Cooperative endurance: A remarkable strategy adopted by symbiotic bacteria to persist in their nematode vector

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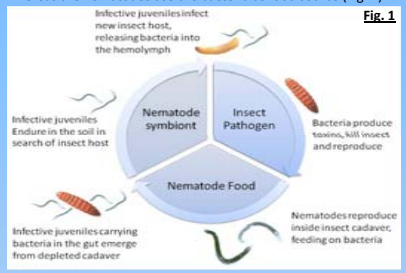
Symbioses between microbes and animals are ubiquitous, yet little is known about the intricate mechanisms maintaining such associations. In an emerging animal-microbe symbiosis model system represented by the partnership between insect-pathogenic bacteria *Photorhabdus temperata* and insect-parasitic nematodes *Heterorhabditis bacteriophora*, we investigated molecular mechanisms adopted by the bacteria to persist in the enduring nematode vector in search of their insect host. Using selective capture of transcribed sequences approach, 50 transcripts were identified to be up-regulated and 56 were down-regulated by the bacteria during persistence in the nematode compared with growth in culture medium. Real-time PCR analysis of 14 representative transcripts displayed 6-12 fold change in expression, reflecting a significant shift in bacterial gene expression in the nematode. The identified transcripts included but not limited to genes involved in proton transport, metabolic pathways, biofilm formation and cell motility, suggesting that the bacteria undergo major transcriptional reshaping in the nematode vector. Besides general starvation mechanisms, the bacteria induce cellular acidification to slow down growth, switch to pentose phosphate pathway to overcome oxidative stress and nutrition limitation, and shed motility but develop biofilm to persist in the nematode intestine until being released into the insect hemolymph. Our mutation data further confirm that such transcriptional reshaping is critical for bacteria to persist in the nematode infective juvenile. These findings demonstrate how the symbiotic bacteria reduce their nutritional dependence on the enduring nematode partner to ensure successful transmission of the couple to the next insect host.

INTRODUCTION

❖Association bet. insect-pathogenic bacteria *Photorhabdus* and insect-parasitic nematodes *Heterorhabditis* represents one of the best-developed systems in symbiosis[1].

❖The association allows the bacteria to promote their transmission among insects by using nematode as a vector whereas the nematodes use the bacteria as food source (Fig. 1).

Fig. 1



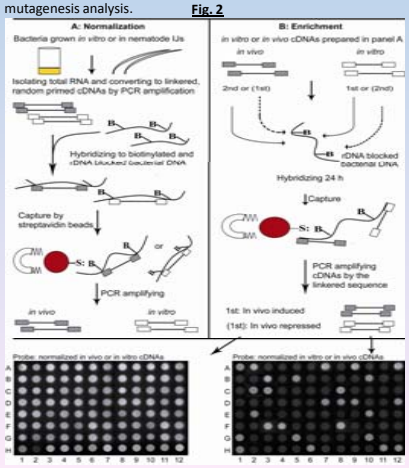
❖We investigated the molecular strategy used by the bacteria to persist in the non-feeding enduring infective juveniles which persist in the soil in search for a suitable host for the couple.

MATERIALS & METHODS

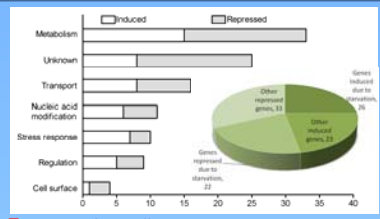
❖Gene expression of *P. temperata* in the enduring infective juveniles of *H. bacteriophora* was profiled with selective capture of transcribed sequences approach followed by southern blot screening[2] (Fig. 2) and qRT-PCR validation.

❖The role of a subset of the identified genes in nematode-bacteria interaction was evaluated through insertion-deletion mutagenesis analysis.

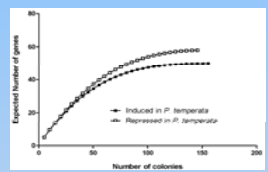
Fig. 2



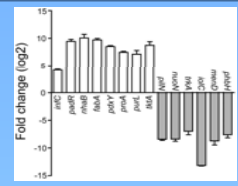
RESULTS



❑The identified differentially expressed genes were distributed in seven functional groups. Screening with bacterial stationary-phase cDNA libraries suggested that about a half of the genes were associated with starvation.



