

Physical and Functional Maps of the Luminescence Gene Cluster in an Autoinducer-Deficient *Vibrio fischeri* Strain Isolated from a Squid Light Organ

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Vibrio fischeri ES114 is an isolate representing the specific bacterial light organ symbiont of the squid *Euprymna scolopes*. An interesting feature of this strain of *V. fischeri* is that it is visibly luminous within the light organ of the squid host but is nonluminous when grown under standard laboratory conditions. Luminescence can be restored in laboratory culture, however, by the addition of autoinducer, a species-specific inducer of the *V. fischeri* luminescence (*lux*) genes. Most other isolates of *V. fischeri* produce autoinducer in sufficient quantities to induce luminescence in laboratory culture. We have cloned an 8.8-kb DNA fragment from *V. fischeri* ES114 that encodes all of the functions necessary for luminescence in *Escherichia coli* in the absence of exogenous autoinducer. This DNA contains both of the recognized *V. fischeri* *lux* regulatory genes, one of which (*luxI*) directs *E. coli* to synthesize autoinducer. The organization of the individual *lux* genes within this DNA fragment appears to be the same as that in the other strains of *V. fischeri* studied; the restriction map of the *V. fischeri* ES114 *lux* DNA has diverged substantially, however, from the largely conserved maps of *V. fischeri* MJ1 and ATCC 7744. Although *E. coli* containing the *V. fischeri* ES114 *lux* DNA synthesizes considerable amounts of autoinducer, *V. fischeri* ES114 synthesizes autoinducer only in small amounts, even when transcription of the *lux* genes, including *luxI*, is activated by the addition of exogenous autoinducer. Nonetheless, transconjugants of *V. fischeri* ES114 that contain multicopy plasmids bearing the ES114 *lux* genes synthesize sufficient autoinducer to induce luminescence. These results suggest that *V. fischeri* ES114 does not lack a functional *luxI*, nor is it deficient in the ability to synthesize metabolic precursors for autoinducer synthesis.

Vibrio fischeri is a luminescent bacterium that occurs in marine environments both as free-living cells and as symbionts within the specialized light organs of a number of animal species (2, 12, 29–32). Luminescence of these bacteria is regulated by the accumulation of autoinducer [*N*-(3-oxohexanoyl) homoserine lactone], a species-specific signaling compound that is required for the transcriptional activation of the luminescence genes (14, 16). Because cells are permeable to autoinducer (23), luminescence occurs only after this compound has accumulated above a threshold concentration in the surrounding medium. As a result, the bacteria produce light only under conditions of relatively high population density, such as those of a light organ symbiosis. Expression of the *V. fischeri* luminescence system is thus regulated by an environmental sensing mechanism that allows the bacteria to respond to changes in their population density (12, 13, 23, 28, 31).

DNA restriction fragments encoding all of the functions necessary for luminescence in *Escherichia coli* have been cloned from *V. fischeri* MJ1 (16), which is the light organ symbiont of the Japanese pinecone fish (31), as well as from the seawater isolate *V. fischeri* ATCC 7744 (5). The luminescence systems of both of these strains consist of seven *lux* genes of known function arranged in two divergently transcribed units (1, 8, 17). One transcriptional unit consists of a single gene, *luxR*, which encodes a protein required for the cell's response to autoinducer. The other unit contains the remaining genes, arranged in the order *luxICDABE*. Of these genes, *luxI* encodes a protein that directs autoinducer syn-

thesis, *luxA* and *luxB* encode the two subunits of the light-emitting enzyme luciferase, and *luxC*, *luxD*, and *luxE* encode components of the fatty acid reductase system responsible for production and recycling of the aldehyde substrate used in the luminescence reaction (17, 27). An additional gene, *luxG*, is located on the *luxICDABE* operon immediately after *luxE* but has no known function at present (39). The arrangement of these genes results in positive autoregulation of the *luxICDABEG* operon such that autoinducer not only activates the luminescence genes (*luxCDABE*) but also activates the autoinducer synthase gene (*luxI*).

The specific light organ symbiont of the squid *Euprymna scolopes* has recently been isolated and characterized as *V. fischeri* (2). Unlike previous isolates of *V. fischeri*, including the Japanese pinecone fish symbiont *V. fischeri* MJ1, the *E. scolopes* symbiont (represented by strain ES114) is essentially nonluminous when grown under standard laboratory conditions. Luminescence of strain ES114 can be restored to a level near that observed in the intact light organ, however, by the addition of either light organ fluid or synthetic *V. fischeri* autoinducer to broth cultures of this organism (2). These results suggest that the squid host may either be providing autoinducer directly to its bacterial symbiont or supplying some factor necessary for the synthesis of autoinducer by the bacterium.

