ELSEVIER

Gene 175 (1996) 89–94

GENE
AN INTERNATIONAL JOURNAL ON GENES AND GENOMES

Construction and symbiotic competence of a *luxA*-deletion mutant of *Vibrio fischeri*

Karen Gillen Visick, Edward G. Ruby *

Department of Biological Sciences University of Southern California, Los Angeles, CA 90089-0371, USA

Received 23 October 1995; revised 19 January 1996; accepted 2 February 1996

Abstract

Bioluminescence by the squid *Euprymna scolopes* requires colonization of its light organ by the symbiotic luminous bacterium *Vibrio fischeri*. Investigation of the genetic determinants underlying bacterial symbiotic competence in this system has necessitated the continuing establishment and application of molecular genetic techniques in *V. fischeri*. We developed a procedure for the introduction of plasmid DNA into *V. fischeri* by electroporation, and isolated a mutant strain that overcame the apparent restriction barrier between *V. fischeri* and *Escherichia coli*. Using the technique of electroporation in combination with that of gene replacement, we constructed a non-luminous strain of *V. fischeri* (*ΔluxA*: :*erm*). In addition, we used the transducing phage rp-1 for the first time to transfer a chromosomal antibiotic resistance marker to another strain of *V. fischeri*. The *luxA* mutant was able to colonize *E. scolopes* as quickly and to the same extent as wild type. This result suggested that, at least during the initial stages of colonization, luminescence per se is not an essential factor for the symbiotic infection.

Keywords: Luminescence; Electroporation; Transduction; ES114; MJ1; *Euprymna scolopes*

1. Introduction

The bioluminescent bacterium *Vibrio fischeri* colonizes the nascent light organ of juveniles of the sepiolid squid *Euprymna scolopes*, and establishes a long-term cooperative symbiosis (McFall-Ngai and Ruby, 1991). This benign infection occurs within a few hours of the squid's hatching, and results in a monospecific population of about a million *V. fischeri* symbionts within 12 h. The progress and extent of colonization can be measured either by counting the number of colonies that arise from a homogenate of the squid's light organ, or by simply monitoring the amount of luminescence emitted by the animal (Ruby and Asato, 1993). This association is an easily studied model for the benign infection of animal tissue, during which both the bacterium (Ruby and Asato, 1993) and the host (McFall-Ngai, 1994) undergo a program of developmental changes. Clearly, both organisms are responding to the presence of the other, presumably through the expression and recognition of specific signaling molecules; however, none of these putative signals has yet been identified.

One way of identifying such factors is to search for bacterial genes that are induced specifically during the symbiotic interaction. Luminescence (or *lux*) genes have been used in other systems to monitor bacterial infection and developmental processes in a non-disruptive manner (Contag et al., 1995; Shaw and Kado, 1986; Wolk et al., 1991). Specifically, the *luxAB* genes of *Vibrio harveyi* have been inserted into transposons for use as promoterless reporter genes (Guzzo and DuBow, 1991; Sohaskey et al., 1992). To apply such a technique in the *V. fischeri*-*E. scolopes* symbiosis, the naturally occurring *luxA* gene would first have to be removed. We report here the construction of a *luxA*-deletion derivative of strain ES114, a *V. fischeri* strain isolated from the light organ of *E. scolopes* (Boettcher and Ruby, 1990). The work presented here (i) demonstrates that no selection for a bioluminescence capacity occurs during the initiation of a light organ symbiosis and (ii) establishes new techniques for DNA transfer, gene replacement, and transduction in symbiotic strains of *V. fischeri*.

* Corresponding author. Tel. +1 213 7405803 Fax +1 213 7408123.

Abbreviations: CFU, colony forming units; DNA, deoxyribonucleic acid; [ec], DNA isolated from *E. coli*; h, hour(s); kV, kilovolt; μg, microgram; ng, nanogram; [vf], DNA isolated from *V. fischeri*.

