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Depressed Light Emission by Symbiotic *Vibrio fischeri* of the Sepiolid Squid *Euprymna scolopes*

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Bioluminescent marine bacteria of the species *Vibrio fischeri* are the specific light organ symbionts of the sepiolid squid *Euprymna scolopes*. Although they share morphological and physiological characteristics with other strains of *V. fischeri*, when cultured away from the light organ association the *E. scolopes* symbionts depress their maximal luminescence over 1,000-fold. The primary cause of this reduced luminescence is the underproduction by these bacteria of luciferase autoinducer, a molecule involved in the positive transcriptional regulation of the *V. fischeri lux* operon. Such an absence of visible light production outside of the symbiotic association has not been previously reported among light organ symbionts of this or any other species of luminous bacteria. Levels of luminescence approaching those of the *E. scolopes* bacteria in the intact association can be restored by the addition of exogenous autoinducer to bacteria in laboratory culture and are affected by the presence of cyclic AMP. We conclude that some condition(s) specific to the internal environment of the light organ is necessary for maximal autoinduction of luminescence in the symbionts of this squid-bacterial association.

The emission of visible light by the marine bacterium *Vibrio fischeri* is dependent upon the enzymatic activity of an inducible luciferase. Its synthesis is regulated at the transcriptional level by a diffusible, sensory autoinducer molecule (7, 22, 28). The autoinducer, which is believed to be species specific (7, 13, 22), has been chemically identified as *N*-(3-oxohexanoyl)homoserine lactone (8). Synthesis of this molecule appears to be continuous (22), and, because it is freely diffusible across the cell membrane, its internal concentration is directly related to the extent of its external accumulation (20). Thus, the autoinducer acts as the sensory component of the autoinduction process, enhancing transcription of the bacterial luminescent system only when *V. fischeri* cells achieve a high density in a confined environment (19, 26, 33). This self-regulation of light production results in visible luminescence when the cells exist either as colonies on the surfaces of decaying organic matter or as symbionts within species-specific light-emitting structures of certain marine fishes and cephalopods (13). Conversely, in seawater and other environments where *V. fischeri* concentrations are rarely more than a few cells per milliliter, autoinducer does not accumulate to a sufficient level for visible luminescence to be expressed (27).

The symbiotic luminescent bacteria that are responsible for light emission by the marine sepiolid squid *Euprymna scolopes* are acquired by the animal early in its development (37). The bacterial culture that develops is maintained in a pair of internal light organs within the mantle cavity (17, 21), where it produces an easily visible luminescence. However, as we demonstrate herein, once removed from the animal, the bacteria become essentially nonluminous. This condition is in marked contrast to the bright luminescence of other described isolates cultured from symbioses with marine fishes and other cephalopods (23, 32, 33) and suggests the presence of an unusual regulatory control of light emission by *E. scolopes* symbionts. We investigated the physiological basis for the depressed light emission of these bacteria grown outside of the symbiosis.

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MATERIALS AND METHODS

Bacterial strains and media. Symbiotic bacteria were isolated from the light organs of *E. scolopes* as described below. A representative symbiotic strain, designated ES114, was used in much of the work described in this study. Other bacterial strains used include *V. fischeri* MJ1, isolated from the light organ of a monocentrid fish (33); *V. fischeri* B61 (1), *Vibrio logei* ATCC 29985 and 35077 (2), and *V. harveyi* B392, all isolated from seawater (22, 29); and two *Photobacterium* species, *P. phosphoreum* NZ-11-D and *P. leiognathi* LN-1a, isolated from the light organs of a macrourid or a leiognathid fish, respectively (5, 32).

Bacteria were grown and maintained on SWT medium containing (per liter) 5 g of Bacto-Tryptone (Difco Laboratories, Detroit, Mich.), 3 g of yeast extract (Difco), 3 ml of glycerol, 700 ml of filtered seawater, and 300 ml of distilled water. Solid medium was prepared by the addition of 1.2% Bacto-Agar (Difco). An artificial-seawater-based basal medium (BM) containing 50 mM Tris hydrochloride buffer adjusted to pH 8.0 with NaOH and 0.3% glycerol was prepared as previously described (29). A complex nutrient BM medium (complex BM) was prepared by the further addition (per liter) of 5 g of Bacto-Tryptone, 3 g of yeast extract, and 3 ml of glycerol. Conditioned medium (CM) was prepared as previously described (22) by growing cells of *V. harveyi* B392 to a concentration of 5.4×10^8 cells per ml in complex BM. The medium was cleared of cells by centrifugation and sterilized by passage through a 0.2-μm-pore-sized filter (Millipore Corp., New Bedford, Mass.).