To investigate the molecular basis for this symbiosis-dependent production of autoinducer, we have isolated the *lux* genes of *V. fischeri* ES114. Our experiments with both *E. coli* and *V. fischeri* containing these genes in multiple copy indicate that *V. fischeri* ES114 possesses a functional *lux* regulon and is fully capable of autoinducer synthesis. These results suggest that ES114 either produces a *luxI* transcript

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TABLE 1. Bacterial strains and plasmids

Bacterial strain or plasmid	Relevant characteristics ^a	Source or reference
Bacterial strains		
<i>V. fischeri</i> MJ1	Japanese pinecone fish light organ symbiont; brightly luminous in laboratory culture	31
<i>V. fischeri</i> ES114	Squid light organ symbiont; nonluminous in laboratory culture	2
<i>E. coli</i> VJS533	<i>ara</i> Δ(<i>lac-proAB</i>) <i>rpsL</i> φ80 <i>lacZ</i> ΔM15 <i>recA56</i>	38
<i>E. coli</i> JM109	<i>endA1 recA1 syrA96 thi hsdR17 relA1 (res mod⁺) supE44 λ⁻ Δ(<i>lac-proAB</i>) [F' <i>traD36 proAB lacI</i>^qZΔM15]</i>	41
<i>E. coli</i> S17-1	RP4-2-Tc::Mu-Km::Tn7 Sm ^r <i>pro (res mod⁺) recA tra⁺</i>	35
Plasmids		
pHG165	ColE1 replicon; Ap ^r <i>lacZ</i> ⁺	36
pBR322	ColE1 replicon; Ap ^r Tc ^r	4
pACYC184	P15A replicon; Cm ^r Tc ^r	6
pSUP102	P15A replicon; <i>mob</i> ⁺ Cm ^r Tc ^r	34
pJE202	8.8-kb <i>SalI</i> fragment containing the <i>V. fischeri</i> MJ1 <i>lux</i> regulon in pBR322; Ap ^r	16
pHK555	<i>luxR luxICDABE</i> ⁺ in pACYC184 (<i>lux</i> DNA from <i>V. fischeri</i> MJ1); Cm ^r	24
pHV100	8.8-kb <i>SalI</i> fragment containing the <i>V. fischeri</i> ES114 <i>lux</i> regulon in pHG165; Ap ^r	This study
pHV200	8.8-kb <i>SalI</i> fragment from pHV100 in pBR322; Ap ^r	This study
pHV202	1.1-kb <i>SalI-PvuII</i> fragment from pHV200 in pBR322; <i>luxR</i> ⁺ ; Ap ^r	This study
pHV300	8.8-kb <i>SalI</i> fragment from pHV200 in pSUP102; Cm ^r	This study
pNL121	8.8-kb <i>SalI</i> fragment from pJE202 in pSUP102; Cm ^r	P. V. Dunlap

^a Ap^r, ampicillin resistant; Cm^r, chloramphenicol resistant; Sm^r, streptomycin resistant; Tc^r, tetracycline resistant.

that is unstable or secondarily processed, translates the *luxI* mRNA poorly, or produces a LuxI protein that is less stable or less active than those of other *V. fischeri* strains for which the *lux* genes have been characterized. As a result, *V. fischeri* ES114 synthesizes insufficient autoinducer for the activation of its *lux* genes at the cell densities normally achieved in laboratory batch culture. Although it cannot be proven, our data support the hypothesis that *V. fischeri*, rather than its host, catalyzes the synthesis of autoinducer in the squid light organ.

MATERIALS AND METHODS

Bacterial strains, media, and culture conditions. The bacterial strains employed in this research are listed in Table 1. *V. fischeri* MJ1 was originally isolated from a Japanese pinecone fish light organ (31), while *V. fischeri* ES114 was isolated from a squid light organ (2). Although later experiments with cloned *V. fischeri lux* DNA were performed using *E. coli* JM109 (41), *E. coli* VJS533 (38) was used for the original isolation and screening of *V. fischeri* ES114 *lux* clones by virtue of its higher transformation competency. *E. coli* S17-1 (35) was employed as a donor strain for the conjugation of *lux* DNA into *V. fischeri* ES114.