0378-1119/96/$15.00 © 1996 Elsevier Science B.V. All rights reserved
PII S0378-1119(96)00129-1

2. Results and discussion

2.1. Electroporation of V. fischeri strains

The source of DNA for our initial electroporation experiments was pSUP102, a small, mobilizable plasmid vector that can be stably maintained in *V. fischeri*. Plasmid DNA isolated from *V. fischeri* strain ESR1 (Graf et al., 1994) carrying pSUP102 (designated pSUP102 [vf]) was used in electroporation experiments designed to establish the optimal conditions for electroporation of ESR1. The highest number of transformants was obtained when *V. fischeri* cells grown at 28°C to an early exponential growth phase (an optical density of 0.3 at 600 nm) were harvested at 4°C and washed twice in a buffered sucrose solution (272 mM sucrose, 1 mM $MgCl_2$, and 7 mM potassium phosphate, pH 7.4). The cells were most efficiently transformed when subjected to electroporation under a range of low voltage conditions (0.60–0.80 kV) at 400 Ω. Amounts of DNA over a 100-fold range (from 2.4 ng to 240 ng) yielded between 10^4 and 10^5 transformants per μg of DNA using plasmid pSUP102 (vf) as a source of DNA.

We investigated whether the electroporation conditions that we had developed for ESR1 would also allow transformation of several natural isolates of *V. fischeri*. ES114, the parent strain of ESR1, was successfully transformed with pSUP102 DNA isolated from ESR1. Transformation was achieved for strain ES213, but not for strain ES235, both of which are wild-type isolates from different adult specimens of *E. scolopes* (Boettcher and Ruby, 1990,Boettcher and Ruby, 1994). Transformation was also not detectable when the recipient was MJ1, a *V. fischeri* strain obtained from the light organ of the fish *M. japonica*. While we found that crucial parameters for efficient electroporation of ESR1 included a rapid growth rate of the cells and harvesting at an early growth phase, it is likely that different conditions are more appropriate for electroporation of other isolates of *V. fischeri*.

2.2. Barrier to foreign DNA uptake by V. fischeri ESR1

There are many procedures for which the direct transfer of DNA from *E. coli* to *V. fischeri* would be of considerable advantage. Thus, it was unfortunate that when plasmid DNA isolated from *E. coli* strain CSH52 (pSUP102 [ec]) was used in the electroporation of ESR1, no transformants were obtained. However, if the DNA was isolated from a *dam*$^-$ *E. coli* strain, which does not methylate its DNA, a low but detectable number of transformants was observed (4.4×10^1 transformants/μg).

In order to increase our effectiveness at transforming *V. fischeri* using *E. coli* DNA, we screened for naturally arising electroporation-mutant strains that had an increased ability to take up DNA. Electroporation transformants of ESR1 that had taken up plasmid DNA (either vf or ec) were isolated, and streaked on a non-selective medium and then screened to obtain isolates that had lost the plasmid. Three ESR1 derivatives were sequentially isolated. The first two strains remained essentially unable to be transformed by pSUP102 (ec) but the third derivative, KV98, could be transformed by DNA isolated from either *dam*$^+$ (4.6×10^3 transformants/μg) or *dam*$^-$ (1.0×10^4 transformants/μg) *E. coli* strains. This strain was subsequently used as a recipient for the introduction of DNA directly from *E. coli* into *V. fischeri*.

Because efficient transformation of wild-type *V. fischeri* occurred only when the source of plasmid DNA was *V. fischeri*, it seems likely that there is a restriction barrier between *V. fischeri* and *E. coli*. We hypothesize that the uncharacterized mutation(s) that allows KV98 to be transformed by *E. coli* DNA is in a restriction system. Ideally, as with *E. coli* restriction$^-$ modification$^+$ cloning strains, it would be useful to have a *V. fischeri* strain through which *E. coli* DNA could be shuttled for appropriate modification before electroporation into the recipient strain of interest. We have been unable, however, to transfer plasmid DNA from KV98 to its parent strain, ESR1. One possibility for these results is that KV98 is defective in genes for both DNA restriction and modification functions.