Stock solutions of hexanoic acid and hexanol (Aldrich Chemical Co., Inc., Milwaukee, Wis.) were prepared in 50% ethanol and added to selected media immediately before use. Methionine, homoserine, homoserine lactone, and cyclic AMP (cAMP) (Sigma Chemical Co., St. Louis, Mo.) were prepared in 10 mM Tris hydrochloride adjusted to pH 8.0 with NaOH. Some media were supplemented with synthetic

autoinducer (19) dissolved in ethyl acetate. When desired, filter-sterilized glucose was added to autoclaved medium as a carbon source. The iron chelator ethylenediamine-di(*o*-hydroxyphenyl)acetic acid was prepared as previously described (15).

Collection of animals and isolation of bacterial symbionts. Symbiotic luminous bacteria were isolated from recently collected *E. scolopes* by cooling the animals to about 8°C before opening the mantle cavity along the ventrum. Two methods were used to obtain samples of symbiotic bacteria: (i) with aseptic precautions, light organ fluid containing bacteria was obtained from the pore that leads into channels within the organ tissue (21) or, (ii) whole light organs were removed by dissection and homogenized in 700 μl of sterile seawater. Material obtained in either of these ways was serially diluted in SWT medium, and samples of the dilutions were both measured for light production and spread on SWT agar plates for the enumeration of bacterial colonies. Alternatively, samples of the dilutions were stained with acridine orange and enumerated by direct counting by using epifluorescence microscopy (18).

Over 200 distinct strains were isolated and stored, including 30 isolates from both the left and right organs of two animals collected in March 1988 in Kaneohe Bay, Hawaii, and 25 isolates from each of four animals collected in March 1989 from either Kaneohe Bay or Niu Bay, Hawaii. One hundred and fifty-five of these strains were taxonomically identified by previously described methods (29, 33).

Measurements of cell density and luminescence. Cell concentrations of *V. fischeri* in liquid medium were determined by using a Perkin-Elmer Lambda 3B spectrophotometer for measurements of the optical density (OD) at 600 nm. An OD of 1.0 corresponded to about 3×10^8 cells of *V. fischeri* per ml of culture as determined by plate counts. Light emission of cell suspensions, recorded as quanta per second, was measured by using a model 110 laboratory photometer (Pacific Instruments, Concord, Calif.) calibrated with the light standard of Hastings and Weber (14). Low levels of light emission were detected by using a model 2000 photometer (Lab-Line Instruments, Inc., Melrose Park, Ill.). In vitro assays of luciferase enzyme activity were performed as previously described (23).

Comparison of induction patterns. *V. fischeri* MJ1, B61, and ES114 were each inoculated into 5 ml of SWT medium and grown overnight to late-log phase. An inoculum of 1.5×10^7 cells of each strain was then transferred to 50 ml of SWT medium in 500-ml Erlenmeyer flasks and shaken continuously at 26°C for the duration of the experiment. Samples of 2 ml were taken periodically for measurements of cell density and luminescence of the culture.

Effect of various additions on luminescence of *V. fischeri* ES114. The relative extent of production and excretion of autoinducer was determined by growing *V. fischeri* MJ1, B61 and ES114 in complex BM to an OD of 0.5. The cultures were centrifuged and filter sterilized to remove cells, and 100 ml of each of these spent media was extracted twice with 200 ml of ethyl acetate (22). The presence of autoinducing activity in light organ tissue was determined by homogenizing in 700 μl of sterile seawater the entire organ dissected from a 22-mm specimen of *E. scolopes* and extracting the homogenate twice with 1 ml of ethyl acetate. The extracts of the media and the light organ were concentrated 400-fold and 20-fold, respectively, by evaporation at 35°C under vacuum. Samples of 100 μl of each extract were placed in small vials, and the ethyl acetate was evaporated under a stream of nitrogen. To each of these vials 1 ml of CM medium, inoculated to an OD of 0.1 with log-phase cells of *V. fischeri* ES114, was added. These suspensions were vigorously shaken at 26°C, and the development of luminescence was monitored.