Unless otherwise indicated, *V. fischeri* strains were grown in an artificial seawater (SWC) broth (23) at 20 to 25°C, while *E. coli* strains were grown on Luria-Bertani (LB) broth (33) at either 30 or 37°C. *E. coli* and *V. fischeri* cultures used for conjugation experiments were grown in 1% tryptone–0.5% yeast extract–0.3% glycerol–350 mM NaCl–50 mM Tris (pH 7.5) (PLBS broth) (10). SWC agar, LB agar, and PLBS agar consisted of the respective broth medium containing a final concentration of 1.25% agar (Difco Laboratories, Detroit, Mich.). Plasmid maintenance was ensured by the addition of ampicillin or chloramphenicol at final concentrations of 200 and 30 μg/ml, respectively, to the medium.

Plasmids, plasmid preparation, cloning, and transformation procedures. The plasmids used are described in Table 1.

Small-scale plasmid preparation was the procedure of Lee and Rasheed (25), while larger amounts of plasmid DNA were prepared by the CsCl gradient method (7). Restriction endonucleases and T4 DNA ligase were purchased from New England Biolabs (Beverly, Mass.). DNA was digested with restriction endonucleases for 2 to 3 h at 37°C, while ligation reactions were allowed to proceed overnight at 14°C; both types of reactions were performed in the buffers supplied with the enzymes. Prior to ligation, restriction enzyme-digested plasmid DNA was treated with calf intestinal phosphatase (Boehringer Mannheim Corp., Indianapolis, Ind.) according to the manufacturer's protocol. DNA restriction fragments were isolated from agarose gels and purified by using a GeneClean kit (Bio 101, La Jolla, Calif.). Transformation of *E. coli* with plasmid DNA was performed either by CaCl₂ shock treatment (20) or by electroporation (9).

Chromosomal DNA from *V. fischeri* ES114 was isolated according to the following procedure (37). *V. fischeri* ES114 was grown overnight in 100 ml of SWC broth and harvested by centrifugation. The cells were washed in 50 ml of cold 150 mM NaCl and resuspended in 2.8 ml of TES buffer (50 mM Tris, 100 mM Na₂EDTA, 20% sucrose [pH 8.0]). The suspension was incubated on ice for 20 min with 13.5 mg of lysozyme (20-mg/ml stock solution in 250 mM Tris, pH 8.0) before adding 75 μg of DNase-free RNase (10-mg/ml stock solution in 10 mM Tris–15 mM NaCl [pH 7.5]), 750 μg of proteinase K (2-mg/ml stock solution in 250 mM Tris, pH 8.0), and 3.75 ml of 1% Sarkosyl in 75 mM Na₂EDTA (pH 8.0). The suspension was then incubated for 4 h at 37°C with gentle agitation, after which 11 g of CsCl and 3.75 ml of water were added and incubation was continued until the CsCl dissolved. After addition of 0.4 ml of ethidium bromide (10-mg/ml stock solution in water), the solution was centrifuged at 55,000 rpm in a TY65 rotor (Beckman Instruments, Palo Alto, Calif.) for 16 h at 20°C. The isolated chromosomal DNA band was then dialyzed four times against 1 liter of TE buffer (10 mM Tris, 1 mM Na₂EDTA [pH 8.2]) at 4°C and

extracted twice with an equal volume of water-saturated butanol to remove the ethidium bromide. The final DNA solution was stored in the dark at 4°C.

Chromosomal DNA prepared by this method was digested with *Sa*I, ligated to *Sa*I-digested pHG165, and then used to transform *E. coli* VJS533. Of 72 recombinant clones obtained, one produced visible levels of light in laboratory culture. In order to provide a basis for comparison with the *V. fischeri* MJ1 *lux* DNA contained in pJE202 (16), the 8.8-kb *Sa*I insert of ES114 *lux* DNA from the recombinant plasmid was cloned in pBR322. *E. coli* JM109 was transformed with this construct, pHV200, for use in all subsequent experiments.