2.3. Plasmid stability in V. fischeri strains

Although it was possible to obtain many transformants of KV98 using pSUP102 DNA, the same was not true when plasmid pSUP202 was used. Plasmid pSUP202 is derived from pBR322, which in *E. coli* is maintained at a higher copy number than is pACYC184, the plasmid from which pSUP102 is derived, and uses a different origin of replication. It was not clear whether the bias observed against pSUP202 was due to a natural instability of this plasmid in *V. fischeri*, or to an enhanced ability of KV98 to replicate pSUP102 due to the manner in which the strain was selected. In order to determine whether KV98 and its parent ESR1 had similar replication biases, we quantified the abilities of these two strains to take up pSUP102 and pSUP202 by conjugation using an established method (Dunlap, 1989; Graf et al., 1994; Simon et al., 1986). The data from three separate experiments showed that both strains have a 100-fold decrease in conjugation efficiency when selected for uptake of pSUP202 DNA as compared to pSUP102 DNA. Interestingly, KV98 exhibited a 2- to 4- fold greater ability than its parent to take up either plasmid.

The stability of the two plasmids was compared by streaking *V. fischeri* cells carrying these plasmids on a non-selective medium, and then screening 100 of the resulting colonies on a selective medium for the presence

of the plasmid. The plasmid-carrying derivatives of ESR1 and KV98 showed a high frequency of plasmid loss. Plasmid pSUP102 was lost by 69% (ESR1) or 75% (KV98) of the colonies examined, while pSUP202 was even less stable, being lost by 99% (ESR1) or 100% (KV98) of them. Perhaps the basis for this differential stability is the different origins of replication of the two plasmids. In any case, the high instability of the pSUP202 plasmid makes it likely that pBR322-based plasmids will be useful as suicide vectors in *V. fischeri*.

2.4. *Gene replacement in a squid-symbiont strain of* V. fischeri

A *luxA* mutant of *V. fischeri* strain MJ1 has been reported (Kuo et al., 1994); however, MJ1 is not useful in the study of the squid symbiosis because it is incapable of successfully colonizing juvenile *E. scolopes* (Ruby and McFall-Ngai, 1992). To obtain a *V. fischeri* squid-symbiont strain that is defective in the ability to produce light, the technique of electroporation that we developed was used to facilitate gene replacement. Plasmid pHV200 is a pBR322-based vector carrying an 8.8-kb region of the *lux* operon of *V. fischeri* strain ES114 that, when introduced into *E. coli*, allows this bacterium to be luminous (Fig. 1) (Gray and Greenberg, 1992). With the exception of the regulatory gene *luxR*, which is transcribed divergently, the eight *lux* genes, encoding the luciferase enzyme, enzymes for the synthesis of its substrates, and a regulatory protein, are grouped in a single operon (Meighen and Dunlap, 1993) (Fig. 1). The *luxA* gene is located in the middle of the *lux* operon and encodes one of the luciferase subunits. Plasmid pKV17, derived from pHV200, contains a replacement of part of the *luxA* gene with the gene for erythromycin resistance along with several kb of unaltered flanking DNA to facilitate homologous recombination (Fig. 1). *V. fischeri* strain KV98 was electroporated with plasmid pKV17, and both large and small colonies were obtained in selections on plates containing erythromycin. We postulate that the small colonies resulted from cells that carry, but have difficulty maintaining, the unrecombined pBR322-based plasmid.

Three out of four large colonies examined appeared to have gained stable erythromycin resistance and were subsequently screened for the ability to produce light. Two of these, KV137 and KV138, were unable to produce light in culture, suggesting that the chromosomal copy of the *luxA* gene had been successfully replaced with the erythromycin resistance gene (*ΔluxA*: :*erm*) (Fig. 1). Light production was restored when a plasmid carrying the $luxA^+$ gene under control of the *lac* promoter was introduced into KV137. In contrast, the third strain, KV139, was erythromycin resistant and visibly more luminous. We believe that this phenotype resulted from a single recombination event in this strain, leading to a tandem duplication of the *lux* region, one copy of which is wild type, while the other copy carries the *ΔluxA*: :*erm* replacement (Fig. 1). As expected for a strain carrying two copies of the *lux* positive regulatory genes (*luxR* and *luxI*), light production of KV139 was induced to a specific luminescence that was 100 times that of wild type.

