Patricia Stock
Department of Entomology, University of Arizona, Tucson AZ 85721, USA

A few studies have shown that the internal environment of an insect host may play a key role in the colonization and reproduction of parasites and entomopathogens. In this study we considered the insect-pathogenic nematodes-symbiotic bacteria system to assess costs and benefits of the physiological condition of one of the three partners (the host insect) and how the insect environment (influenced by diet changes) may affect the system as

a whole. The tobacco horn worm, *Manduca sexta* (Lepidoptera: Sphingidae), was considered as the insect host. Two entomopathogenic nematode spp.: *Steinernema carpocapsae* ALL strain and Heterorhabditis sonorensis Caborca strain, and their cognate bacterial symbionts, *Xenorhabdus nematophila* and *Photorhabdus luminescens*, respectively, were examined to assess the effects of insect host diet on pathogen/symbiont performance. *M. sexta* was reared on an artificial diet under low and high quality conditions. We evaluated the impact of insect diet choices on nematode fitness and bacterial symbiont colonization and proliferation. Insect host mortality, EPN time to emergence, nematode progeny production and number of bacterial cfu/ IJ were assessed. Insect host mortality was generally higher for *M. sexta* reared on a low quality diet relative to those reared on the optimal, high quality diet. In addition, *M. sexta* exposed to *S. carpocapsae* had significantly higher mortality than those exposed to *H. sonorensis*. The EPN progeny production as well as time to emergence did not differ based on insect host diet; however, there were significantly fewer IJs per cadaver for *S. carpocapsae* compared *to H. sonorensis*. Finally, the average number of bacterial symbionts per IJ is not different between treatment groups, but there is more variation within the low diet treatment group.

***Towards a SNP map for* Heterorhabditis bacteriophora**

Hillel Schwartz and Paul Sternberg
Division of Biology and HHMI, California Institute Of Technology, Pasadena, CA

Heterorhabditis bacteriophora is a species of insect-parasitic nematode that lives in mutually beneficial symbiosis with pathogenic bacteria of the genus *Photorhabdus*. *Photorhabdus* bacteria are lethal to insects and to other nematodes but are required for *H. bacteriophora* growth. The symbiosis between *H. bacteriophora* and *Photorhabdus* bacteria therefore offers the potential to study the molecular genetic basis of their cooperative relationship. We are developing tools to make such studies more feasible, and especially seek to develop tools and methods to make the nematode *H. bacteriophora* a more tractable system for molecular and genetic analysis. In particular, we are working on improved protocols for cryopreservation, mutagenesis, and transgenesis of *H. bacteriophora* and on the construction of a molecularly linked genetic map of *H. bacteriophora*. We have demonstrated that six independent isolates of *H. bacteriophora* are interfertile and are currently inbreeding them and characterizing their interactions with wild-type and mutant *Photorhabdus* bacteria. From these studies we will select an isolate or isolates and will use next-generation high-throughput sequencing technology for the purpose of refining the existing draft genome sequence and for the creation of a SNP map. We anticipate that this SNP map will enable us and the wider insect-parasitic nematode community to identify induced mutations and natural variations affecting the symbiotic interactions between *H. bacteriophora* nematodes and pathogenic *Photorhabdus* bacteria.

Characterizing a novel entomopathogenic partnership between* Serratia *spp. and Rhabditid nematodes.