Measurement of culture density and luminescence. Growth and luminescence of either *V. fischeri* or *E. coli* containing *V. fischeri lux* DNA were monitored in 50-ml broth cultures contained in 500-ml Erlenmeyer flasks. These cultures were inoculated as previously described (11), incubated with shaking (200 rpm) at 25°C, and sampled periodically for optical density and luminescence measurements. Optical density was measured with a Spectronic 20D spectrophotometer (Milton Roy Co., Rochester, N.Y.) at a wavelength of 660 nm. The equipment and calibration standard employed for luminescence measurements have been described elsewhere (11, 21).

Autoinducer extractions and bioassays. Autoinducer was extracted from broth cultures of either *E. coli* JM109 or *V. fischeri* ES114 containing *V. fischeri lux* genes on multicopy plasmids. These cultures were grown to an optical density of 1.0 in the appropriate medium at 25°C, the cells were removed by centrifugation, and the supernatant medium was filter sterilized and extracted with ethyl acetate (28). Bioassays of the ethyl acetate extracts for autoinducer were performed as previously described (22) except that the bioassay strain used was *V. fischeri* ES114. This strain was grown to an optical density of 1.0 and diluted to an approximate density of 6×10^5 cells per ml in autoinducer bioassay medium (22). Luminescence measurements were made by using a single-photon-counting program on a model LS 1800 scintillation counter (Beckman Instruments).

Southern analysis of *lux* gene organization. The arrangement of *V. fischeri* ES114 *lux* genes was in part determined by Southern hybridization analysis (3) of pHV200 by using gene-specific oligonucleotide probes derived from the published sequence of *lux* DNA from *V. fischeri* ATCC 7744 (1). The oligonucleotide probes used for each gene were as follows: *luxR*, 5'-AATGCCGACGACACTTACAG-3' (nucleotides 758 to 739 from the *V. fischeri* ATCC 7744 sequence); *luxI*, 5'-GCAATTCCATCGGAGGAGTA-3' (nucleotides 1022 to 1041); *luxC*, 5'-TACCGTTGGTCAACGATGGA-3' (nucleotides 1815 to 1834); *luxD*, 5'-CATGTCATTGCTACGATTC-3' (nucleotides 3274 to 3293); *luxA*, 5'-ACAGCTCATCCTGTTTCGACA-3' (nucleotides 4270 to 4289); *luxB*, 5'-CGTCACATTCCATCAAGGCA-3' (nucleotides 5492 to 5511); *luxE*, 5'-GCTCTCTATGGCGAATGTCA-3' (nucleotides 7287 to 7306); and *luxG*, 5'-GCATCTAACTCAATTGCGAC-3' (nucleotides 7613 to 7594). All of these probes were synthesized at the University of Iowa DNA Core Facility.

We also isolated a 440-bp *Hind*III-*Bgl*II restriction fragment from pJE202 as a probe for *luxD*, while a 750-bp *luxA*-specific probe obtained from *V. fischeri* ES114 DNA by polymerase chain reaction (PCR) amplification was provided by C. F. Wimpee (40). These double-stranded DNA probes were radiolabeled and hybridized with the pHV200 DNA according to the conditions outlined by Sambrook et al. (33).

Transfer of *lux* DNA into *V. fischeri* ES114. Spontaneous mutants of *V. fischeri* ES114 resistant to nalidixic acid (Nal^r) were isolated by plating broth cultures on SWC agar containing nalidixic acid (15 µg/ml) and incubating the plates at room temperature (20 to 25°C) for 2 to 3 days. The isolated Nal^r mutants were then conjugated with *E. coli* S17-1 containing either pSUP102, pNL121, or pHV300 according to the following method (10).

Overnight cultures of donor *E. coli* S17-1 were grown at 30°C in LB broth containing chloramphenicol (30 µg/ml). Overnight cultures of the recipient *V. fischeri* ES114 were grown at 30°C in SWC broth containing nalidixic acid (15 µg/ml). These cultures were diluted 1:20 into PLBS broth without antibiotics and grown for 4 h at 30°C. Samples of 20 µl from each culture were spotted together on PLBS agar plates and incubated at 30°C overnight. The plates were then flooded with 5 ml of SWC broth, and the cells were suspended, spread on SWC agar containing both nalidixic acid and chloramphenicol, and incubated at room temperature for 2 to 3 days. Colonies were picked, and stocks were maintained on SWC agar containing chloramphenicol. These *V. fischeri* ES114 transconjugants contained the appropriate plasmid, as indicated by restriction analysis of plasmid preparations.

