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, 26, 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 synthesis, 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 luxICDABE 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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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 bac-
terial 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 experi-
ments 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
were composed 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
digestive 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 enzy-
me-digested plasmid DNA was treated with calf intestinal
phosphatase (Boehringer Mannheim Corp., Indianapolis,
Ind.) according to the manufacturer’s protocol. DNA restric-
tion 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 cen-
trifuged at 55,000 rpm in a Ty25 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

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**TABLE 1. Bacterial strains and plasmids**

<table>
<thead>
<tr>
<th>Bacterial strain or plasmid</th>
<th>Relevant characteristics*</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. fischeri MJ1</td>
<td>Japanese pinecone fish light organ symbiont; brightly luminous in laboratory culture</td>
<td>31</td>
</tr>
<tr>
<td>V. fischeri ES114</td>
<td>Squid light organ symbiont; nonluminous in laboratory culture</td>
<td>2</td>
</tr>
<tr>
<td>E. coli VJS533</td>
<td>ara Δ(lac-proAB) rpsL φ80 lacZAM15 recA456</td>
<td>38</td>
</tr>
<tr>
<td>E. coli JM109</td>
<td>endAl recA1 syrA96 thi hsdR17 relA1 (res mod+) supE44 Δ(lac-proAB) [F' traD36 proAB lacPZAM15]</td>
<td>41</td>
</tr>
<tr>
<td>E. coli S17-1</td>
<td>RF4-2-Tc::Mu-Km::Tn7 Sm' pro (res mod+) recA4 tra+</td>
<td>35</td>
</tr>
</tbody>
</table>

Plasmids

- pHG165: ColE1 replicon; Ap' lacZ*
- pBR322: ColE1 replicon; Ap' Te'
- pACYC184: P1A replicon; Cm' Te'
- pSUP102: P1A replicon; mob' Cm' Te'
- pIE202: 8.8-kb SalI fragment containing the V. fischeri MJ1 lux regulon in pBR322; Ap'
- pHK555: luxR luxICDABE* in pACYC184 (lux DNA from V. fischeri MJ1); Cm'
- pHV100: 8.8-kb SalI fragment containing the V. fischeri ES114 lux regulon in pHG165; Ap'
- pHV200: 8.8-kb SalI fragment from pHV100 in pBR322; Ap'
- pHV202: 1.1-kb SalI-PvuII fragment from pHV200 in pBR322; luxR+; Ap'
- pHV300: 8.8-kb SalI fragment from pHV200 in pSUP102; Cm'
- pNLI121: 8.8-kb SalI fragment from pJE202 in pSUP102; Cm'

* Ap', ampicillin resistant; Cm', chloramphenicol resistant; Sm', streptomycin resistant; Te', tetracycline resistant.
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 SauI, ligated to SauI-digested pHG165, and then used to transform E. coli V18533. 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 SauI 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 Erlmeneyer 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 filtered 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 × 10⁵ 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'-AATGCCGACGACTTACAG-3' (nucleotides 758 to 739 from the V. fischeri ATCC 7744 sequence); luxI, 5'-GCAATTCATCGAGAGAT-3' (nucleotides 1022 to 1041); luxC, 5'-TACGGTGTGCAACGATGGA-3' (nucleotides 1815 to 1834); luxD, 5'-CATGCTTCGATCTAGAC-3' (nucleotides 4270 to 4289); luxB, 5'-CTCAGATCCTACGATCA-3' (nucleotides 5492 to 5511); luxE, 5'-CAGCTATTAGGCGA-3' (nucleotides 7287 to 7306); and luxG, 5'-CATCTTAGCATTAGCAGA-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 HindIII-BglII 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). The 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') 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' mutants were then conjugated with E. coli S17-1 containing either pSUP101, 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 [³²P]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 ultrapure C₁₈ reverse-phase column (4.6 mm by 25 cm; Beckman Instruments), from which 0.5-ml fractions were collected. A sample of [³³P]-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 SauI 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-
constructed 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 pJE202, which contained a 1.1-kb SalI-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,
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 luxICD-ABE/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 luxICDABEG 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 luxCDABE/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 luxCDABE/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 (10^6 to 10^9 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.

ACKNOWLEDGMENTS

We thank E. G. Ruby for providing us with V. fischeri ES114 and E. P. Greenberg and G. S. Shadel for V. fischeri MJ1. We also thank P. V. Dunlap for providing us with pNL121 in advance of publication.

This work was supported by the Office of Naval Research (N00014-80-K-0570).

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