Feseha Abebe-Akele[1], Louis Tisa[1]; Eyualem Abebe[2], Vaughn Cooper[1], Kelley Thomas[1].
[1]Department of Molecular, Cellular, and Biomedical Sciences, University of New Hampshire, Durham, NH, 03824.
[2]School of Mathematics, Science & Technology, Department of Biology, ESCU, Elizabeth City, NC, 27909

The classic entomopathogenic nematodes (EPN) and their symbiotic bacteria exhibit a high degree of specificity in their association that has been tweaked through a long history of co-evolution. At present only three such associations are documented. Given the natural abundance of all three players – insect hosts, carrier nematodes and pathogenic bacteria, the number of such associations in nature is very low and all three systems described in the literature seem to be terminally evolved systems with no trace of their early adaptational paths. Using the classic galleria trap, we have discovered an EPN association that involves the well known freeliving nematode of the genus *Caenorhabditis: C. briggsae*. The symbiotic bacterial strain, which has been identified as a member of the genus *Serratia* by 16S rDNA sequencing, has also been shown to transform other free living nematodes into efficient EPNs. This generic ability of the bacterial isolate to transform a wide range of nematodes into potent EPNs may represent the early stages of evolution in EPN establishment and it is a very promising model to study the early events in the establishment of obligatory EPN associations in the wild. Recently, we have sequenced the genome of the symbiotic bacteria and comparative genomic studies are underway to determine its genetic similarities and differences to both the bacterial species in the established EPNs and to other *Serratia spp* that do not have the symbiotic predilection and kill the nematodes. Salient genomic differences and features that set apart the bacteria from both EPN bacteria and other members of the genus *Serratia* will be presented.

***Investigations of symbiotic interactions between soil bacteria and the free-living nematode* Acrobeloides maximus.**

Sammy Sedky[1], Jean-Paul Baquiran[2], Brian Thater[2], David E. Crowley[2], Paul De Ley[1], Paul M. Orwin[3]
[1]Department of Nematology, University of California at Riverside
[2]Environmental Sciences Department, University of California at Riverside
[3]Biology Department, California State University at San Bernardino

We have introduced large numbers of the free-living bacterivorous nematode *Acrobeloides maximus* into a rhizosphere soil sample after monoxenic culture on *Escherichia coli*. The nematodes were extracted after 24h and the

bacterial population was analyzed using culture dependent and culture-independent techniques. Long term co-cultures of nematodes and soil bacteria from this exposure were established as well. Based on these initial studies, two major likely bacterial symbionts were identified, an alpha-proteobacterium in the genus *Ochrobactrum*, and a Sphingobacterium in the genus *Pedobacter*. We have been able to isolate and culture a representative Ochrobactrum species, most closely related to *Ochrobactrum tritici*, for further study. Efforts to isolate other symbionts, including those present in smaller numbers, are ongoing. We will present qPCR and fluorescence in situ hybridization data supporting the close association of these bacteria with *Acrobeloides maximus*. We hypothesize that several potential positive interactions may be taking place. The bacterial symbiont may be defending the nematode from pathogenic bacteria or fungi, it may be aiding in the digestion of certain bacterial prey, or the symbiont may be providing an essential metabolic precursor (such as cholesterol) for worm growth. Using our cultured species of *Ochrobactrum*, we will present data on these potential interactions. We must also consider a parasitic relationship, and in particular we have designed enrichment experiments to isolate chitinophagous bacteria, whose presence is suggested by our diversity studies (the genus *Chitinophaga*). Overall, these studies suggest that the microbial population associated with these nematodes is specific, and functions as an ecosystem similar to the microbiomes of other metazoans.

Towards a worm-free world- using bacteria to cure human nematode diseases

Yan Hu, Sophia Georghiu, Alan Kelleher, Cheng-Yuan Kao, Ferdinand C.O. Los, and Raffi V. Aroian.
Section of Cell and Developmental Biology, University of California, San Diego CA 92093

Intestinal parasitic roundworms (nematodes) are leading causes of disease burden in humans worldwide, infecting 2.3 billion people. New cures are urgently needed as current medicines lack strong efficacy and are increasingly targets of parasite resistance. We are using the soil bacterium *Bacillus thuringiensis* and its invertebrate-specific Crystal proteins as a source for new, potent drugs against human intestinal roundworms. In this presentation, I will talk about how research on bacterial-nematode interactions is being applied to cure one of the great diseases of our time and futhermore how pathogenic bacterial-nematode interactions can be used to teach us about pathogenic bacterial-human interactions.

