Multiple Repetitive Elements and Organization of the *lux* Operons of Luminescent Terrestrial Bacteria

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The complete nucleotide sequences of the luxA to luxE genes, as well as the flanking regions, were determined for the lux operons of two Xenorhabdus luminescens strains isolated from insects and humans. The nucleotide sequences of the corresponding lux genes (luxCDABE) were 85 to 90% identical but completely diverged 350 bp upstream of the first lux gene (luxC) and immediately downstream of the last lux gene (luxE). These results show that the luxG gene found immediately downstream of luxE in luminescent marine bacteria is missing at this location in terrestrial bacteria and raise the possibility that the lux operons are at different positions in the genomes of the X. luminescens strains. Four enteric repetitive intergenic consensus (ERIC) or intergenic repetitive unit (IRU) sequences of 126 bp were identified in the 7.7-kbp DNA fragment from the X. luminescens strain isolated from humans, providing the first example of multiple ERIC structures in the same operon including two ERIC structures at the same site. Only a single ERIC structure between luxB and luxE is present in the 7-kbp lux DNA from insects. Analysis of the genomic DNAs from five X. luminescens strains or isolates by polymerase chain reaction has demonstrated that an ERIC structure is between luxB and luxE in all of the strains, whereas only the strains isolated from humans had an ERIC structure between luxD and luxA. The results indicate that there has been insertion and/or deletion of multiple 126-bp repetitive elements in the lux operons of X. luminescens during evolution.

The lux genes of luminescent bacteria have been isolated from marine bacteria of the Vibrio and Photobacterium genera (3, 5, 6, 14, 15, 18) and from terrestrial bacteria of the Xenorhabdus genus (9, 26, 29). lux genes that code for luciferase (luxAB) and the fatty acid reductase complex (luxCDE) involved in the synthesis of the fatty aldehyde substrate for the luminescence reaction have been detected (17) in all cases. In certain Photobacterium species, an additional gene (luxF), homologous in sequence to the luciferase genes, is also present (1, 12, 23). A new gene, luxG, closely linked to luxE, has been discovered in Vibrio and Photobacterium species (14, 24, 25). The order of the lux genes in these species is luxCDAB(F)EG, with the luciferase genes flanked by the luxCD and luxE genes.

Significant differences do, however, exist between the *lux* systems of the marine luminescent bacteria owing to the presence of other *lux* genes. Upstream of *luxC* in *Vibrio fischeri* species are two regulatory genes, *luxI* and *luxR* (6, 7), whereas in *Vibrio harveyi* and *Photobacterium* species, an AT-rich region of more than 500 bp with no extended open reading frames is present (19, 20). Downstream of *luxG* in *V. fischeri* is the *lux* operon termination site, while the *V. harveyi lux* operon contains another *lux* gene, *luxH*, before termination (24, 25). The roles of the *luxG* and *luxH* gene products in luminescence are not known. Genetic experiments using transposon insertion have not provided any evidence concerning their possible involvement in the emission of light (16).

Recently, lux genes have been cloned and transferred into Escherichia coli from three strains (Hb, Hm, and Hw) of the enteric terrestrial bacterium Xenorhabdus luminescens (9, 26, 29). Two of the strains, Hb and Hm, are very similar and can be isolated from the gut tracts of nematodes or from the hemocoel of insects (21). The nucleotide sequences of the luxA and luxB genes from these two strains (13, 26), as well

as the Hm luxD gene (2), have been determined, and the sequences of the luciferase genes are almost identical. A transcribed sequence capable of forming a strong hairpin loop (-20.5 kcal [1 cal = 4.184 J]) was identified downstream of luxB in the insect Hb DNA (26). This sequence has recently been recognized as part of a 126-bp enterobacterial repetitive intergenic consensus (ERIC) sequence or intergenic repetitive unit found in multiple copies in the genomes of a number of enterobacteria, including E. coli and Salmonella typhimurium (11, 22, 28). However, such a sequence has not been found in multiple copies in one operon or at one site.

The nucleotide sequences of the *luxCDAB* genes, but not those of the *luxE* gene or flanking regions, have also been reported for the Hw strain (29). This strain is of particular interest, since it was isolated from a human wound rather than from a nematode and/or insect (4, 8). Although the nucleotide sequences of the intergenic regions flanking the luciferase genes were determined, repetitive structures were not recognized.

