Nova Acta Leopoldina NF *88*, Nr. 333, 93–100 (2004)

Tn*luxAB* Insertion Mutants of *Vibrio fischeri* with Symbiosis-regulated Phenotypes

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With 3 Figures and 1 Table

Abstract

The symbiosis between the luminous marine bacterium *Vibrio fischeri* and the Hawaiian sepiolid squid *Euprymna scolopes* provides a model system for the study of signal exchange between bacteria and the hosts they colonize. To identify bacterial genes specifically induced in response to colonization of host tissue, transposon mutagenesis using luminescence (*lux*) as a reporter of gene expression was performed in a *luxA*-mutant (i. e., dark) strain of symbiotically competent *V. fischeri*. The transposon was inserted randomly into the bacterium's chromosome, and the resulting transconjugants exhibited a wide range of luminescence levels when grown in culture. The luminescence levels of approximately 1,500 individual insertion mutants were determined for cells growing in culture and in the light organ of juvenile *E. scolopes*. About 40 % of these mutants produced detectable amounts of luminescence in the squid, and of these, three strains were identified that appeared to have an induced expression of luminescence during colonization of the host. The regions of DNA flanking the transposon in each of these mutants have been sequenced, revealing two identifiable (*glpD* and *iucA*) and one unknown (*cinA*) gene that were disrupted by an insertion. These results indicate that during the first 24 hours following entry into the light organ *V. fischeri* increases the expression of loci involved in both lipid metabolism and siderophore production, as well as other, as yet undefined, functions. The increased expression of these metabolic activities is consistent with previous studies of both the physiological conditions in the light organ crypts, and colonization-induced genes in a diversity of bacterial pathogens.

Zusammenfassung

Die Symbiose zwischen dem lumineszierenden, marinen Bakterium *Vibrio fischeri* und dem in Hawaii heimischen Tintenfisch *Euprymna scolopes* dient als Modellsystem zur Erforschung des Signalaustauschs von Bakterien mit den von ihnen kolonisierten Wirten. Um bakterielle Gene zu identifizieren, die speziell während der Kolonisierung des Wirtes induziert werden, wurde eine Transposon-Mutagenese in einer *luxA*(nicht lumineszierenden)-Mutante des symbiotisch kompetenten Bakteriums *V. fischeri* durchgeführt, die Luziferase-Gene *luxA* und *luxB* dienten dabei als Reporter der Genexpression. Die Transposition erfolgte wahllos in die genomische DNA des Bakteriums, die Lichtemissionswerte der resultierenden Transkonjuganten in Kultur variierten daher erheblich. Die Lumineszenzcharakteristik von circa 1500 individuellen Insertionsmutanten wurde sowohl in Kultur als auch im Lichtorgan von *E. scolopes* bestimmt. Etwa 40 % dieser Mutanten produzierten nachweisbare Lumineszenzwerte im Wirt, von diesen wurden drei Stämme identifiziert, deren spezifische Lumineszenz während des Kolonisierungsprozesses anstieg. Die das Transposon flankierende DNA dieser drei Mutanten wurde sequenziert, durch die Sequenz konnten zwei Gene identifiziert werden, die eine Transposoninsertion aufwiesen (*glpD* und *iucA*), ein Gen war jedoch nicht identifizierbar (*cinA*). Diese Ergebnisse deuten darauf hin, daß während der ersten 24 h nach dem Kontakt von *V. fischeri* mit dem Lichtorgan von *E. scolopes* die Expression von Genen, die mit Fettmetabolismus, Siderophoreproduktion und anderen, nicht definierten, Funktionen verbunden sind, ansteigt. Die gesteigerte Expression dieser meta-

bolischen Aktivitäten steht in Übereinstimmung mit vorhergehenden Studien von sowohl den physiologischen Bedingungen im Lichtorgan von *E. scolopes* als auch von kolonisierungsinduzierenden Genen einer Vielzahl pathogener Bakterien.