A Southern blot analysis was performed to confirm the mutations that occurred in strains KV137, KV138, and KV139. Digestion of chromosomal DNA from parent strains ESR1 (not shown) or KV98 with the *Sal*I enzyme yields an 8.8-kb DNA fragment that contains the known *lux* operon. Insertion of the *erm* gene into the *luxA* gene resulted in the introduction of a second *Sal*I site, such that digestion with *Sal*I results in a 4.7-kb DNA doublet. The data (Fig. 2) was consistent with the occurrence of double (KV137 and KV138) and single (KV139) recombinational events in the *lux* region. These data confirm the usefulness of pBR322-based plasmids as suicide vectors for performing gene replacement in *V. fischeri*.

2.5. *Symbiotic competence of the* luxA *mutants*

To determine whether derivatives of the electroporation variant strain KV98 would be useful in our studies of the symbiotic infection of *E. scolopes*, seawater containing individual juvenile squid was inoculated with cells of either wild-type ES114 or KV98. The average extent of colonization (Ruby and Asato, 1993) by the two strains after 24 h was determined to be 7.3×10^5 (ES114) and 7.2×10^5 (KV98) CFU per animal. Thus, there was no evidence that KV98 was unable to infect *E. scolopes* in a normal manner.

To determine whether the production of light by *V. fischeri* is required for successful colonization of *E. scolopes*, the dark mutants KV137 and KV138 were tested for their ability to symbiotically infect the light organ of juvenile squid. Although infection is typically accompanied by the onset of the production of host bioluminescence, no light was produced by *E. scolopes* juveniles that had been exposed to the *ΔluxA*: :*erm* strains (Fig. 3). Therefore, we assayed the extent of colonization by determining the numbers of CFU of *V. fischeri* in light organ homogenates of these juveniles after 24 h of infection. The data (Fig. 4) indicated that KV137 and KV138 cells were able to colonize *E. scolopes* to an extent that is similar to the colonization that occurs with the luminous strain ESR1. The wide range of colonization levels observed in Fig. 4 are typical for experiments like these (Lee and Ruby, 1994; Ruby and Asato, 1993) and are probably due to the relatively small sample sizes and the variations in the light organ size of individual juveniles (M. McFall-Ngai, personal communication). The data also suggested that the production of light is not a necessary prerequisite either for the initial infection, or for the rapid bacterial growth phase