In this report, the complete nucleotide sequences of a 7-kbp DNA fragment of the Hb strain and a 7.7-kbp DNA fragment of the Hw strain that contains the *luxCDABE* genes, as well as the flanking regions, are described, including corrections to the previous Hw *lux* DNA sequence. As a result, four ERIC sequences have been discovered in the Hw DNA, including three ERIC sequences flanking the luciferase genes within the *lux* operon. Comparison of the nucleotide sequences of the Hw and Hb DNAs not only demonstrates that the organization of *lux* operons of terrestrial bacteria differs from that of marine luminescent bacteria but suggests that the *lux* operons are at different genomic locations in the *X. luminescens* strains.

MATERIALS AND METHODS

Materials. Restriction enzymes were from Bethesda Research Laboratories. [35S]dATP (1,400 Ci/mol) was from

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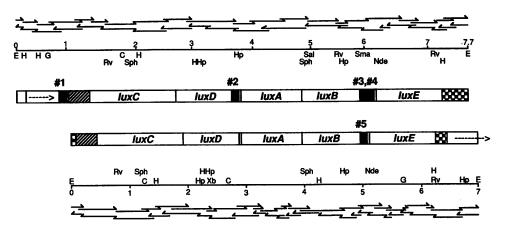


FIG. 1. Organization, restriction maps, and sequencing strategy for *EcoRI* DNA fragments containing the *lux* structural genes from *X. luminescens* Hw (upper) and Hb (lower). Open reading frames corresponding to the *lux* genes are labelled, and the direction of transcription is from left to right. Two additional open reading frames are indicated by the boxes with arrows. The numbered black areas correspond to the five repetitive ERIC sequences. The checkered areas upstream and downstream of the *lux* genes are noncoding regions that cannot be aligned, whereas the areas labelled with diagonal lines are noncoding regions with a high degree of sequence homology between the two *X. luminescens* DNAs. Common restriction sites between the two DNAs are given closest to the center of the diagram, whereas restriction sites unique to the Hw and Hb DNAs are given immediately adjacent to the respective linear scales of 0 to 7.7 kbp and 0 to 7 kbp for the respective DNAs. All restriction sites for *BgIII* (G), *ClaI* (C), *EcoRI* (E), *EcoRv* (Rv), *HindIII* (H), *HpaI* (Hp), *NdeI* (Nde), *SaII* (Sal), *SmaI* (Sma), *SphI* (Sph), and *XbaI* (Xb) are given. There are no sites in either DNA fragment for *BamHI*, *NcoI*, *PstI*, *SacI*, *SnaB*, *StuI*, or *XhoI*. A single *PvuI* site (not shown) is located between the *EcoRI* and *HindIII* sites at the start of the Hw DNA.

Dupont. X. luminescens Hb (ATCC 29999) and three other strains (ATCC 29304, ATCC 48949, and ATCC 48951) were obtained from the American Type Culture Collection, while strain Hw (ATCC 48950) was the kind gift of P. Colepicolo and J. W. Hastings (Harvard University). The lux genes of the Hw DNA were cloned as previously described for strain Hb (26). The genomic DNA was restricted with EcoRI, ligated into plasmid pT7-5, transformed into E. coli HB101, and screened for light.

DNA sequence analysis. Subfragments of the lux DNAs created by restriction digestion with four- and six-base cutters were inserted into M13mp18 or M13mp19 and analyzed by sequencing with dideoxynucleotides. In a few instances, synthetic oligonucleotides (McGill Biotechnology Centre) were used to complete missing regions. In this manner, the sequences of the entire lengths of both EcoRI fragments from strains Hb and Hw were determined in both directions with overlaps at each junction. Analysis of nucleotide and amino acid sequence data was performed by using the DNASIS and PROSIS programs provided by Hitachi Software Engineering Co. Ltd.

The sequence data for the Hw and Hb *lux* DNAs of the *X. luminescens* strains can be obtained under GenBank accession numbers M90092 and M90093, respectively.

Polymerase chain reaction (PCR). The PCR mixture (50 µl) contained 50 pmol of each primer, 300 ng of genomic DNA, a 1.25 mM concentration of each of the four deoxynucleoside triphosphates, and 0.25 U of Vent DNA polymerase

(New England BioLabs) in the standard buffer supplied with the polymerase. PCR was performed in an automated thermal cycler with cycles of 95°C for 1 min for denaturation, 55°C for 1 min for annealing, and 72°C for 1 min for extension. After 35 cycles, an aliquot (10 µl) of the PCR mixture was resolved by electrophoresis on 3% agarose (Nu-Sieve) gels in Tris-borate-EDTA buffer containing 0.5 µg of ethidium bromide per ml.