1. Introduction

In their natural state all animals and plants maintain associations with a specific and often obligate microbiota. While for a small number of microorganisms the interactions are pathogenic, the vast majority are benign or even beneficial to their host. The nature of these associations is often complex, both in the number of participants and the communication by which the partners recognize and cooperate with each other. Thus, with only a few exceptions (e. g., GAGE and MARGOLIN 2000, KOLENBRANDER et al. 2002), only recently have the biochemical and molecular mechanisms characterizing these interactions been accessible for study (MCFALL-NGAI 2002).

When juveniles of the bioluminescent squid *Euprymna scolopes* first hatch, their nascent light-emitting organs are bacteria-free; however, if *Vibrio fischeri* cells are present in the ambient seawater, the organ will rapidly and specifically become colonized (MCFALL-NGAI 1999). These bacteria colonize the tissue by entering pores on the surface of the organ that lead to epithelium-lined crypt spaces in which the inoculum proliferates (NYHOLM et al. 2000). Within 12 h the growing population of *V. fischeri* induces the expression of its *lux* operon, which encodes the light-emitting, oxygen-requiring, enzyme luciferase, and the host becomes bioluminescent. As the colonization persists the bacteria undergo additional differentiation: they become smaller and at least some of the cells no longer produce polar flagella (RUBY and ASATO 1993). The bacteria also trigger a specific program of developmental changes in the tissues of the host (MCFALL-NGAI 2002), and each morning 95 % of the bacterial population is expelled into the environment, leaving the remaining cells to repopulate the light organ by nightfall (BOETTCHER et al. 1996, LEE and RUBY 1994a). Thus, both of the partners differentiate and change aspects of their cellular behavior in response to the symbiosis. To better understand the basis for these changes, and the signals underlying their action, we have developed a method for identifying *V. fischeri* genes that are preferentially induced when the bacterium initiates the colonization of the light organ.

In recent years a number of genetic tools have been developed to reveal genes that are induced as a result of either pathogenic or cooperative bacterial colonization of host tissue (LEE and CAMILLI 2000). Luminescence has been used effectively as a reporter of gene expression in several systems, either to identify environmentally regulated genes, or to characterize the expression patterns of specific genes (STEWART et al. 1996, KRICKA 2000). Because the squid light organ is ideally suited for reporting the emission of luminescence, we chose the promoterless luciferase genes, *luxAB* (VISICK and RUBY 1998, D. MILLIKAN personal communication), to report the expression pattern of *V. fischeri* genes during the first 24 h of the symbiotic interaction. Using this approach as a screen, several genes were identified, whose expression appeared to be increased as the bacteria initiate the colonization of the light organ crypts. The nature of these genes, and their encoded enzymes, was consistent with other information obtained about the conditions present in the light-organ environment.

2. Results and Discussion

To adapt a luciferase-based detection system to the study of a luminous bacterium it was first necessary to construct a *luxA*-null mutant of a symbiotic strain of *V. fischeri*. In this way there would be no intrinsic light emission from the bacterium that would confound the screen for induced promoters. Thus, the first step was to construct a Δ*luxA* mutant of *V. fischeri* ES114 by gene replacement with an erythromycin resistance (*erm*) gene (Fig. 1*A*) and test this mutant for the retention of symbiotic competence (VISICK and RUBY 1996). We found that during the first 24 h after symbiosis the initiated level of colonization by the Δ*luxA*::*erm* mutant (strain KV150) was similar to that of its parent (VISICK and RUBY 1996, VISICK et al. 2000). This result suggested that the mutant's use in the preliminary identification of early symbiosis-induced genes would be feasible. In addition we found that complementation with a plasmid carrying wild-type *V. fischeri luxAB* genes under the control of the *lac* promoter restored luminescence to the mutant (VISICK and RUBY 1996, and data not shown). This latter finding indicated that the *erm* insertion created no significant polar effect on the downstream (*luxE*) gene of the *lux* operon (Fig. 1*A*), which is required for the synthesis of aliphatic aldehyde, a substrate of the luminescence reaction (HASTINGS and NEALSON 1977).