In vivo autoinducer biosynthesis assay. Synthesis of autoinducer by *V. fischeri* strains in vivo was measured according to the procedure of Eberhard et al. (15), except that 200 nM synthetic autoinducer (22) was added to each culture and 10 µCi of uniformly labeled [¹⁴C]methionine (New England Nuclear; >225 mCi/mmol) was used as substrate for autoinducer biosynthesis. For high performance liquid chromatography (HPLC), we used a 110B pump fitted with an ultrasphere C₁₈ reverse-phase column (4.6 mm by 25 cm; Beckman Instruments), from which 0.5-ml fractions were collected. A sample of ³H-labeled synthetic *V. fischeri* autoinducer (22) was used as a standard.

RESULTS

Characterization of *V. fischeri* ES114 *lux* DNA. The *V. fischeri* ES114 *lux* gene cluster was isolated as an 8.8-kb *Sa*I fragment contained in pHV200. This DNA fragment directed *E. coli* to produce similar amounts of light regardless of its orientation within the plasmid vector.

The luminescence of *V. fischeri* in laboratory batch culture characteristically exhibits a lag during the initial stages of growth, followed by a rapid increase as autoinduction of the *lux* genes occurs. This same pattern of autoinducible luminescence occurs in *E. coli* containing the *V. fischeri* MJ1 *lux* genes on pJE202 (16) (Fig. 1) and was also observed with *E. coli* containing the *V. fischeri* ES114 *lux* genes on pHV200 (Fig. 1). Luminescence of *E. coli* containing pHV200 did not require an exogenous source of autoinducer, although it was affected by temperature, showing an approximately 50-fold decrease in maximal luminescence at 30°C compared with that at 25°C. This result contrasts with that of *E. coli* containing pJE202, which is equally luminescent at both temperatures, but is similar to the results reported for *E. coli* containing the *lux* genes from *V. fischeri* ATCC 7744 (5). Although the maximal luminescence directed by pHV200 was comparable to that directed by pJE202 (Fig. 1), quantitative bioassays (22) showed that *E. coli* containing pHV200 synthesized only about 10% of the amount of autoinducer produced by *E. coli* containing pJE202. These assays were performed using extracts of media from cultures grown to an optical density (660 nm) of 1.0; at this density the lumines-

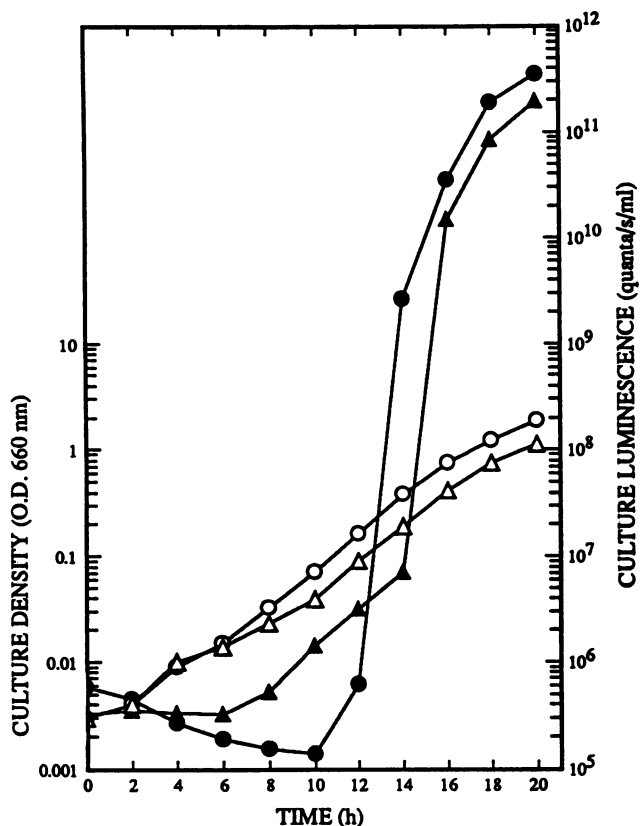


FIG. 1. Growth and luminescence of *E. coli* JM109 containing *V. fischeri* lux genes. Cells containing the *V. fischeri* ES114 lux genes on pHV200 (● and ○) or the *V. fischeri* MJ1 lux genes on pJE202 (▲ and △) were grown at 25°C (culture density, open symbols; luminescence, closed symbols).

cence of both cultures was approximately 2×10^{11} quanta/s/ml (Fig. 1).