Oligonucleotides for PCR. Synthetic oligonucleotides were synthesized by the McGill Biotechnology Centre. The ERIC primer was designed from the most highly conserved ERIC sequences within the Hw and Hb lux DNAs so that it would direct transcription in the coding direction of the lux operon. Three places of degeneracy in the sequence [GTGAC(T/C)GGGGTG(A/C)GTGAA(A/C)GCAGCCAAC] were introduced so that it would be identical to the ERIC structures in both the Hw and Hb lux DNAs immediately in front of luxE and luxA. The reverse luxA (CCAAACTCCGTGAAAT GATGCTC) and luxE (TGTGCCTGACAGTAGTGACG) primers were selected so that they were completely complementary to a region in close proximity to the 5' terminus of the coding strands for these genes in both the Hw and Hb lux DNAs.

Expression of lux DNA in the pT7 plasmids. Plasmids pT7 (ampicillin resistance) and pGP-1-2 (kanamycin resistance) were the generous gifts of S. Tabor and C. Richardson (27). E. coli K38 cells were transformed with plasmid pGP-1-2, which codes for T7 RNA polymerase and plasmid pT7,

FIG. 2. Nucleotide sequences of the Hw and Hb DNAs containing the *lux* genes. The upper sequence, corresponding to the Hw DNA, extends from nucleotides 1 to 7669; the lower sequence, corresponding to the Hb DNA, extends from nucleotides 1 to 6960. Spaces have been left before and after the initiation and termination codons of the *lux* genes. The sequences have been aligned starting at nucleotide 82 and extending to nucleotide 6258 of the Hb DNA, and identical nucleotides are denoted by colons. A few gaps have been introduced for maximum alignment and are indicated by spaces for long stretches and hyphens for short stretches. Sequence homologies were not detected outside these regions. Differences previously reported for the Hw DNA between nucleotides 1182 and 6508 are indicated above the line in lowercase with additional nucleotides indicated by a cicumflex and missing nucleotides indicated by crosses. Frameshifts in coding regions in the Hw DNA occur between nucleotides 1471 and 1498 in *luxC* and from residue 4840 to the end of *luxA*. The sequences corresponding to the repetitive ERIC structures are indicated above and below the Hw and Hb DNAs, respectively, by double or single dashes.