In the second step, a mini-transposon, Tn*10* (ALEXEYEV and SHOKOLENKO 1995), was modified to carry a promoterless copy of the *Vibrio harveyi luxAB* genes and a chloramphenicol (Cam) resistance marker (VISICK and RUBY 1997), as well as a R6K origin suitable for use in *V. fischeri*. When introduced by conjugation on a suicide vector, this transposon inserted into the chromosome of *V. fischeri* KV150. The resulting transconjugant strains were found to produce a range of levels of luminescence that presumably depended upon promoter activity levels in the locus of the insertion (Fig. 1*B*).

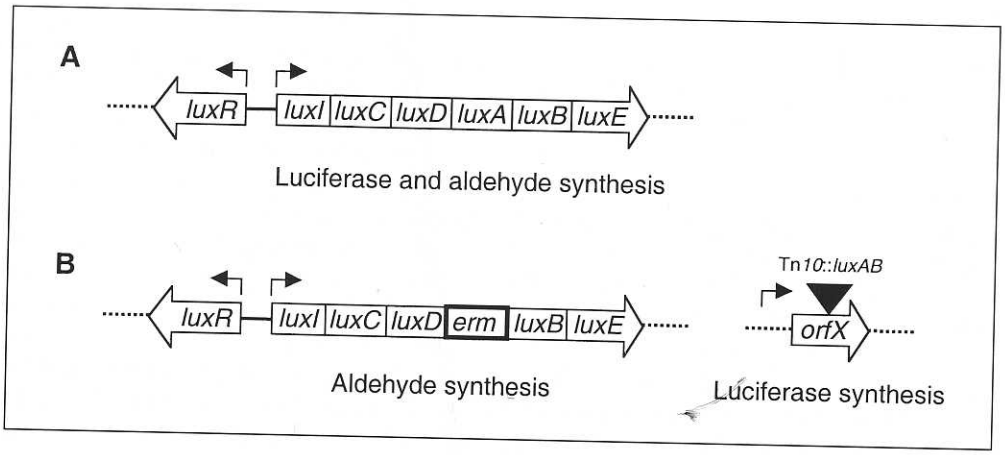

Fig. 1 Schematic illustration of the *V. fischeri lux* operon. (*A*) The wild-type operon, composed of two divergently transcribed units, *luxICDABE* and *luxR*. Bacterial luciferase is encoded by *luxAB*, and enzymes required for the synthesis of the luciferase's aldehyde substrate are encoded by *luxCDE*. The functions of *luxI* and *luxR* are regulatory. (*B*) A Δ*luxA* mutant strain (KV150) was created by gene replacement with an erythromycin resistance gene (*erm*). KV150 was the parent strain for mutagenesis by random insertion of a miniTn*10*::*luxAB* cassette that provided the *luxA* function under the control of the promoter of the interrupted gene (*orfX*).

Using this system of mutagenesis 1.500 individual Tn*luxAB* mutants were isolated, and subsequent Southern analysis of a subset of them was consistent with a random pattern of insertion (data not shown). The relative luminescence level of each of these mutants during growth in a conditioned medium (CM) (Boettcher and Ruby 1990) was determined and compared to that during initiation of colonization in juvenile squid. While about 40 % of the strains produced detectable levels of light emission in the host, only a small number of these appeared to have a markedly increased luminescence activity relative to that in culture. The genetic locus in which several of these insertions occurred was determined by partial sequencing of the regions of DNA flanking the transposon, and comparing these results to the sequence of the *V. fischeri* genome (http://ergo.integratedgenomics.com/Genomes/VFI/). Strains with insertions in homologs of two described genes (*iucA* and *glpD*), and one, colonization-induced gene A (*cinA*), without a clear ortholog, were identified (Tab. 1).