***The regulation of pathogenicity and mutualism in* Photorhabdus.**

David J. Clarke.
Department of Microbiology and Alimentary Pharmabiotic Centre
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. How does *Photorhabdus* control these different relationships and make the appropriate life-style decisions? Our observation that pathogenicity is correlated with the rate of *Photorhabdus* exponential growth inside the insect whilst mutualism (i.e. the support of nematode growth and reproduction) is associated with the post-exponential phase of growth suggested that life-style decisions might be linked to bacterial metabolism. *Photorhabdus* elaborates an extensive secondary metabolism during post-exponential growth that is also associated with mutualism. Products of this secondary metabolism include an antibiotic called 3-5-dihydroxy-4-isopropylstilbene (ST), an anthraquinone pigment (AQ) and bioluminescence. We identified a mutant that was unable to produce ST, AQ and light and this mutation was found to be in the mdh gene, encoding malate dehydrogenase, a key enzyme in the TCA cycle. The mdh mutant was unaffected in virulence but, importantly, the mdh mutant was not able to support nematode growth and development *in vivo* or *in vitro*. Furthermore the construction of mutations in key genes in other central metabolic pathways confirmed the critical role for the TCA cycle in both secondary metabolism and mutualism, but not in virulence. Therefore the TCA cycle is required for the transition of *Photorhabdus* from pathogen to mutualist suggesting that a metabolic switch plays a key role in the regulation of life-style decisions in this bacterium.

Bacterial-responsive transcriptomes of rhabditid soil nematodes

Michael A. Herman, Brian Darby and David Wheeler
Ecological Genomics Institute, Division of Biology, Kansas State University, Manhattan, KS 66502

Soil bacteria are an important part of the biotic environment for bacterial feeding soil nematodes, serving not only as food sources but also as potential pathogens. Interactions between nematodes and bacteria, therefore, are of significant importance to understand the ecological dynamics of the soil community. Our previous work using *C. elegans* to model the interactions of native soil nematodes with soil bacteria indicated that differential gene expression is a good predictor of the importance of gene function for life history traits (Coolon et al, 2009 PLoS Genet 5(6): e1000503). To test this idea we isolated four rhabditid nematodes (*Oscheius tipulae, Oscheius* sp. 2,

Rhabditis sp., and *Mesorhabditis monhystera*) from soils collected at Konza Prairie Biological Station and used transcriptome sequencing to investigate their responses to native soil bacteria. Specifically, we grew nematodes on a mix of gram-positive and gram-negative bacteria and sequenced normalized cDNA libraries using a Roche GS-FLX Genome Sequencer. We obtained 400,000- 600,000 reads per species and assembled them into 20,000 to 25,000 unique contigs of 500-600 bp average length. 40-65 % of each transcriptome was conserved in the *C. elegans* genome. The remaining contigs are likely to be either short, untranslated regions (UTRs) of genes otherwise known in *C. elegans*, or possibly lineage specific genes, as suggested by the observation that the degree of conservation of these sequences among the four taxa followed phylogenetic expectations. Our short-term goal is to use this information to determine patterns of gene expression in response to soil bacteria in the lab and in the field. In addition, these transcriptome sequences will complement genomic information available within the Rhabditid 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.

The involvement of the Wolbachia WSP-like and the ankyrin domain containing proteins in the endosymbiotic relationship with Brugia malayi

Elena Melnikow[1*], Thomas Unnasch[2], Elodie Ghedin[3], Sara Lustigman[1]

[1]Lindsley F. Kimball Research Institute, New York Blood Center, New York, NY 10065 USA

[2]Department of Global Health, University of South Florida, Tampa, FL 33612 USA

[3]Center for Vaccine Research, University of Pittsburgh School of Medicine, 3501 5th Ave, Pittsburgh, Pennsylvania 15261 USA