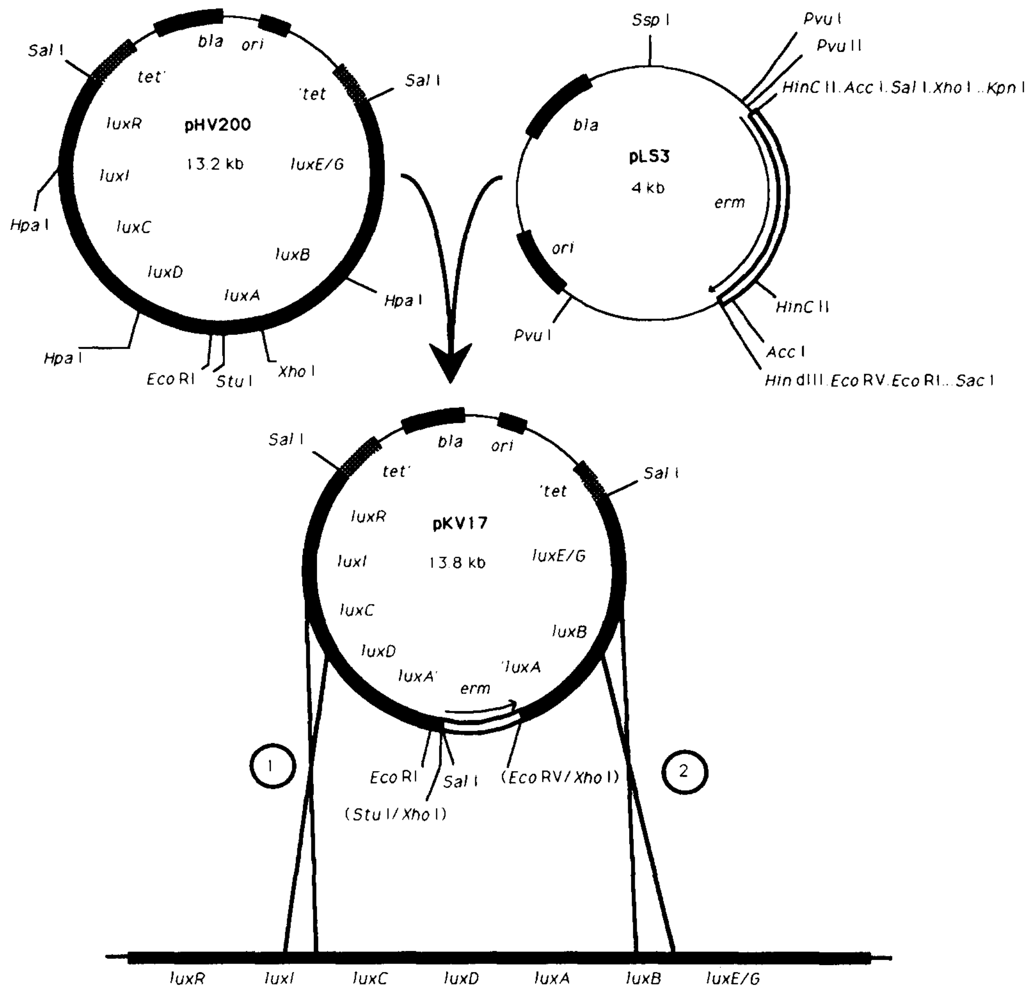

Fig. 1. Construction of the *ΔluxA::erm* gene replacement mutant. The *V. fischeri lux* genes are arranged in two divergent transcriptional units, *luxR* and *luxICDABEG*, and are contained on an 8.8-kb DNA segment in pHV200. To construct a *luxA* deletion mutant, plasmid pKV17 was constructed from pHV200 and pLS3 by: (i) deletion of the *Stu*I-*Xho*I segment (approximately 500-bp) internal to *luxA*; and, (ii) replacement of this segment with a 1.1-kb DNA fragment carrying the gene for erythromycin resistance from pLS3 digested with *Eco*RV and *Xho*I. The DNA fragments were separated by gel electrophoresis, purified using GeneClean (Bio101, Inc. Vista, CA) and the single-stranded ends of the DNA were filled in by treatment with the Klenow fragment of DNA polymerase (Sambrook et al., 1989). Following blunt-ended ligation of the two fragments, $CaCl_2$-competent cells of *E. coli* strain DH5α were transformed with the resultant mixture, and an ampicillin-containing medium was used to select for cells carrying the correct recombinant plasmid. Ampicillin-resistant colonies were replica-printed to plates containing erythromycin to identify clones carrying the erythromycin resistance marker. Plasmid pKV17, purified from one clone, was then electroporated into strain KV98. Recombination into the *V. fischeri* chromosome at either site 1 or site 2 creates a tandem duplication, while recombination at both sites results in replacement of *luxA* with *ΔluxA::erm*. The arrows indicate the direction of transcription of the gene for erythromycin resistance. Gene names are indicated.

that occurs in the first 24 h of infection (Ruby and Asato, 1993), and thus, the *ΔluxA::erm* mutant is more than adequate to use as a recipient in our search for symbiosis genes of *V. fischeri* that are activated within the first 24 h of the symbiosis.

While it would be interesting to determine the colonization ability of strain KV139, which carries a chromosomal duplication of the *lux* operon, (one copy of which was wild type and the other of which carried the *ΔluxA::erm* mutation), we were unable to do so accurately. The large (8.8 kb) region of identity resulting from the duplication created a high potential for recombination in this strain, and our preliminary results suggested that during colonization of the light organ, additional recombination events did occur at a sufficiently high frequency that a mixture of strains, bright, dark or wild type for luminescence, resulted. This inherent genetic instability made it difficult to accurately determine the colonization phenotype of the duplication strain and may speak to the conditions the cells encounter while in the light organ.