Organization of *V. fischeri* ES114 lux genes. The restriction map of pHV200 (Fig. 2) has diverged substantially from the nearly identical restriction maps of lux DNA from *V. fischeri* MJ1 (16) and ATCC 7744 (5). This finding suggests that there might be further divergence in either the number of genes present or their organization within the lux DNA of *V. fischeri* ES114; consequently, we identified the presence and relative positions of the individual lux genes within pHV200. Oligonucleotide probes based on the published sequence of the lux regulon from *V. fischeri* ATCC 7744 (1) were con-

structed for all eight of the lux genes. The luxI, luxC, luxB, luxE, and luxG probes hybridized with specific restriction fragments of lux DNA from pHV200 (Fig. 2).

Because the luxR, luxD, and luxA probes failed to hybridize with any specific *V. fischeri* ES114 lux DNA fragment, a 440-bp luxD-specific HindIII-BglII restriction fragment was isolated from pJE202 and used as a probe to locate the position of luxD within pHV200, while a 750-bp luxA-specific PCR product derived from *V. fischeri* ES114 DNA (40) was used as a probe for luxA. These luxA and luxD probes hybridized to the pHV200 restriction fragments indicated in Fig. 2. The luxR gene was localized by testing the ability of subclones of pHV200 to complement the luxR mutation of pHK555 in *E. coli*. This luxR mutation was complemented by pHV202, which contained a 1.1-kb Sall-PvuII fragment of lux DNA from pHV200 (Fig. 2). Therefore, the gene order of *V. fischeri* ES114 lux DNA appears to be luxR, luxI, luxC, luxD, luxA, luxB, and luxE/G. Although the relative positions of luxE and luxG in *V. fischeri* ES114 cannot be determined from this analysis, the gene order is indistinguishable from that found in both *V. fischeri* MJ1 and ATCC 7744 (1, 17). Sequence analysis of the *V. fischeri* ES114 DNA containing luxR and luxI (18) indicates that luxR is divergently transcribed from luxI, as it is in the two *V. fischeri* strains previously studied.

***V. fischeri* ES114 makes substrate for autoinducer biosynthesis.** Because *V. fischeri* ES114 contains a functional luxI gene but still requires exogenous autoinducer for luminescence in laboratory culture, it was possible that this strain did not make the required substrates for autoinducer synthesis. To test this hypothesis, we supplied *V. fischeri* ES114 with multiple copies of the *V. fischeri* MJ1 lux genes carried on pNL121. Cultures of *V. fischeri* ES114 containing pNL121 were visibly luminous and, as determined by autoinducer bioassay, produced autoinducer in amounts similar to those produced by *E. coli* containing pJE202. Thus, we conclude that *V. fischeri* ES114 is not deficient for any metabolic precursors required for autoinducer synthesis.

Similar results were obtained when the *V. fischeri* ES114 lux genes were supplied in multiple copy on pHV300. Maximal luminescence of *V. fischeri* ES114 transconjugants containing pHV300 was approximately 25% of that of cultures containing the *V. fischeri* MJ1 lux genes on pNL121; similarly, autoinducer levels produced by these transconjugants were only about 25% of those of equally dense cultures of ES114 containing pNL121. These results, which are consistent with those of autoinducer synthesis directed by pHV200 and pJE202 in *E. coli*, suggest either that the ES114 luxI gene is transcribed less efficiently than the MJ1 gene, that its message is less stable or is translated less efficiently,

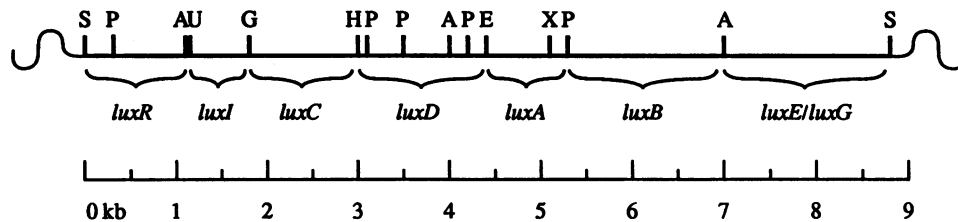


FIG. 2. Restriction map and organization of lux genes in pHV200. The 8.8-kb Sall fragment cloned from *V. fischeri* ES114 DNA directs autoinducible luminescence in *E. coli*. Restriction sites are S, Sall; P, PstI; A, HpaI; U, PvuII; G, BglII; H, HindIII; E, EcoRI; and X, XhoI. There are no restriction sites for BamHI, KpnI, or XbaI. With the exception of luxR, the relative positions of the lux genes on pHV200 were determined by Southern analysis in which gene-specific probes hybridized to the individual restriction fragments indicated. luxR was localized to the designated Sall-PvuII fragment by the ability of this DNA to complement a luxR mutation in trans.