The parasitic nematode *Brugia malayi* causes Lymphatic Filariasis and harbors the endosymbiotic intracellular bacteria *Wolbachia* that are required for their development and reproduction. Our study aims to identify proteins that have an essential function in this endosymbiotic relationship. We hypothesize that surface and/or secreted proteins of the endosymbiont are most likely to be involved in this interaction. In this study we focused on the *Wolbachia* surface proteins (WSP) and the ankyrin domain proteins known to mediate specific protein-protein interactions essential for many cellular processes. The *Wolbachia* genome contains six WSP-like proteins and five major ankyrin domain family proteins, each with 1-8 ankyrin repeats. Localization studies with anti-WSP antibodies confirmed that the protein is not only present on the surface of *Wolbachia*, but that it is also secreted. The protein was found in the body cavity within the uterine wall of the adult worms, in the eggshells surrounding the developing microfilaria, and in the hypodermal region of the cuticle. To explore whether the WSP-like and the ankyrin domain containing proteins interact with proteins produced by the filarial host, we expressed them as GST or HIS tagged recombinant proteins. When tested using a modified ELISA assay, four of the WSP and one of the two ankyrin proteins tested bound specifically to *B. malayi* crude extracts. Their putative target proteins were identified by panning of a *B. malayi* cDNA phage library. The putative *B. malayi* target protein found to bind to WSP (Wbm0284) was named WSP/G2 and contains a BTB/POZ-like domain known be involved in protein-protein interactions. The specific interactions between the *B. malayi* and *Wolbachia* proteins will be presented.

The Root-Knot Nematode genome encodes suites of plant peptide-hormone ligands

Peter M. DiGennaro[1] , Elizabeth H. Scholl[1] , David McKenzie Bird[1,2]

[1]Plant Nematode Genomes Group and [2]Bioinformatics Research Center, NC State University, Raleigh NC 27695

It is widely accepted that plant parasitic nematodes have acquired cell-wall degrading enzymes from soil-borne bacteria by ancient horizontal gene transfer. We speculate that other cross-kingdom exchanges of genetic information may have occurred. Indeed, the plant parasitic, soybean cyst nematode (SCN: *Heterodera glycines*) contains a gene of similar sequence and equivalent function to a plant peptide hormone called Clavata-Like Element (CLE). Because a full SCN genome is not available, we elected to mine the fully-sequenced genome of the root-knot nematode (RKN) *Meloidogyne hapla*, revealing numerous genes with sequence similarity to plant peptide hormones, including eight CLEs and nine members of the C-terminally Encoded Peptide (CEP) family. Because of their roles in regulating plant cell development, growth and responses to environment, the RKN-encoded CLE and CEP ligands are obvious candidates for playing a central role in the host-parasite interaction. Plant-encoded CLEs and CEPs are expressed as pre-pro-proteins for secretion and proteolytic activation in the extracellular space (apoplast). The RKN genes lack the pro-protein domain, consistent with secretion of active peptides directly into the host apoplast. To dissect the biological role of the RKN-encoded CEPs/CLEs, we have synthesized a panel of wild type and mutant peptides for testing in a plant bioassay we developed. This assay also permits antagonistic and agonistic effects on RNK infection to be measured. It also is amenable to studying the many peptide-hormone receptor mutants available in plants. Using this approach we hoped to elucidate the role of plant peptide hormones in parasitic nematode biology. We also remain acutely interested in the origin of plant-like genes in

parasitic nematodes; did they arise as a result of horizontal gene transfer from their host, or as mimics due to convergence? Ongoing genome analysis will shed light on this question.