2.6. Transduction of markers in V. fischeri

While the uncharacterized mutation(s) present in electroporation strain KV98 has appeared to be silent

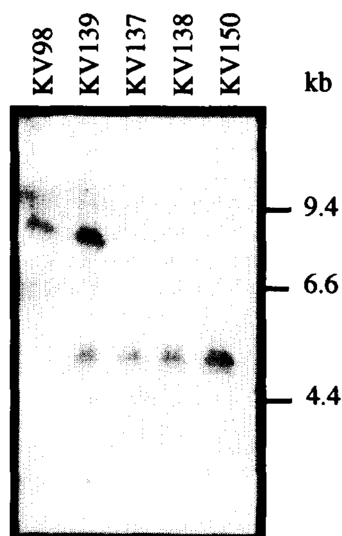

Fig. 2. Southern blot analysis of the *ΔluxA*: :*erm* mutants. Lane 1, KV98 (*luxA*$^{+}$); Lane 2, KV139 (*luxA*$^{+}$/*ΔluxA*: :*erm*); Lane 3, KV137 (*ΔluxA*: :*erm*); Lane 4, KV138 (*ΔluxA*: :*erm*); Lane 5, KV150 (*ΔluxA*: :*erm*). **Methods:** The Southern blot procedure (Ausubel et al., 1987; Southern, 1975) was performed as follows: Chromosomal DNA was prepared (Graf et al., 1994) and digested with *Sal*I. The DNA fragments were separated on a 0.6% agarose gel and transferred to a Nylon 66 plus (Hoeffer, San Francisco, CA) membrane. Prehybridization was carried out at 42°C in 5 × SSC, 50% formamide, 10 × Denhardt's solution, and 250 μg/ml herring sperm DNA for 4 h. Hybridization was carried out in the same solution, with the addition of dextran sulfate (to a final concentration of 10%) and ^{32}P-labeled probe DNA. The probe was a 3-kb *Hpa*I fragment of DNA containing the *luxD*, *luxA*, and *luxB* genes (Fig. 1).

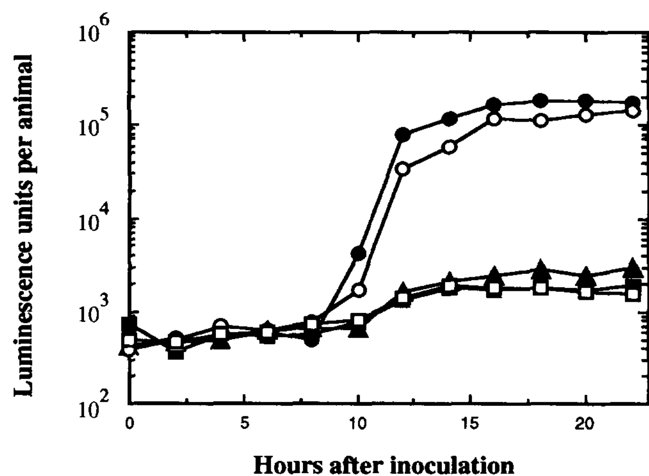

Fig. 3. Luminescence of *ΔluxA*: :*erm* mutants after colonization of juvenile *E. scolopes* light organs. Relative levels of light emission are shown for representative *E. scolopes* juveniles infected with ES114 (*lux*$^{+}$, ○); KV98 (*lux*$^{+}$, ●); KV137 (*ΔluxA*: :*erm*, □); KV138 (*ΔluxA*: :*erm*, ■); or an uninfected animal (▲). **Methods:** To initiate a symbiotic light organ infection (Ruby and Asato, 1993), newly hatched *E. scolopes* juveniles were exposed to seawater containing cells of specific strains of *V. fischeri* for 16 h. Light emission from the juvenile *E. scolopes* was monitored every 2 h using an automated photometer. Background (dark current) values ranged from 500 to 3000 luminescence units over the course of the experiment.