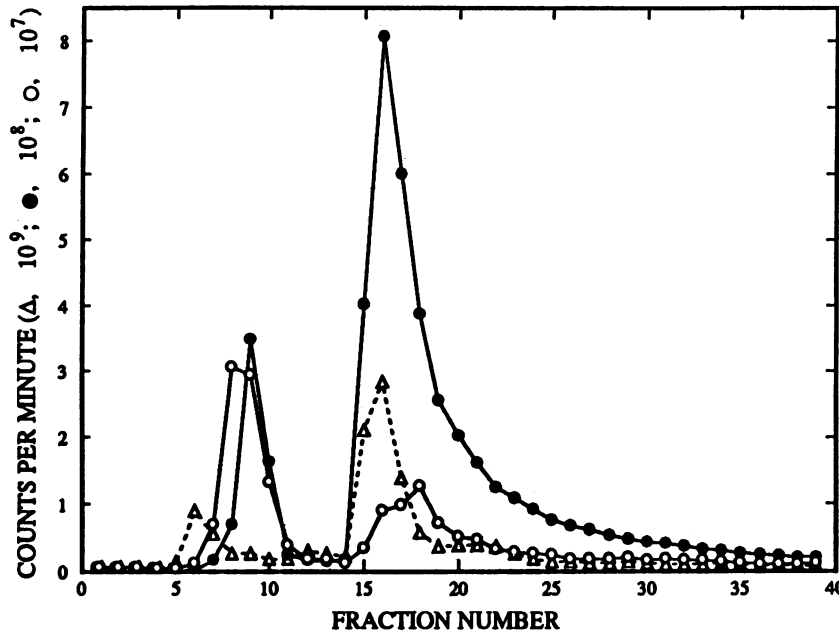


FIG. 3. HPLC analysis of ³H-labeled synthetic *V. fischeri* autoinducer (Δ) and ¹⁴C-labeled material extracted from cultures of *V. fischeri* MJ1 (●) and *V. fischeri* ES114 (○) incubated with [¹⁴C]methionine.

or that its translation product is less stable or less active than the *luxI* product of *V. fischeri* MJ1.

Autoinducer synthesis by induced *V. fischeri* ES114. The autoinducer requirement of *V. fischeri* ES114 in laboratory batch culture might result from a low basal level of *luxI* transcription such that autoinducer does not accumulate in sufficient amounts to induce luminescence before the onset of stationary phase. If so, induction of the luminescence system of *V. fischeri* ES114 by the addition of exogenous autoinducer should lead to positive autoregulation of the *luxI* gene and the de novo synthesis of autoinducer in amounts comparable to those synthesized by *V. fischeri* MJ1. To test this hypothesis, however, the autoinducer synthesized by the culture must be distinguished from the autoinducer added to activate the transcription of *luxICDABE/G*.

To accomplish this, we added a radiolabeled substrate that is known to be incorporated into autoinducer during its synthesis in *V. fischeri* (15) and used HPLC to compare the relative amounts of label incorporated by *V. fischeri* ES114 and MJ1. *V. fischeri* ES114 grown in the presence of exogenous autoinducer was luminescent, indicating activation of the *luxICDABE/G* operon. Although these cells incorporated radiolabeled methionine into material that eluted with autoinducer, the amount of this radiolabeled material was only about 1 to 2% of that from a similarly grown culture of *V. fischeri* MJ1 (Fig. 3). Sequence and genetic analyses indicate that the *V. fischeri* ES114 *luxI*, *luxC*, *luxD*, *luxA*, *luxB*, and *luxE* genes are transcribed as a single unit, as in other *V. fischeri* strains (18, 19). Therefore, the fact that the transcription of these genes in induced cultures of *V. fischeri* ES114 is sufficient for visible luminescence indicates that the small amount of autoinducer produced by these cultures is not due to a lack of *luxI* transcription.