Log-phase cells of strain MJ1 or ES114 were diluted to an OD of 0.05 in 25 ml of either CM or SWT medium. Cells inoculated into CM medium do not exhibit the lag in induction of luminescence that is observed in other complex media, which contain an unidentified inhibitor that is removed during the growth of the culture (7, 22). Synthetic autoinducer was added to a concentration of 100 ng/ml, a level that was determined to provide the maximum induction activity (data not shown). All cultures were shaken continuously at 26°C, with periodic removal of 1-ml samples for cell density and luminescence measurements.

It has been hypothesized that short-chain aliphatic acids may be precursors in the biosynthesis of the acyl moeity of autoinducer of *V. fischeri* (A. Eberhard, personal communication). Therefore, sodium hexanoate (0.03%) or hexanol (0.003%) was added to cultures of *V. fischeri* ES114 in CM medium at the maximum concentration that did not inhibit growth to determine their effect on the development of luminescence capability. Similarly, 20 μg of either methionine, homoserine, or homoserine lactone was added per ml of fresh culture of *V. fischeri* ES114 in CM medium as a potential precursor for the synthesis of the cyclic portion of the autoinducer molecule.

Effect of physiological growth conditions on luminescence. Cells of strains MJ1 and ES114 were grown in complex BM medium at 30°C with shaking in the presence of synthetic autoinducer at a concentration of 100 ng/ml. The specific activity of light production was monitored as a function of cell growth under conditions of reduced iron availability, produced by the addition of 200 μM ethylenediamine-di(*o*-hydroxyphenyl)acetic acid as described previously (15).

The effect of glucose availability on the development of luminescence was determined in 30 ml of CM broth containing 0.2% (wt/vol) glucose or glycerol. These media were inoculated to an OD of 0.01 with a 1% inoculum of log-phase cells of *V. fischeri* MJ1 and ES114 growing in SWT broth. Cultures were shaken vigorously at 24°C and monitored for increasing OD and luminescence (33).

A comparison of the effect of oxygen availability on the luminescence of *V. fischeri* MJ1 and ES114 was made by growing the bacteria in SWT as previously described (25) and measuring the amount of light emitted per cell as a function of culture oxygen concentration.

RESULTS

Characterization of light organ symbionts. All of the 155 bacterial symbionts isolated from eight light organs of six *E. scolopes* collected at two different locations over a 13-month period were identified as *V. fischeri* (Table 1). Although the diagnostic traits that distinguish between the species *V. fischeri* and *V. logei* are few in number (2, 3), the *E. scolopes* symbionts most closely resembled *V. fischeri*. In a past review (13), an unpublished observation was made that *V. logei* was responsive to the addition of a crude preparation of *V. fischeri* autoinducer. In the present study it was observed that pure, synthetic autoinducer did not enhance light production by two strains of the closely related (2) species *V. logei*. Instead, this autoinducer appeared to be a species-specific luminescence regulator (Table 1). Thus, the strong response of the *E. scolopes* strains to added autoinducer (see ahead) further substantiated their identity as *V. fischeri*.

TABLE 1. Taxonomic characteristics of *E. scolopes* symbionts and other bacteria

Species[a]	Auto-inducer[b]	Luci-ferase[c]	Pig-ment[d]	Flagella[e]	Lipase excre-tion	Growth at:		Growth on:				
						4°C	35°C	Mannitol	Maltose	Cellobiose	Lactate	Gluconate
E. scolopes symbionts	+	F	Y	ST	+	−	+	+	+	+	−	−
V. fischeri	+	F	Y	ST	+	−	+	+	+	+	−	−
V. logei	−	F	Y	ST	+	+	−	+	+	+	−	+
V. harveyi	−	S	B	SP	+	−	+	+	+	+	+	+
P. phosphoreum	−	F	W	NP	−	+	−	−	−	−	−	+
P. leiognathi	−	F	W	NP	−	−	+	−	−	−	+	−

[a] Consensus characteristics of luminous species were described previously (23).
[b] Luminescence increased (+) or not (−) by *V. fischeri* autoinducer.
[c] F, Fast turnover kinetics; S, slow turnover kinetics.
[d] Colony pigmentation was yellow (Y), brown (B), or white (W).
[e] Flagellation was sheathed tuft (ST), sheathed single polar (SP), or unsheathed single polar (NP).