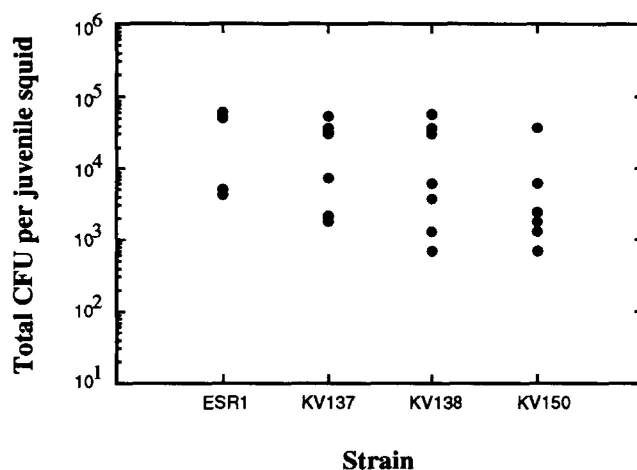

Fig. 4. Extent of colonization of juvenile *E. scolopes* after inoculation with *ΔluxA*: :*erm* mutants. The total number of *V. fischeri* cells colonizing individual juvenile squid light organs at 24 h post-inoculation was determined for squid infected with strains ESR1, KV137, KV138 or KV150. **Methods:** Juvenile *E. scolopes* were infected as described in Fig. 3 legend. Twenty-four hours later, the animals were washed by passage through sterile seawater and homogenized, and aliquots were plated onto SWT agar to determine the number of CFU as a measure of the extent of light organ colonization (Ruby and Asato, 1993).

with respect to colonization of *E. scolopes*, it is possible that the mutation(s) may affect a characteristic of the symbiosis that has not yet been recognized. In order to circumvent any future problems resulting from using an uncharacterized strain background, we have utilized the generalized transducing phage, rp-1 (Levisohn et al., 1987), to move the *ΔluxA*: :*erm* mutation present in KV138 into ESR1. The transduction was carried out using exponentially growing ESR1 cells essentially as described (Levisohn et al., 1987), except that the rp-1 phage was not UV-irradiated. The resulting strain, KV150, was non-luminous in culture. Southern blot data (Fig. 2) show that the transduced strain had the same 4.7-kb *lux* DNA fragments as did its parent, KV138. Squid infection assays demonstrated that a similar pattern of colonization occurs when KV150 cells were used for inoculation (Fig. 4). This is the first report of the use of phage rp-1 since it was described in 1987, and both confirms the transducing character of this phage (by Southern analysis of the transductant), and demonstrates that a 1-kb region of non-homology (from the antibiotic resistance marker) can be successfully transduced. The use of rp-1 to move genetic markers in *V. fischeri* will greatly enhance our ability to isolate and characterize the effect of mutations, such as those generated by transposon mutagenesis.

3. Conclusions

We have developed conditions for successful electroporation of *V. fischeri* strains ESR1, ES114, and ES213.

The same conditions apparently do not work for strains MJ1 or ES235.

A restriction barrier exists that prevents transfer of foreign (*E. coli*) DNA into *V. fischeri* strain ESR1. Only DNA isolated from a *dam*⁻ strain of *E. coli* was able to transform ESR1. The restriction barrier was overcome by the selection of mutant strain, KV98, that is transformed by plasmid DNA isolated from *E. coli*.

The stability of pACYC184- and pBR322-based plasmids in ESR1 and KV98 was investigated. The relative instability of pBR322-based plasmids made it likely that they would be useful as suicide vectors in *V. fischeri*.

Electroporation was used to introduce into *V. fischeri* a pBR322-based plasmid carrying a *ΔluxA*: :*erm* cassette for gene replacement. Both single and double recombinant strains were obtained and confirmed. The double recombinant strains were non-luminous in culture.

The symbiotic competence of the *ΔluxA*: :*erm* mutant strains was assayed, and found to be essentially normal (although non-luminous) after 24 hours of colonization. Thus, the *ΔluxA*: :*erm* mutant can be used as a recipient for a transposon mutagenesis experiment to hunt for *V. fischeri* genes important in the early stages of the symbiosis.

Transduction using the generalized transducing phage rp-1 was developed as a technique for use in *E. scolopes*-symbiont strains of *V. fischeri*. The *ΔluxA*: :*erm* allele was successfully transduced into strain ESR1, allowing this mutation to be isolated from potentially complicating mutations in the electroporation-variant strain KV98.

Acknowledgement

We thank Daniel O'Sullivan for his generous gift of plasmid pTRKH2, from which the *erm* gene was cloned into pLS3, and Lisa Shiroishi for her work in constructing plasmid pLS3. We thank Kenneth Nealson for his generous gift of phage rp-1, and Mauricio Mejia for his preliminary work with rp-1. In addition, we thank Magdalena Martinez-Cañamero for her preliminary work on the *luxA*-deletion project, and Joerg Graf for valuable discussions.