Tab. 1 Extent of colonization by Tn*10*::*luxAB* insertion strains of *V. fischeri*

Strain	Genotype	Putative Tn*10*-interrupted gene function	CFU/organ[1] ($\times 10^5$)
KV 150	Δ*luxA* (no Tn*10* insertion)	(Parent strain)	1.6 +/– 0.9
KV495	Δ*luxA*;Tn*10*::*iucA*	Siderophore synthesis	1.2 +/– 0.4
KV496	Δ*luxA*; Tn*10*::*carB*	Arginine/pyrimidine synthesis	1.0 +/– 0.4
KV504	Δ*luxA*; Tn*10*::*glpD*	Aerobic glycerolipid catabolism	1.5 +/– 0.7
KV511	Δ*luxA*; Tn*10*::*cinA*	Unknown	1.4 +/– 0.6

[1] Determined as previously described (Ruby and Asato 1993), at approximately 22 h after the initiation of colonization. Values are the averages of nine animals per treatment, plus or minus the standard error of the mean.

To verify that these three promoters were activated during colonization relative to their level of expression in culture, each candidate strain was matched with another transposon-insertion strain that had the same growth rate and expressed approximately the same level of luminescence in culture. The levels of luminescence of squids infected with these matched strains were then compared during the first 22 h of colonization (Fig. 2). Insertions in all three of these genes were induced about 8- to 10-fold relative to their matched partner strain, while other genes (like *carB*) were not (Fig. 3).

To determine whether the insertion had an effect on the level of colonization, we quantified the number of *V. fischeri* cells present in the light organ 22 h after colonization (Tab. 1). There was no significant difference between the number of symbionts in the light organs of animals colonized by any of the insertion mutants relative to the parent Δ*luxA* strain, KV150. This result also indicates that the activities of the proteins encoded by these induced genes are not required to achieve normal levels of colonization during at least the initial phase of the association.

The enzymes encoded by both *iucA* and *glpD* are involved in metabolic pathways found in *V. fischeri*, and are consistent with what is known about the nutrition of this bacterium in the symbiosis. For instance, the *V. fischeri* genome encodes a number of putative siderophore synthetases, including IucA, homologs of which catalyze aerobactin synthesis in *Escherichia coli* and other bacteria (Vokes et al. 1999). We determined that *iucA* was re-