Exploiting Caenorhabditis elegans as a heterologous expression system for the functional characterisation of surface coat genes of plant parasitic nematodes

Keith Davies[1], Maria Gravato-Nobre[2], Jonathan Hodgkin[2], and Valerie Williamson[3]
[1]Plant pathology and Microbiology, Rothamsted Research, Harpenden, AL5 2JQ UK
[2]Laboratory of Genes and Development, University of Oxford, Oxford, OX1 UK
[3]Department of Nematology, University of California, Davis, USA

Pasteuria species of bacteria infect nematodes and can be deployed as biological control agents of plant parasitic nematodes. However, they exhibit a wide range of different specificities showing that the surface of the cuticle is variable. The mechanism by which spores of Pasteuria can adhere to the cuticle is poorly understood. *Caenorhabditis elegans* is a model organism and is recently being used to study innate immunity against a wide range of pathogens. *Microbacterium nematophilum* is a bacterium that adheres to the rectal canal of *C. elegans* and causes a swelling that deforms the anal region. Mutagenesis experiments have identified genes that confer resistance to *M. nematophilum* and these genes also affect the surface coat of the nematode. Using BLAST we have identified several orthologous genes in *Meloidogyne hapla*. Constructs have been made that link the tissue specific gene promoter from *C. elegans* to the plant parasitic nematode ortholog amplified from a cDNA library. The results of micro-injection experiments to rescue the original *C. elegans* phenotypes will be discussed.

Characterizing the involvement of Lrp in an X. nematophila phenotypic variation pathway

Elizabeth Hussa [1] and Heidi Goodrich-Blair [1]
[1]Department of Bacteriology, University of Wisconsin-Madison, Madison WI 53706, USA.

Phenotypic variation occurs when a clonal population exhibits two or more distinct phenotypes. Though recently discovered in bacteria, it appears that phenotypic variation occurs in many species, and often influences a bacterium's ability to interact with a host. In *Xenorhabdus nematophila*, a clonal, wild-type population of cells includes both a virulent subpopulation and a subpopulation with decreased virulence toward *Manduca sexta* insect larvae. Our studies indicate a somewhat surprising correlation between this phenotypic variation pathway and expression of the global regulatory protein Lrp; the subpopulation of cells with reduced virulence exhibit higher levels of Lrp protein relative to the virulent population. Previous studies indicate that Lrp is required for optimal virulence, however. Taken together, these data indicate that intermediate levels of Lrp protein correlate with optimal virulence. Given that Lrp also regulates a number of genes involved in mutualistic association with nematodes (such as the nil genes), this phenotypic variation pathway may be important in the transition between insect and nematode hosts. Further studies will focus on this question, as well as determining whether Lrp regulates the switch between phenotypes, or if there are additional, upstream factors involved in this process.

Asymmetric Wolbachia segregation during early Brugia malayi embryogenesis.

Frederic Landmann[1], Barton Slatko[2], Jeremy Foster[2], William Sullivan[1]
[1]Department of Molecular, Cell and Developmental Biology, Sinsheimer Labs, University of California, Santa Cruz, CA 95064, USA
[2]New England Biolabs. Inc., 240 County Road, Ipswich, MA 01938. USA

Filarial nematodes are the causative agents of human filariasis, affecting over 150 million individuals. The most pathogenic diseases, lymphatic filariasis and onchocerciasis (river blindness) comprise a major cause of global morbidity in the tropics, with over 1 billion people at risk of these arthropod-transmitted infections. Three filarial nematode species are responsible for lymphatic filariasis: *Wuchereria bancrofti, Brugia malayi and Brugia timori*, causing lymphatic pathologies that include hydrocoele and lymphoedema (elephantiasis). These parasitic nematodes rely on alpha-proteobacterial *Wolbachia* endosymbionts for development, viability and fertility. Antibiotic treatments deplete *Wolbachia*, resulting in embryonic arrest and a decrease in microfilarial (larval) production. Human trials with doxycycline or rifampicin provide evidence for long-term sterilization and macrofilaricidal (adulticidal) effects against both lymphatic filariasis and onchocerciasis. *Wolbachia* also play a significant role in the pathogenesis of filarial disease. One feature of the symbiotic relationship left unresolved is the localization and segregation patterns of *Wolbachia* during embryogenesis, which is essential to understand its specific localization in adult somatic tissue and the germline. To address this issue, we developed fixation, immunofluorescent staining and imaging protocols to characterize *Wolbachia* in whole-mount *B. malayi* embryos and adult specimens at the tissue, cellular and sub-cellular levels. We will present the asymmetric and lineage-specific segregation patterns of