GAATTCTCAGACTCAAATAGAACAGGATTCTAAAGACTTAAGAGCAGCTGTAGATCGTGATTTAGTACGATAGAGCCAACATTGA 100 200
GARATTATGGGGCAACGGAAGCACAACTTGAAGACGCCAGAGCCAAAATACACAAGCTTAACCAAGACAGAGCTTATACAAATGACAGTTAATACAGAGGCACTAATAAACAGCCTAGG 350 400
TGAACGGAATAGTAAAATATTAAACGAAATTACTTTAAGATTGCTTAGAGACGATAAAGCTTTGTTTATATTTCCAAATGAATTGCCATCACCGTTGAAGCATTCTATGGATAGGGGATG $\begin{array}{c} \textbf{GAATTCTTCTTTAGAAATCTGCCGGTAAAAATTAGATTGCTATTCAATCTATTTCTATCGGTATTTGT} \\ \textbf{1} \end{array}$ CCACC
CCACC
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ATGAACAAAAAAATTCATTCATTATTAACGGCCAGGTTGAAATATTTCCTGAAAGTGAT
ATGACTAAAAAAAATTCATTCATTATTATCACGGCCAGGTTGAAATCTTTCCCGAAAGTGAT 2250 2350
TTTGCTGGATTAAAAGTAGAGGTTGATCATCAGCGCTGGATGGTTATTGAGTCAAATGCGGGTGTAGAACTAAATCAACCACTTGGCAGATGTGTGTATCTTCATCACGTCGATAAT
TTTGCTGGATTAAAAGTAGAGGTGGATATTCATCACCACTTGGATGATTATTGAGTCAAATGCAGGTGTGGAATTTAATCAACCACTTGGCAGATGTGTGACCTTCATCACGTCGATAAT
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GCGGTCGAAATAGAACAGACTCGATTCCTGGAAGAAGATAAGTTCCTGGTATTTGTCC	
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$luxD^{1850}$	1900
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ATTATTGCGTCTGGTTTTGCCCGCAGGATGGATCATTTTGCTGGTCTGGCGGAATAT	TA TCGCGGAATGGATTTCATGTGATCCGCTATGATTCGCTTCACCACGTTGGATTGAGTTCA 2100 2150
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	HITTGGTTAACTACACGAAAAATAAATAACTTCGGTATGTTGGCTTCAAGCTTATCTGCGCGG
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	SAA GTTATTACATTACTATCAAGCATCCGTAGTCATCAATGTAAGATATATTCTTTACTAGGA
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AGCTCACATGATTTGGGTGAGAACTTAGTGGTCCTGCGCAATTTTTATCAATCGGTT	ACG AAAGCCGCTATCGCGATGGATAATGGTTGTCTGGATATTGATGTCGATATTATTGAGCCG
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TCATTCGAACATTTAACCATTGCGGCAGTCAATGAACGCCGAATGAAAATTGAGATTY TCATTTGAACATTTAACTATTGCGACAGTCAATGAACGCCGAATGAAAATTGAGATTY 2800 9	3590 AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCGAGTTGC AATC
TCATTCGAACATTTAACCATTGCGGCAGTCAATGAACGCCGAATGAAAATTGAGATTY TCATTTGAACATTTAACTATTGCGACAGTCAATGAACGCCGAATGAAAATTGAGATTY 2800 9	3590 AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCGAGTTACA AACCTATACCAATAGATTTCGAGTTACA AACCTATACCAATAGATTTCGAGTTACATACCAACCTCCCCAATTTTCTCAAACAGAGTTAA ATGAAATTTGGAAACTTTTTTGCTTACATACCAACCTCCCCCAATTTTCTCAAACAGAGGTA AATGAAATTTGGAAACTTTTTACTTACATACCAACCTCCCCCAATTTTCTCAAACAGAGGTA AATGAAATTTGGAAACTTTTTACACTACCAACCTCCCCCAATTTTCTCAAACAGAGGTA AATGAAATTTGGAAACTTTTTACACGAGGTTTGGTTT
TCATTCGAACATTTAACCATTGCGGCAGTCAATGAACGCCGAATGAAAATTGAGATTC TCATTTGAACATTTAACTATTGCGACAGTCAATGAACGCCGAATGAAGAATTCAAGTTC 2800 3750 CTCTATCACTCAAAATAGCAATATAAGGACTCTCT 2880 t	3590 AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCGAGTTGC AATC AATC AATC AATC AATC AACCTATACCAATAGATTTCGAGTTGC AATC AATTC AATCAAATTTCCCAAACCACCCCCCCAATTTTCTCCAAACCAGGGTT AATCAAATTTCCCAAAACCAACCTCCCCCCAATTTTCTCCAAACCAGGGTT AATCACAATTTCCCAAACCACCTCCCCCAATTTTCCCAAACCAGGGTT AACCTATACCAAATTCCCCCCCCCC
TCATTCGAACATTAACCATTGCGGCAGTCAATGAACGCCGAATGAAAATTGAGATTA TCATTTGAACATTTAACTATTGCGACAGTCAATGAACGCCGAATGAAAATTGAGATTA 2800 g	3590 AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCCGAGTTGC AACCTATACCAATAGATTTCCGAGTTGC AACCTATACCAATAGATTTCCGAGTTACA AACCTATACCAATAGATTTCCGAGTTACA AACCTATACCAATAGATTTCCGAGTTACATACCAACCGCCCCCAATTTTCCTCAAACAGAGTTAA ATGAAATTTGGAAACTTTTTTCCTTACATACCAACCCCCCCC
TCATTCGAACATTTAACCATTGCGGCAGTCAATGAACGCCGAATGAAAATTGAGATTY TCATTTGAACATTTAACTATTGCGACAGTCAATGAACGCCGAATGAAAATTGAGATTY 2800 9	3590 AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCGAGTTGC AATC AATC AACCTATACCAATAGATTTCGAGTTGC AATC AATC AACCTATACCAATAGATTTCGAGTTGC AATCAAATCA
TCATTCGAACATTTAACCATTGCGGCAGTCAATGAACGCCGAATGAAAATTGAGATTY TCATTTGAACATTTAACTATTGCGACAGTCAATGAACGCCGAATGAAAATTGAGATTY 2800 9	3590 AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCGAGTTGC AACCTATACCAATAGATTTCCGAGTTGC AACCTATACCAATAGATTTCCGAGTTGC AACCTATACCAATAGATTTCCGAGTTACA AACCTATACCAATAGATTTCCGAGTTACA AACCTATACCAATAGATTTCCGAGTTACATACCAACCGCCCCCAATTTTCCTCAAACAGAGTTAA ATGAAATTTGGAAACTTTTTTCCTTACATACCAACCCCCCCC