Luminescence of *E. scolopes* symbionts. Light emission from the organ of *E. scolopes* is sufficiently bright to be visually detected in a darkened room when the animal is disturbed in its aquarium. The light is due entirely to the presence of luminous bacteria at a concentration of approximately 10^{11} cells per ml of light organ fluid, as determined by plating of fluid dilutions on SWT agar medium. Plating efficiency on SWT medium was between 85 and 97% as determined by comparison with direct microscopic counts of fluorescently stained cells.

Because the light organs contain between 1 and 10 μl of fluid volume, visible light emission must result from the activity of between 10^8 and 10^9 bacterial cells, or approximately the number found in a 1-mm-diameter colony growing on an agar plate. However, bacterial colonies that developed from the initial plating of light organ fluid were not visibly luminous at any time during their growth. Nevertheless, when exposed to a sensitive photometer, each one of the colonies was revealed to be producing a low but detectable level of luminescence. Thus, when cultured outside of the light organ the symbiotic bacteria produced a significantly diminished level of luminescence that continued throughout subsequent transfers as well as during growth on a variety of complex and minimal media. This behavior was in marked contrast to that of luminous bacteria cultured from other symbioses such as the monocentrid fish light organ isolate *V. fischeri* MJ1 (33). A comparison of the luminescence of strain MJ1 and strain ES114, isolated from *E. scolopes*, illustrates the unusual nature of the squid symbionts (Table 2).

Pattern of induction of the *V. fischeri* luminescent system. The development of luminescence in strain ES114 was compared with that of the visibly luminous strains *V. fischeri* MJ1 and B61 during growth in a liquid broth. When log-phase cells were inoculated into fresh SWT medium, the light emission of all three strains demonstrated the typically observed initial decrease (22, 28), followed by a logarithmic increase, in the level of luminescence per cell (Fig. 1). At a culture density of about 2.0 OD units the cultures reached a maximum luminescence level that was characteristic for each strain. Although the cell density continued to increase, there was no further increase in light emission per cell.

Because the increase in in vivo luminescence occurred at approximately the same cell density in each culture, all of the strains apparently have a similar ability to remove the inhibitor of luminescence induction present in SWT medium (7, 22). When cells were grown in CM medium, from which the inhibitor had been removed, the point of induction of luminescence appeared to be directly related to capacity for autoinducer production; i.e., strain MJ1 induced at a lower cell density than did other strains that produce lower amounts of autoinducer (22; data not shown). Although the three strains of *V. fischeri* had quantitatively similar patterns of luminescence expression, the maximum light emission of strain ES114 was between 2 and 5 orders of magnitude lower than that of either of the other two strains. This low level of

TABLE 2. Bioluminescence of symbiotic bacteria

Treatment	Quanta/s per cell	
	MJ1	ES114
Freshly collected from light organ	ND[a]	819
Cultured[b]	2,600	0.8
Cultured with autoinducer (200 ng/ml)	2,000	325
Cultured with ES114 medium extract	ND	1.0
Cultured with MJ1 medium extract	ND	169
Cultured with light organ extract	ND	111

[a] ND, Not determined.
[b] Strains were grown in CM medium as described in the text.

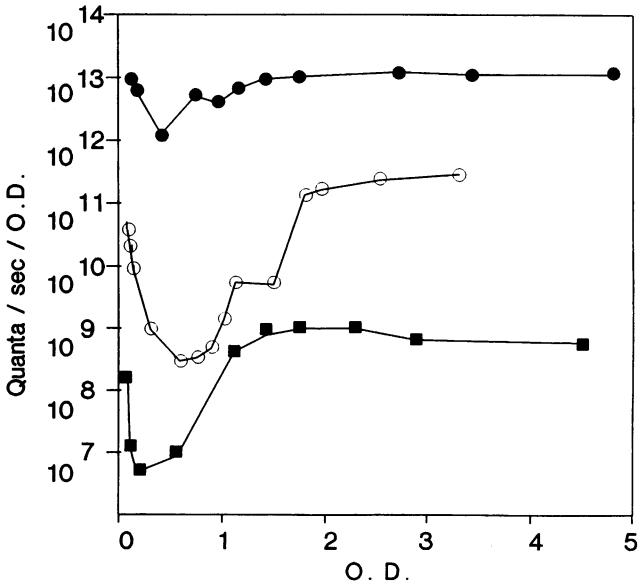

FIG. 1. Comparison of the intensity of light production by growing cultures of three strains of *V. fischeri*. The specific activities of light production (in quanta per second) of strains MJ1 (●), B61 (○), and ES114 (■) were measured during growth in SWT broth (1 unit of OD at 600 nm is equivalent to about 3×10^8 cells per ml).