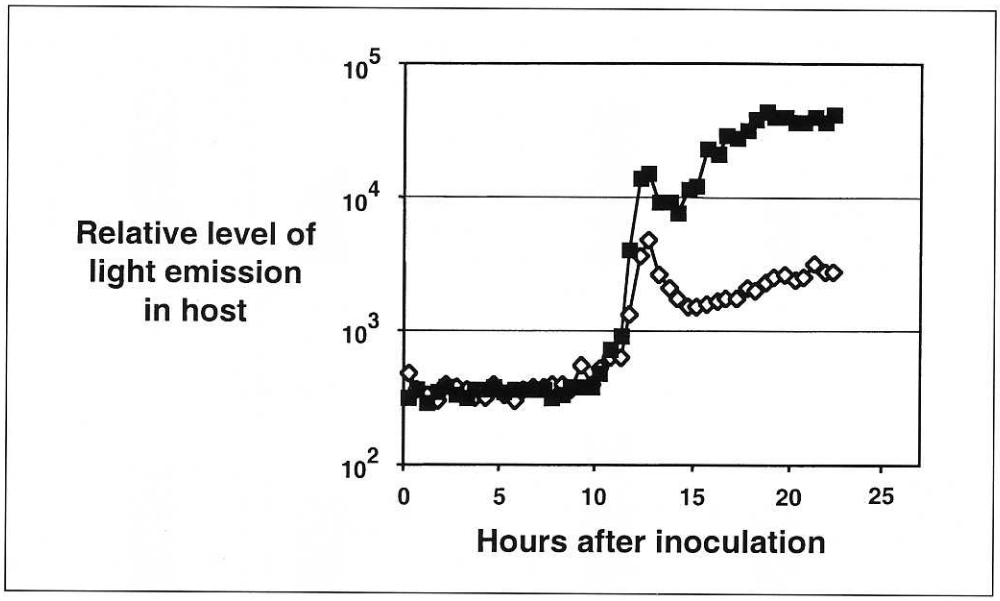

Fig. 2 Bioluminescence of strain KV504 (Δ*luxA*; Tn*10*::*glpD*) during colonization of juvenile *E. scolopes* squids. Animals were exposed to an inoculum of either KV504 (solid squares) or another transposon insertion mutant strain that expressed a similar level of luminescence in culture (open diamonds). The light emission of the animals was measured periodically over the first 22 h after inoculation, providing an indication of the interrupted gene's promoter activity during colonization. Each point is the mean value of the light levels of nine animals.

sponsible for siderophore synthesis in *V. fischeri* as well: growth on low-Fe medium containing the siderophore indicator CAS (Lee and Ruby 1994b) revealed the absence of detectable Fe-scavenging activity in the *V. fischeri iucA* transposon mutant (data not shown). The ability to synthesize siderophores is known to be a required activity during tissue colonization in a number of pathogenic bacteria (Crosa and Walsh 2002). Previous work with a regulatory gene mutation that led to decreased siderophore synthesis in *V. fischeri* similarly showed that persistence of a normal symbiotic colonization required this activity (Graf and Ruby 2000). Thus, an increased expression of the *V. fischeri iucA* homolog during symbiosis would not be unexpected. Interestingly, while other siderophore synthetases are present, no homolog for *iucA* occurs in the genome of the sequenced strain of the closely related pathogenic species *Vibrio cholerae* (Heidelberg et al. 2000).

V. fischeri is a facultative aerobe that grows well in either the presence or absence of oxygen; however, its luminescence activity in the symbiosis is an indication that oxygen must be present, at least at low levels, in the light-organ crypt environment (Boettcher et al. 1996, Ruby and McFall-Ngai 1999). The aerobic form of glycerol-3-phosphate dehydrogenase is encoded by *glpD* in *E. coli* (Lin 1987, Walz et al. 2002) and other bacteria. This enzyme is induced under conditions of aerobic glycerol utilization, such as during growth on host membrane phospholipids by pathogenic bacteria (Schmiel and Miller 1999). In *E. coli* the synthesis of this enzyme is normally highly repressed; similarly, the *glpD* transposon-insertion mutant in *V. fischeri* produced relatively low lev-