This work was supported by National Science Foundation grant IBN 92-20482 to E.G.R. and M.J. McFall-Ngai (USC) and Office of Naval Research grant N0014-93-1-0846 to E.G.R.

References

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. (1987) Current Protocols in Molecular Biology. Wiley, New York.

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

Boettcher, K.J. and Ruby, E.G. (1994) Occurrence of plasmid DNA in the sepiolid squid symbiont *Vibrio fischeri*. Curr. Microbiol. 29, 279–286.

Contag, C.H., Contag, P.R., Mullins, J.I., Spillman, S.D., Stevenson, D.K. and Benaron, D.A. (1995) Photonic detection of bacterial pathogens in living hosts. Mol. Micro. 18, 593–603.

Dunlap, P.V. (1989) Regulation of luminescence by cyclic AMP in *cya*-like and *crp*-like mutants of *Vibrio fischeri*. J. Bacteriol. 171, 1199–1202.

Graf, J., Dunlap, P.V. and Ruby, E.G. (1994) Effect of transposon-induced motility mutations on colonization of the host light organ by *Vibrio fischeri*. J. Bacteriol. 176, 6986–6991.

Gray, K.M. and Greenberg, E.P. (1992) Sequencing and analysis of *luxR* and *luxI*, the luminescence regulatory genes from the squid light organ symbiont *Vibrio fischeri* ES114. Mol. Mar. Biol. Biotechnol. 1, 414–419.

Guzzo, A. and DuBow, M.S. (1991) Construction of stable, single-copy luciferase gene fusions in *Escherichia coli*. Arch. Microbiol. 156, 444–448.

Kuo, A., Blough, N.V. and Dunlap, P.V. (1994) Multiple *N*-acyl-L-homoserine lactone autoinducers of luminescence in the marine symbiotic bacterium *Vibrio fischeri*. J. Bacteriol. 176, 7558–7565.

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

Levisohn, R., Moreland, J. and Nealson, K.H. (1987) Isolation and characterization of a generalized transducing phage for the marine luminous bacterium *Vibrio fischeri* MJ-1. J. Gen. Microbiol. 133, 1577–1582.

McFall-Ngai, M.J. (1994) Animal-bacterial interactions in the early life history of marine invertebrates: the *Euprymna scolopes*/*Vibrio fischeri* symbiosis. Am. Zool. 34, 554–561.

McFall-Ngai, M.J. and Ruby, E.G. (1991) Symbiont recognition and subsequent morphogenesis as early events in an animal-bacterial mutualism. Science 254, 1491–1494.

Meighen, E.A. and Dunlap, P.V. (1993) Physiological, biochemical and genetic control of bacterial bioluminescence. Adv. Microbial. Physiol. 34, 1–67.

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

Ruby, E.G. and McFall-Ngai, M.J. (1992) A squid that glows in the night: development of an animal-bacterial mutualism. J. Bacteriol. 174, 4865–4870.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor Laboratory, NY.

Shaw, J.J. and Kado, C.I. (1986) Development of a *Vibrio* bioluminescence gene-set to monitor phytopathogenic bacteria during the ongoing disease process in a non-disruptive manner. BioTechnology 4, 560–564.

Simon, R., O'Connell, M., Labes, M. and Puhler, A. (1986) Plasmid vectors for the genetic analysis and manipulation of rhizobia and other gram-negative bacteria. Meth. Enzymol. 118, 640–659.

Sohaskey, C.D., Im, H. and Schauer, A.T. (1992) Construction and application of plasmid- and transposon-based promoter-probe vectors for *Streptomyces* spp. that employ a *Vibrio harveyi* luciferase reporter cassette. J. Bacteriol. 174, 367–376.

Southern, E.M. (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98, 503–517.

Wolk, C.P., Cai, Y. and Panoff, J.-M. (1991) Use of a transposon with luciferase as a reporter to identify environmentally responsive genes in a cyanobacterium. Proc. Natl. Acad. Sci. USA 88, 5355–5359.