DISCUSSION

The cloning and characterization of the *lux* regulon from the squid symbiont *V. fischeri* ES114 answer several questions concerning the molecular basis for the lack of autoinducer synthesis by this strain in laboratory culture. Because the cloned ES114 *lux* DNA contains all of the genes normally present within the *lux* regulon of autoinducer-producing strains of *V. fischeri*, with the genes organized in the same arrangement found in those other strains (Fig. 2), we can rule out the possibility that ES114 does not possess a *luxI* gene or has otherwise grossly rearranged its *lux* regulon. The fact that the *V. fischeri* ES114 DNA directs autoinducer synthesis in *E. coli* eliminates the possibility that the *luxI* gene or its product is inactive. In addition, the synthesis of autoinducer by *V. fischeri* ES114 harboring a multicopy plasmid containing the ES114 *lux* regulon suggests that the protein encoded by the ES114 *luxI* functions within its native background as well as in *E. coli*. The possibility that defects specific to the *luxR* gene are responsible for depressed autoinducer synthesis was already largely discounted by the ability of *V. fischeri* ES114 to respond to exogenous additions of autoinducer and was further corroborated by our complementation in *E. coli* of a *V. fischeri* MJ1 *luxR* deletion by an ES114 *lux* DNA fragment.

A central issue to which this research pertains is whether the squid or the bacterium is responsible for autoinducer synthesis in the *E. scolopes* light organ (2). Our data are consistent with the hypothesis that, as in other light organ symbioses involving *V. fischeri*, it is the bacterium that synthesizes autoinducer. We still do not know why the squid light organ isolates make insufficient autoinducer to activate the luminescence genes when grown in laboratory culture, although for the representative strain, ES114, several possible explanations can be discounted on the basis of our results.

For example, we know that *V. fischeri* ES114 produces the substrates or metabolic precursors required for autoinducer synthesis because transconjugants containing multiple copies of either the ES114 or the MJ1 *lux* regulon synthesize sufficient autoinducer to induce luminescence. We also know that *luxI* is transcribed and its message is translated in *V. fischeri* ES114 because transconjugants containing the ES114 *lux* regulon in multiple copies on pHV300 clearly express the *luxI* gene by producing relatively high levels of autoinducer. Finally, we know that the limited autoinducer synthesis by *V. fischeri* ES114 in laboratory batch culture is not the result of an inherently low basal rate of *luxI* transcription because even cultures in which the *luxICDABE/G* operon has been fully induced synthesize little or no autoinducer (Fig. 3).

There are several alternative explanations still remaining, however, that cannot be discounted by the data currently available. For example, the *luxI* portion of the *luxICDABE/G* transcript could be processed or preferentially degraded in *V. fischeri* ES114 before translation. Alternatively, the LuxI protein itself may be unstable or turned over at an enhanced rate in this strain. Another explanation for the lack of *luxI* function in ES114 is that the *luxI* message could be translated at a low efficiency.

It should be remembered that the luminescence system of *V. fischeri* is adapted for a symbiotic habitat and that the environmental conditions of laboratory batch culture are not encountered by these bacteria in nature. If the environmental sensing system defined by autoinduction is designed to distinguish between the very high population densities (about 10^{11} cells per ml) found within the light organ of an animal host (2, 31) and the very low population densities (≤ 10 cells per ml) found in seawater (30, 32), then the intermediate cell densities achieved in laboratory batch culture (10^9 to 10^{10} cells per ml) may by coincidence be sufficient for autoinduction in some but not all *V. fischeri* strains. Consequently, the luminescence of *V. fischeri* MJ1 in laboratory culture might be considered an artifact of a relatively high basal rate of autoinducer synthesis in this strain. In the case of *V. fischeri* ES114, autoinducer is produced in small amounts. Perhaps the luminescence system is induced only when the bacteria have achieved population densities that, though common for the light organ habitat, are not easily attained in laboratory batch culture. The squid host may thereby be activating the luminescence system of *V. fischeri* ES114 by providing a growth environment that is capable of supporting the dense bacterial populations required for autoinduction of the *lux* genes in this strain.

The methods described here and our analysis of the *V. fischeri* ES114 *lux* genes provide a foundation for future investigations of symbiont recognition in the early development of this symbiotic association. Squid hatchlings must initially acquire their bacterial symbionts from seawater, but only a specific subset of cultured *V. fischeri* strains appears competent to establish such an infection (26). By using a molecular genetic approach, it should now be possible to investigate the genetic and biochemical basis for this apparent strain discrimination by the animal host.

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