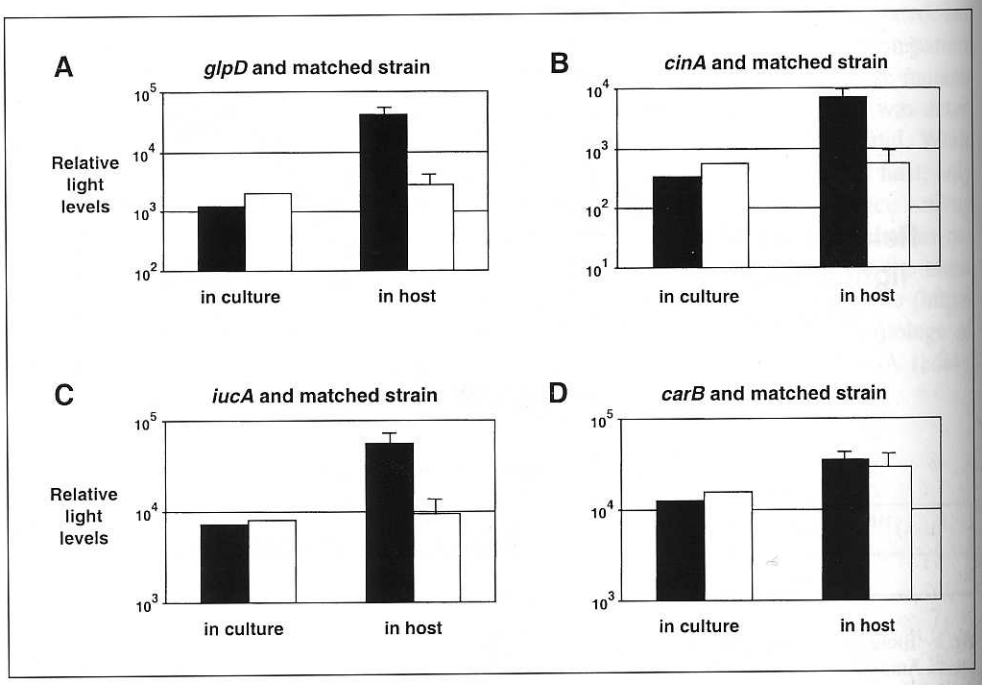

Fig. 3. Bioluminescence of transposon mutants in culture and in the host. The relative levels of light emission of strains growing in culture medium were measured at an optical density of 1.0 (about 10^9 cells per ml). The light levels of four strains, (*A*) KV504, (*B*) KV511, (*C*) KV495, and (*D*) KV496, with insertions in *glpD*, *cinA*, *iucA*, or *carB*, respectively (solid bars), were each matched to one of four other transposon mutants (open bars) that were found to have similar bioluminescence levels in culture. When the light emission of these pairs of strains was compared during colonization, *glpD*, *cinA* and *iucA*, each induced luminescence about eight- to ten-fold more than their matched strain (e. g., see Fig. 2). Such a relative increase was not observed with insertions in genes like *carB*, which apparently is not specifically induced during colonization. Values for the light levels in the host after 22 h are the means of nine animals per treatment, and the standard errors of the means are indicated with error bars.

els of light in the tryptone-based CM broth (data not shown). However, after the first 12 h of light organ colonization the expression of this promoter is apparently induced (Fig. 2), suggesting the presence in the light organ crypts of both aerobic conditions, and this enzyme's substrate. It may be significant that the epithelial cells lining the light organ crypts slough off periodically, releasing a large quantity of lipid vesicles into the crypt environment of the bacterial symbionts (NYHOLM and MCFALL-NGAI 1998). This event occurs at daybreak, which first occurs about 12 h after colonization is initiated, a time that is consistent with when *glpD* induction first becomes apparent (Fig. 2).

There are no recognizable homologs of the third colonization-induced gene, *cinA*, in the public database. Nevertheless, it is interesting that the results of a Pfam analysis (SONNHAMMER et al. 1997) of *cinA* suggest that it may encode a protein containing a lipid hydrolase domain. *V. fischeri* produces in culture an extracellular lipase activity (REICHELT and BAUMANN 1973) which is still present in the *cinA* mutant (data not shown); however, conclusions based on this result should be mindful that other genes encoding putative lipase activities can be found in the *V. fischeri* genome.

While at least two of the three colonization-induced genes described here encode functions that not only are consistent with what is known about the environment of the light organ crypts, but also similar to functions reported for other tissue-colonizing bacteria, the role of these genes in symbiosis is only presumptive. Further studies will be required in which the promoter region for each gene is placed on the chromosome in single copy in front of a different reporter fusion (e. g., to a promoterless green-fluorescent protein gene), in the wild type *V. fischeri* Tn*7* site (VISICK and RUBY 1997, MCCANN et al. 2001). It would then be possible to determine the nature of, and possible requirement for, the activity of these promoters in a wild-type (lux^+) background, which would allow a more accurate and long-term evaluation of their dynamics and function in the symbiosis (VISICK et al. 2000).

Acknowledgements

We would like to thank the members of the E. G. R. laboratory for valuable advice during the development of this project. C. WHISTLER tested the *iucA* mutant for siderophore activity, and C. LUPP kindly provided German translation. K. L. V. was supported by National Research Service Award GM17424 from the National Institutes of Health. This work was supported in part by NIH grant RR12294 to E. G. R. and M. MCFALL-NGAI, and National Science Foundation grant IBN-0211673 to M. M-N. and E. G. R. Genomic sequence information was made possible by the W. M. KECK Foundation through funding to E. P. GREENBERG and E. G. R. for the *Vibrio fischeri* Genome Project.