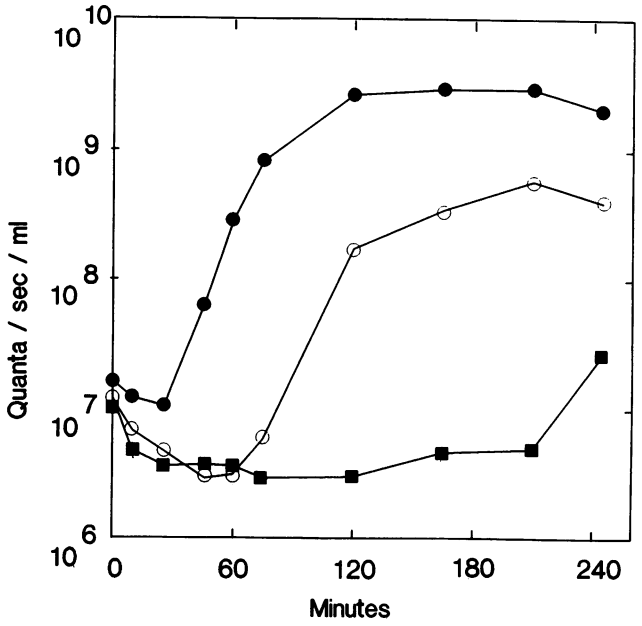

FIG. 2. Effect of the addition of concentrated extracts of spent growth medium of *V. fischeri* MJ1 (●), B61 (○), and ES114 (■) on the development of luminescence of ES114 cells. Light production (in quanta per second) was measured in cultures grown in CM broth to which small amounts of an ethyl acetate extract of spent medium were added.

in vivo luminescence in ES114 was due primarily to a relatively low luciferase content as revealed by in vitro analyses of enzyme-specific activities of strains ES114 (0.0075 quanta per s per ml) and MJ1 (6.9 quanta per s per ml) grown to a culture density of 1.0 OD unit in SWT medium.

Direct addition of exogenous aldehyde, a substrate in the luciferase-catalyzed luminescence reaction, stimulates the light emission of aldehyde-deficient luminous bacteria within seconds (30, 36). To test for such an aldehyde deficiency in ES114, a 5-μl drop of either decanal or dodecanal, both as 0.001% (vol/vol) solutions in water, was added to 24-h-old colonies of *V. fischeri* ES114 growing on SWT agar. Although two aldehyde mutants of *V. fischeri* MJ1 isolated in our laboratory gave a strongly positive reaction to such an addition, there was no response by ES114 to this treatment.

Effect of medium extracts containing autoinducer. Previous studies investigating the reason behind the variation in luminescence intensity observed among different strains of *V. fischeri* have demonstrated the direct correlation between the luminescence capability of a given strain and production and secretion of autoinducer (22). Thus, one explanation for the low light emission of strain ES114 is that it is an underproducer of *V. fischeri* autoinducer. This hypothesis was tested by incubating strain ES114 in extracts of the spent media of strains MJ1 and B61, which are known to contain high and low concentrations of autoinducer, respectively. Upon transfer of log-phase strain ES114 cells to broth containing a culture medium extract from strain MJ1, induction of luminescence was observed almost immediately (Fig. 2). Induction in the presence of medium extract from strain B61 was delayed, beginning 60 min after addition. The increase in luminescence of cells of strain ES114 exposed to their own spent medium extract was detectable only after 4 h of incubation, suggesting that strain ES114 produces significantly less autoinducer than does strain B61. This conclusion was supported by the absence of any enhancement of luminescence induction in a culture of strain B61 cells to which was added a medium extract from strain ES114 (data not shown). Therefore, the apparent effectiveness of medium extracts to induce luminescence (Fig. 2) reflected the relative luminescence intensity of these strains (Fig. 1).

TABLE 3. Effect of additions on ES114 luminescence in culture

Compound added	Maximum light production[a] (quanta/s per cell)
None	0.8
cAMP (10 mM)	6.8
Autoinducer (200 ng/ml)	195
Autoinducer plus cAMP	208
Potential autoinducer precursors	
Methionine (20 μg/ml)	1.0
Homoserine (20 μg/ml)	0.8
Homoserine lactone (20 μg/ml)	1.0
Hexanoate (0.03%)	1.0
Hexanol (0.003%)	0.6

[a] Light emission of a culture after 5 h.