Wolbachia during the initial stages of embryogenesis that resemble that of some *Caenorhabditis elegans* polarity and lineage-specific determinants, and suggest that *Wolbachia* may interact with the counterparts of these determinants in *B. malayi*.

RpoS-dependent resistance to reactive oxygen species is necessary for* Xenorhabdus nematophila *mutualistic colonization of its nematode host

Xiaojun Lu, Aaron Andersen, Heidi Goodrich-Blair

Department of Bacteriology, University of Wisconsin-Madison

The entomopathogenic bacteria *Xenorhabdus nematophila* can form specie-specific mutualistic relationship with the nematode *Steinernema carpocapsae* by colonizing inside the nematode receptacle, a specialized structure developed at the anterior end of the nematode intestine. A previous study showed that the stress response regulator RpoS is necessary for *X.nematophila* to colonize and develop a mutualistic relationship in its nematode host. In our study we demonstrated that the RpoS-dependent nematode colonization capability of *X.nematophila* is due to RpoS-dependent resistance to reactive oxygen species.

Phase variable expression of* Photorhabdus luminescens *TTO1 mad fimbriae inside the nematode Heterorhabditis bacteriophora

Vishal S. Somvanshi and Todd A. Ciche

Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI 48824

The symbionts *Photorhabdus luminescens* TTO1 (TTO1) bacterium and *Heterorhabditis bacteriophora* nematode cooperate to kill insects. The symbiosis is established through a series of sophisticated and selective bacterial and nematode life cycle events. Offspring nematodes acquire the *Photorhabdus* symbionts maternally. A key initial step in symbiosis is formation of a persistent biofilm on posterior intestinal cells of maternal nematode by *Photorhabdus* cells expressing maternal adherence defective (mad) fimbriae. The expression of mad fimbriae is regulated by a phase variable ON/OFF invertible promoter switch (madswitch); mad fimbriae are expressed only when the madswitch is ON. To investigate the regulation of madswitch inside the nematodes and in cultures, a TTO1 madswitch::GFP reporter strain was constructed. Using recombineering and site specific recombinase technology, the GFP reporter was precisely inserted downstream of madswitch between madA and madB in a way that expression of mad fimbriae was not affected. The flipping ON of the madswitch was directly observed in single cells inside the nematode intestines and in cultures by detecting GFP by fluorescence microscopy. We determined that mad fimbriae are expressed only in a minority of *Photorhabdus* cells present in the nematode gut and in 24 h old cultures. However, madswitch was ON in the cells in the persistent biofilm on the maternal nematode intestine. Interestingly, madswitch turned OFF in the *Photorhabdus* cells present in the gut of infective juveniles (IJs). In summary, we determined that the madswitch first turns ON in the persistent biofilm cells in the maternal nematodes and then turns OFF in the IJs during the nematode life cycle. The regulation of madswitch in the nematode life cycle, insect infection and different environmental conditions are currently being investigated.

Bet-hedging strategy employed by* Photorhabdus *to initiate symbiosis.

Vishal S. Somvanshi, Anthony J. Heidt, Bettina, Kaufmann-Daszczuk, Kwi-suk Kim, and Todd A. Ciche.

Department of Micobiology and Molecular Genetics and the Center for Microbial Pathogenesis, Michigan State University.