References

ALEXEYEV, M. F., and SHOKOLENKO, I. N.: Mini-Tn*10* transposon derivatives for insertion mutagenesis and gene delivery into the chromosome of Gram-negative bacteria. Gene *160*, 59–62 (1995)

BOETTCHER, K. J., and RUBY, E. G.: Depressed light emission by symbiotic *Vibrio fischeri* of the sepiolid squid *Euprymna scolopes*. J. Bacteriol. *172*, 3701–3706 (1990)

BOETTCHER K. J., RUBY, E. G., and MCFALL-NGAI, M. J.: Bioluminescence in the symbiotic squid *Euprymna scolopes* is controlled by a daily biological rhythm. J. Comp. Physiol. *179*, 65–73 (1996)

CROSA, J. H., and WALSH, C. T.: Genetics and assembly line enzymology of siderophore biosynthesis in bacteria. Microbiol. Mol. Biol. Rev. *66*, 223–249 (2002)

GAGE, D., and MARGOLIN, W.: Hanging by a thread: invasion of legume plants by rhizobia. Curr. Opin. Microbiol. *3*, 613–617 (2000)

GRAF, J., and RUBY, E. G.: Novel effects of a transposon insertion in the *Vibrio fischeri glnD* gene: defects in iron uptake and symbiotic persistence in addition to nitrogen utilization. Mol. Microbiol. *37*, 168–179 (2000)

HASTINGS, J. W., and NEALSON, K. H.: Bacterial bioluminescence. Annu. Rev. Microbiol. *31*, 549–595 (1977)

HEIDELBERG, J. F., EISEN, J. A., NELSON, W. C., CLAYTON, R. A., GWINN, M. L., DODSON, R. J., HAFT, D. H., HICKEY, E. K., PETERSON, J. D., UMAYAM, L., GILL, S. R., NELSON, K. E., READ, T. D., TETTELIN, H., RICHARDSON, D., ERMOLAEVA, M. D., VAMATHEVAN, J., BASS, S., QIN, H., DRAGOI, I., SELLERS, P., MCDONALD, L., UTTERBACK, T., FLEISHMANN, R. D., NIERMAN, W. C., WHITE, O., SALZBERG, S. L., SMITH, H. O., COLWELL, R. R., MEKALANOS, J. J., VENTER, J. C., and FRASER, C. M.: DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae*. Nature *406*, 477–483 (2000)

KOLENBRANDER, P. E., ANDERSON, R. N., BLEHART, D. S., EGLAND, P. G., FOSTER, J. S., and PALMER, R. J. Jr.: Communication among oral bacteria. Microbiol. Mol. Biol. Rev. *66*, 486–505 (2002)

KRICKA, L. J.: Applications of bioluminescence and chemiluminescence in biomedical sciences. Meth. Enzymol. *305*, 333–345 (2000)

LEE, S. H., and CAMILLI, A.: Novel approaches to monitor bacterial gene expression in infected tissue and host. Curr. Opin. Microbiol. *3*, 97–101 (2000)

Lee, K.-H., and Ruby, E. G.: Effect of the squid host on the abundance and distribution of symbiotic Vibrio fischeri in nature. Appl. Environ. Microbiol. *60*, 1565–1571 (1994a)

Lee, K.-H., and Ruby, E. G.: Competition between *Vibrio fischeri* strains during initiation and maintenance of a light organ symbiosis. J. Bacteriol. *176*, 1985–1991 (1994b)

Lin, E. C. C.: Dissimilatory pathways for sugars, polyols, and carboxylates. In: Neidhardt, F. C. (Ed.): *Escherichia coli* and *Salmonella typhimurium* Cellular and Molecular Biology; pp. 244–284. Washington, D. C.: American Society for Microbiology Press 1987