Response to synthetic autoinducer. Purified synthetic autoinducer increased the maximum level of luminescence in cells of strain ES114 approximately 1,000 times above that of a control culture to which no additions were made (Table 2). Similar data were obtained when eight other strains of *E. scolopes* isolates were tested for their response to autoinducer (data not shown), indicating that this sensitivity to autoinducer is a general trait in these symbionts. Increasing the concentration of autoinducer above 100 to 200 ng/ml did not further increase the maximum light output above about 300 quanta per s per cell. Cells of strain ES114 that had been exposed to autoinducer became visibly luminescent, producing light levels about 40% that of cells freshly removed from the light organs (Table 2). This luminescence by induced cells of strain ES114 was about 15% that of MJ1 cells that had been fully induced in culture with or without exogenous autoinducer.

The preceding data suggested that cells of ES114 grown outside of the symbiosis may simply have a defect in the metabolic pathway that leads to the synthesis of autoinducer. In an attempt to stimulate production of autoinducer by providing possible metabolic precursors for its synthesis, cells of strain ES114 were grown in medium supplemented with potential sources of either the aliphatic (hexanoate or hexanol) or cyclic (methionine, homoserine, or homoserine lactone) portion of the autoinducer molecule. None of these additions was successful in increasing luminescence significantly above control levels, indicating continued low production of autoinducer under these conditions (Table 3).

Response to cAMP. The addition of exogenous cAMP increased luminescence of ES114 cells by a factor of 8 to 9 times that of control cell cultures (Table 3). Although this effect could indicate that strain ES114 is an adenylate-cyclase-defective mutant (6, 11), the production of acid on galactose-maltose plates by this strain is consistent with the presence of a functional adenylate cyclase and thus does not support this hypothesis. Although it increased the development of luminescence somewhat, the addition of cAMP was not as effective as the addition of autoinducer and did not increase levels of luminescence significantly above those achieved with autoinducer alone (Table 3).

Effects of iron, glucose, and oxygen. Although the presence of 200 μM ethylenediamine-di(*o*-hydroxyphenyl)acetic acid

in a culture of ES114 reduced the concentration of free iron, resulting in a 50% decrease in growth rate, it had only a minor effect on the onset of induction. This response differed markedly from the distinct enhancement of light emission that accompanied diminished iron concentration in the medium of strain MJ1 (15; data not shown). Although the induction of luminescence of *V. fischeri* MJ1 was delayed significantly by the addition of glucose to the growth medium, no such effect was observed with strain ES114 grown under identical conditions. Similarly, limiting the availability of oxygen to a growing culture of strain ES114 depressed the growth rate but had no measurable effect on either the rate of luminescence induction or the specific activity of light emission (data not shown).

Taken together, these results indicate two points: (i) in culture the requirement for exogenous autoinducer could not be overcome by the limitation of iron, oxygen, or glucose; and (ii) these physiological effectors had no significant effect on the induction or level of luminescence in the presence or absence of added autoinducer.

DISCUSSION

The shallow-water sepiolid squid *Euprymna scolopes* maintains dense cultures of luminescent bacteria in specialized light-emitting structures within its mantle cavity. This association is species specific: over 150 representative isolates from six animals were identified as *V. fischeri*. *V. fischeri* has also been reported as the light organ symbiont of two species of monocentrid fishes (10, 33). Thus, this species of luminous bacteria shares with *Photobacterium leiognathi* the characteristic of including strains adapted to specific, morphologically complex associations with both vertebrate and invertebrate hosts (12, 13). At this time it is not possible to say that any single *V. fischeri* strain can initiate a successful symbiotic association with either fish or squid hosts or that there is a bacterial strain specificity expressed by different host species, as is the case for the *Rhizobium*-legume symbiosis (16). However, the physiological and biochemical controls of expression of luminescence in *V. fischeri* isolated from *E. scolopes* are distinct from those characteristic of *V. fischeri* isolated from fish light organs.