Bacteria are known to utilize a bet-hedging strategy whereby phenotypic variants are stochastically generated in a minority of cells. The utility of this may be to survive rapid and severe changes in environmental conditions such as antibiotic exposure or immunogenic selective sweeps. Phenotypic variant forms such as small colony variants (SCVs) are known to occur during host-bacteria associations and can confer antibiotic tolerance and lead to chronic infections. *Photorhabdus* exhibits two different lifestyles, that of a voracious insect pathogen and harmless nematode symbiont. Phenotypic variants of *Photorhabdus* are known to occur although little is known about what regulates their formation and biological function in nematode or insect hosts. Recently, it was determined that *Photorhabdus* cells that initiate symbiosis as a persistent biofilm in maternal nematode intestines are phase variants. The persistent cells have an invertible ON/OFF promoter switch, madswitch, controlling expression of the adhesive fimbrial organelle, ON relative to most other wild type cells. Surprisingly, these cells are also SCVs drastically different from the wt primary forms in many respects. Furthermore, expression of the mad genes is essential for the phenotypic switch to SCVs because most insertional mutants in mad genes are defective in phenotypic switching to SCVs. Additional insights into the regulation of the switch and speculation of why *Photorhabdus* gambles with an essential symbiotic step will be discussed. In summary, the madswitch appears to regulate a Dr. Jekyll to Mr. Hyde like switch, changing pathogen to mutualistic symbiont.

A sensory code for host seeking in parasitic nematodes.

Elissa A. Hallem, Adler R. Dillman, and Paul W. Sternberg

Howard Hughes Medical Institute and Division of Biology, California Institute of Technology, Pasadena, California 91125

How do parasites encode information about host cues, and how are responses to different cues integrated to generate host-seeking behavior? We are addressing these questions using three species as models: *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, and *Caenorhabditis elegans*. We are comparing host-seeking behaviors in response to a large and diverse panel of ecologically-relevant odorants in parasitic infective juveniles (IJs) and *C. elegans* dauers. We find that host seeking involves a broad host signal (carbon dioxide), as well as a broad range of host odors. Overall, the parasitic nematodes respond more similarly to each other than to *C. elegans* despite their phylogenetic distance, supporting a key role for olfaction in their convergently evolved parasitic lifestyles. We showed previously that *C. elegans* adults avoid CO_2, and that this response requires the BAG neurons (Hallem and Sternberg, 2008). In contrast to adult *C. elegans*, we find that *C. elegans* dauers and parasitic IJs are robustly attracted to CO_2. BAG neurons are required for CO_2 attraction in all three species, as well as for attraction of *H. bacteriophora* to *Galleria mellonella* hosts. BAG neurons are also required for CO_2-evoked jumping in *S. carpocapsae*. Thus BAG neurons contribute to multiple aspects of host seeking in parasitic worms. We are now examining the contribution of bacterial symbionts to host-seeking behaviors in parasitic nematodes.

Whole genomic sequencing of Steinernematids Illuminates parasitism and mutualism in nematodes

Adler R. Dillman, Ali Mortazavi, and Paul W. Sternberg.

Howard Hughes Medical Institute, Division of Biology, California Institute of Technology, Pasadena, California 91125

Rapidly lethal endoparasitism of insects enhanced by obligate mutualism with Gram-negative enteric bacteria has arisen at least twice in Nematoda; within the Steinernematidae and Heterorhabditidae. Between these, not only does *Steinernema* appear to be an older lineage, it is also more speciose. We utilize the robust phylogenetic framework and wealth of ecological data of *Steinernema* to inform the sequencing of four whole nematode genomes (*S. carpocapsae, S. scapterisci, S. monticolum*, and *S. glaseri*) along with their respective bacterial symbionts using the Solexa platform. Steinernematid genomes prove amenable to Solexa sequencing due to their size (~95 Mb) and high G+C content (~45%). Having the genomic sequence of four closely related species will increase the resolution of comparative studies within *Steinernema* and allow for powerful comparisons to other genera such as *Caenorhabditis*. We explore the utility of these genomes by examining the conservation of biological pathways (RNAi and dauer juvenile), and identify candidate genes involved in niche partitioning, host range, and mutualism within *Steinernema*. We report the progress and state of these genomes.