McCann, J. R., Stabb, E. V., and Ruby, E. G.: Characterization of the early stages of infection in the cooperative association between *Vibrio fischeri* and *Euprymna scolopes*. Abstr. Genl. Meet. Amer. Soc. Microbiol. *101*, 504 (2001)

McFall-Ngai, M. J.: Consequences of evolving with bacterial symbionts: insights from the squid-vibrio associations. Annu. Rev. Ecol. Syst. *30*, 235–256 (1999)

McFall-Ngai, M. J.: Unseen forces: the influence of bacteria on animal development. Dev. Biol. *242*, 1–14 (2002)

Nyholm, S. V., and McFall-Ngai, M. J.: Sampling the microenvironment of the *Euprymna scolopes* light organ: description of a population of host cells with the bacterial symbiont *Vibrio fischeri*. Biol. Bull. *195*, 89–97 (1998)

Nyholm, S. V., Stabb, E. V., Ruby, E. G., and McFall-Ngai, M. J.: Establishment of an animal-bacterial association: recruiting symbiotic vibrios from the environment. Proc. Natl. Acad. Sci. USA *97*, 10231–10235 (2000)

Reichelt, J. L., and Baumann, P.: Taxonomy of the marine, luminous bacteria. Arch. Mikrobiol. *94*, 283–330 (1973)

Ruby, E. G., and Asato, L. M.: Growth and flagellation of *Vibrio fischeri* during initiation of the sepiolid squid light organ symbiosis. Arch. Microbiol. *159*, 160–167 (1993)

Ruby, E. G., and McFall-Ngai, M. J.: Oxygen-utilizing reactions and symbiotic colonization of the squid light organ by *Vibrio fischeri*. Trends Microbiol. *7*, 414–420 (1999)

Schmiel, D. H., and Miller, V. L.: Bacterial phospholipases and pathogenesis. Microbes Infect. *1*, 1103–1112 (1999)

Sonnhammer, E. L., Eddy, S. R., and Durbin, R.: Pfam: a comprehensive database of protein domain families based on seed alignments. Proteins *28*, 405–420 (1997)

Stewart, G. S. A. B., Loessner, M. J., and Scherer, S.: The bacterial *lux* gene bioluminescent biosensor revisited. ASM News *62*, 297–301 (1996)

Visick, K. L., Foster, J., Doino, J., McFall-Ngai, M., and Ruby, E. G.: *Vibrio fischeri lux* genes play an important role in colonization and development of the host light organ. J. Bacteriol. *182*, 4578–4586 (2000)

Visick, K. L., and Ruby, E. G.: Construction and symbiotic competence of a *luxA*-deletion mutant of *Vibrio fischeri*. Gene *175*, 89–94 (1996)

Visick, K. L., and Ruby, E. G.: New genetic tools for use in the marine bioluminescent bacterium *Vibrio fischeri*. In: Hastings, J. W., Kricka, L. J., and Stanley, P. E. (Eds.): Bioluminescence and Chemiluminescence; pp. 119–122. New York City: Wiley and Sons 1997

Visick, K. L., and Ruby, E. G.: Tn*luxAB* insertion mutants of *Vibrio fischeri* with symbiosis-regulated phenotypes. Abstr. Genl. Meet. Amer. Soc. Microbiol. *98*, 277 (1998)

Vokes, S. A., Reeves, S. A., Torres, A. G., and Payne, S. M.: The aerobactin iron transport system genes in *Shigella flexneri* are present within a pathogenicity island. Mol. Microbiol. *33*, 63–73 (1999)

Walz, A. C., Demel, R. A., Kruijff, B. de, and Mutzel, R. Aerobic sn-glycerol-3-phosphate dehydrogenase from *Escherichia coli* binds to the cytoplasmic membrane through an amphipathic alpha-helix. Biochem. J. *365*, 471–479 (2002)

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