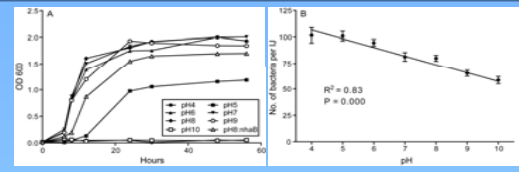
❑Rarefaction analysis curves demonstrating coverage of cDNA libraries for genes identified from *P. temperata* during persistence in *H. bacteriophora*.



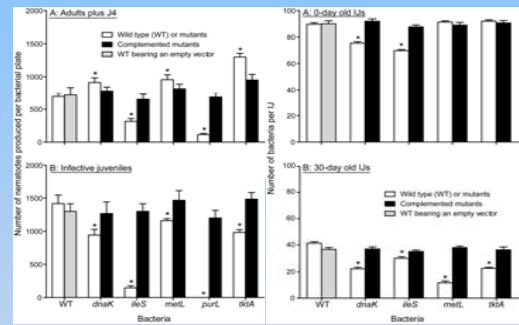
❑Differential gene expression was confirmed by qRT-PCR analysis of 14 randomly selected genes.

A subset of differentially expressed genes

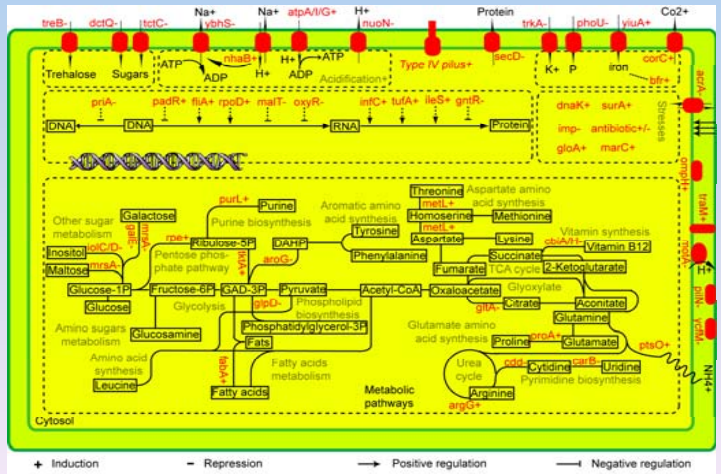
Gene	Possible function	Expression
tktA	Transketolase	+ (induced)
gltA	Citrate synthase	- (repressed)
argG	Argininosuccinate synthase	+
purL	FGAM synthase	+
nhaB	Sodium/proton antiporter	+
secD	Protein-export transporter	-
phoU	Phosphate transporter	-
tctC	Tricarboxylic transporter	-
corC	Mg^{2+}/Co^{2+} efflux protein	+
trkA	K^+ importer	-
gloA	Glyoxalase resistance protein	-
fliA	Flagella synthesis sigma factor	+
motA	Chemotaxis protein	-



(A): *P. temperata* growth in-vitro under different pH conditions, suggesting that up-regulation of *nhaB* gene enables the bacteria to reduce growth. (B): Bacterial persistence in the infective juvenile exposed to different pH conditions.



Left: Reproduction of *H. bacteriophora* (adults, 4th stage and infective juveniles) with *P. temperata* mutants of the identified genes. Right: Persistence of *P. temperata* mutants in freshly-produced and 30-day-old *H. bacteriophora* infective juveniles.



❑Conceptual molecular model showing contributions of differentially expressed genes during symbiotic persistence of the bacteria in the infective juveniles. Key features include bacteria maintaining intracellular H^+ level by regulation of proton transport systems, limiting carbon metabolism by repression of glucose conversion genes, and switching from TCA cycle to pentose phosphate pathway.

DISCUSSION & CONCLUSIONS

❖This is the first comprehensive global profile of differentially regulated "symbiosis genes", providing insights into the molecular mechanisms by which bacteria persist in their nematode vector.

❖The bacteria make major physiological shifts in the enduring nematode infective juvenile representing a form of "cooperative endurance" to ensure their transmission to a new insect host.

❖The bacteria induce cellular acidification via regulation of proton transport systems, switch to pentose phosphate pathway, shed motility but form biofilm to persist in the nematode intestine.

❖More details are available from "An R. & Grewal P.S. (2010) PLoS One 5 (10):e13154".

ACKNOWLEDGMENTS

This project was supported by interdisciplinary grant from the Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio.

[1] Ruby E.G. (2008) Nat Rev Microbiol 6: 752-762.

[2] Graham J.E. & Clark-Curtiss J.E. (1999) Proc Natl Acad Sci USA 96: 11554-11559.