The development of luminescence by *V. fischeri* MJ1 has been shown to be depressed by several physiological effectors, including a relatively high ambient concentration of dissolved iron (15), glucose (11, 33), or oxygen (25). When subjected to these same conditions, strain ES114, a representative *E. scolopes* symbiont, did not exhibit any measurable response in the normal onset or range of light production. Thus, the greatly diminished luminescence by the squid symbionts in culture is not simply a severe example of the response of strain MJ1 to environmental concentration of iron, oxygen, or glucose. In addition, these data suggest that the squid symbionts are adapted to a different set of physiological conditions in their light organ environment than that proposed for the symbionts of monocentrid fishes (24).

Immediately upon removal from the light organ environment, the *E. scolopes* symbionts are visibly luminous; however, these bacteria subsequently exhibit a rapid and severe decrease in light emission that is not reversed by culturing the bacteria on any of a variety of laboratory media. This behavior contrasts sharply with the maintenance of bright luminescence of all other luminous bacteria (regardless of species) that have been successfully cultured from any light organ association (13, 22). The low level of light emission by *E. scolopes* symbionts in laboratory culture has been shown by in vitro assay to be the result of a depressed synthesis of luciferase, the inducible enzyme responsible for bacterial luminescence. Levels of luminescence approximating those of freshly collected *E. scolopes* symbionts can be restored in culture by the addition of luciferase autoinducer. *V. fischeri* autoinducer is a diffusible sensory molecule that is normally produced during growth of *V. fischeri* cells and acts to increase transcription both of the luciferase genes and of a gene responsible for the production of autoinducer itself (4, 9). Once a critical concentration of autoinducer is reached, induction of the luminescence system occurs, resulting in an exponential increase in light emission per cell until a strain-specific maximum is reached (22; Fig. 1).

The variation in luminescence of different strains of *V. fischeri* appears to be due primarily to their different rates of autoinducer synthesis (22), rather than to variations on the general mechanism for *lux* operon regulation. The roles of autoinducer and cAMP as transcriptional activators of the *lux* operon of strain MJ1 have been described (4, 6). The response of *E. scolopes* symbionts like ES114 to externally added autoinducer and cAMP suggests that this aspect of the regulatory organization of the *lux* operon does not differ from that of other described *V. fischeri* strains. In addition, the similarity in pattern (although not extent) of increase in light emission per cell shared by strain ES114 and other autoinducer-synthesizing *V. fischeri* strains (Fig. 1) is consistent with the hypothesis that strain ES114 does synthesize autoinducer. However, if strain ES114 synthesizes any autoinducer in laboratory culture, it is at a considerably decreased level compared with that of light organ symbionts from monocentrid fishes and is among the lowest recorded for any *V. fischeri* strain (22).

One hypothesis for their low rate of autoinducer production is that the *E. scolopes* strains have a biochemical lesion in its synthesis. The biosynthetic pathway for the synthesis of autoinducer is not known, but it has been suggested to involve short-chain aliphatic acids and methionine among its possible precursors (Eberhard, personal communication). It is conceivable that when these bacteria are cultured outside of the light organ, synthesis of autoinducer is limited by the availability of such precursors. The lack of stimulation of ES114 luminescence by a number of compounds (Table 3) indicated that the availability of any of these potential precursors probably does not limit the synthesis of autoinducer in *E. scolopes* symbionts in culture.

There is an alternative hypothesis to explain the different rates of autoinducer synthesis among strains of *V. fischeri*. If the amount of *luxI* gene product controls the rate of autoinducer synthesis proportionally, then different strains may differ in their rates of transcription and/or translation of the *luxI* gene. Although there is a good general molecular model for the regulation of the *lux* operon in this luminous species, the potential for strain-specific differences in *luxI* transcription has yet to be adequately addressed.

The unusually dim luminescence of *E. scolopes* symbionts outside of the light organ has implications for the study of the ecology of the luminous bacteria as well. Our current understanding of the distribution of luminous bacteria in the marine environment has been based on the selection and taxonomic identification of bacteria that form visibly luminous colonies upon isolation (31, 34). The contribution of non-visibly luminous *V. fischeri* (or other species) would have been missed because of the dependence of this protocol on the single property of visible luminescence for primary selection (29). This may explain why no *V. fischeri* were reported from an extensive sampling of coastal Hawaiian

waters (29), even though the *E. scolopes* strains of *V. fischeri* are likely to be abundant there because of the presence of large populations of these animals (35). Thus a new approach to the study of luminous bacterial ecology will require the use of a more direct identification of bacteria carrying specific luminescence genes, regardless of their expression in culture.

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