***Development of* Heterorhabditis bacteriophora *in rich broth medium using feed of luminous signals produced by* Photorhabdus luminescens.**

Eric Moreau, Sunita Singh, Floyd Inman, and Holmes Leonard.

Biotechnology Center, University of North Carolina, Comtech, Pembroke, North Carolina, USA

The development and reproduction of *Heterorhabditis bacteriophora* was studied using luminous food signal from *Photorhabdus luminescens*. For this the bacteria *P luminescens* was used to produce bioluminescent (food signals) on Carolina nutrient broth, rich medium, to feed infective stage juveniles of *H. bacteriophora*. The study showed that the bacterial luminescence increased only till a specific period. Luminosity was a phenomenon associated to quorum signaling in the bacteria and thus was available for a certain period. The luminosity produced on this medium (CNB) with peptone, yeast extract, glycerol and salt remained at 104 level of bioluminescence in 7-8 days. Also the medium with cellular growth when red pigmentation developed, was fed, from the medium to the juveniles of *H. bacteriophora*. Ringers solution (pH 7.0), was used for growing *H. bacteriophora* with feedings ~(in 48 h), from bacteria and periodical filtration of medium. This helped to keep away protozoa that rapidly developed in the solution with *H. bacteriophora*. The food signals thus produced by the bacteria favored the *H. bacteriophora* development. Though a high frequency of the juveniles did not reach reproductive stage, a few of the adults released eggs, but were lost by breakage after release. Also some crystalline structures were released into the medium after this stage. The osmolarity required for their development; the period for signal requirement in the life cycle during broth culture may still need confirmation. The last instars of the lepidopteran insect *Galleria mellonella* showed bioluminescence after they were injected with the same food signal from CNB medium directly. The bioluminescent signals play a critical role in *H. bacteriophora* development an important intermediate in biocontrol.

***Symbiotic properties of the entomopathogenic bacterium*, Photorhabdus luminescens*, and its nematode partner*, Heterorhabditis bacteriophora**

Len Holmes and Floyd L. Inman III
UNCP Biotechnology Research and Training Center
The University of North Carolina at Pembroke
Pembroke NC 28372

Photorhabdus luminescens is a biphasic, Gram-negative, bioluminescent enteric bacterium that maintains a mutualistic relationship with its nematode host, *Heterorhabditis bacteriophora*. Not only does the bacterium maintain this close relationship, it also exhibits pathogenicity towards a diverse group of insects. Reports suggest that the phase I variant signals nematode development and that its virulence may provide an excellent breeding ground for nematode reproduction inside of the insect carcass. Initial investigations began when attempting to culture the nematodes in liquid media. Reported liquid media were used and of those, only one medium exhibited nematode growth and development. Investigational data suggests that strain specificity of the bacterium is necessary for nematode reproduction. It was determined more research was needed to understand how this bacterium can support the growth and development of the nematode. One investigation studied the antimicrobial pigment produced by *P. luminescens* and it was shown that eleven species and strains of bacteria were determined to be sensitive to this pigment. This suggested that *P. luminescens* may have the capability to fight off many other competing microbes. Another investigation studied the effects of carbohydrate utilization on the stability and bioluminescence of the phase I variant. Preliminary results showed that trehalose, the blood sugar of insects, increased stability and bioluminescence of the phase I variant for seven days in liquid culture media. Genetic investigations are currently being performed on the isolated bacterial strain, *P. luminescens* ARB. Recent results show that this strain carries a plasmid that is approximately 29 kilobases in size and is in the process of being sequenced. Plasmid electro-transformation results of *Escherichia coli* DH10α suggest that this isolated plasmid may confer kanamycin resistance. Experimental studies of pathogenicity and symbiosis are currently being designed to utilize the electro-transformed *E. coli* as a bacterial replacement for *P. luminescens* ARB.