4600 4650 TGATGATTCAGACAAAACAAAGGGCTATGATTTCAATAAAGGACAATGGCGCGACTTTGTCTTAAAAGGACATAAAAATACTAATCGTCGCGTTGATTACAGTTACGAAATCAATC
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3700 3750
4700 g 4750
GGGAACCCCCGCAGGAATGTATTGATATAATTCAAACAGACATTGACGCCACAGGÁATATCAAATATTTGTTGTGGGTTTGAAGCTAATGGAACAGTAGATGAAATTATTTCTTTC
a a 800 ^ 4850
GCTCTTCCAGTCTGATGTAATGCCGTTTCTTAAAGAAAACAACGTTCGCTATTATATTAG CTAAGGAAAATGAA
GCTCTTCCAGTCTGATGTCATGCCATTTCTTAAAGAAAAACAACGTTCGCTATTATATTAG CTAAGGAGAAAGAA 3950
U X B
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5000 5050 5100
GTGTGAAAATCATTTTTCAGATAATGGTGTTGTCGGGCGCTCCTTTGACTGTTTCTGGTTTTTACTTGGCCTAACAGAAAAAATTAAAATTGGTTCATTGAATCATGTCATTACAACT
GTATGAAAATCATTTTTCAGATAATGGTGTTGTCGGCGCTCCTCTGACTGTTTCTGGTTCTGGTTCTGCTCGGTTTAACAGAGAAAATTAAAATTGGTTCATTAAATCACATCATTACAACT 4150 4200
5150 5200 ca TCATCCTGTCCGCATAGCGGAAGAAGCGTGCTTATTGGATCAGTTAAGCGAAGGAAG
TCATCCTGTCGCCATAGCGGAGGAGAAAAAAAAGCTTACTTA
5250 5350 a 5350
ACANTACCAGCAGCATTATTTGAAGAATGCTATGACATTATTAACGATGCTTTAACA ACAGGCTATTGTAATCCAAATGGCGATTTTTATAATTTCCCCAAAATATCCGTGAATCCC !!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
5400 TGCTTATACGCAAAACGGGCCTCGGAAATATGTAACAGCAACAAGTTGTCATGTTGTTGAGTGGGCTGCTAAAAAAAGGCATTCCTCTAATCTTTAAGTGGGATGATTCCAATGAAGTT
TGCTTATACGCCAGGCGGACCTCGGAAATATGTAACAGCAACCAGTCATCATATTGTTGAGTGGGCGGCCAAAAAAGGTATTCCTCATCTTTAAGTGGGATGATTCTAATGATGTT
4500 4550
5500 5550 ACATGAATATGCGAAAAGATATCAAGCCATAGCAGGTGAATATGGTGTTGACCTGGCA GAGATAGATCATCAGTTAATGATATTGGTTAACTATAGTGAAGACAGTGAGAAAGCTAAA
HATATGAATATGCTGAAAGATATAAAGCCGTTGCGGATAAATATGACGTTGACCTATCA GAGATAGACCATCAGTTAATGATATTAGTTAACTATAACGAAGATAGTAATAAAGCTAAA 4600 4650 4700
5600 5650 5700
5650 5700 SGAAACGCGTGCATTTATAAGTGATTATATTCTTGCAATGCACCCTAATGAAAATTTC3AAAAGAAACTTGAAGAAATAATCACAGAAAACTCCGTCGGAGATTATATATGGAATGTACA :::::::::::::::::::::::::::::::::::
GGAAACGCGTGCATTTATAAGTGATTATATTCTTGCAATGCACCCTAATGAAAATTTC3AAAAGAAACTTGAAGAAATAATCACAGAAAACTCCGTCGGAGATTATATGGAATGTACA 1
GGAAACGCGTGCATTTATAAGTGATTATATTCTTGCAATGCACCCTAATGAAAATTTC3AAAAGAAACTTGAAGAAATTATCACAGAAAACTCCGTCGGAGATTATATGGAATGTACA AGAGACGCGTGCATTTATTAGTGATTATGTTCTTGAAATGCACCCTAATGAAAATTTC3AAAATAAACTTGAAGAAATAATTGCAGAAAACGCTGTCGGAAATTATACGGAGTGTATA 4750 4800
GGAAAGGCGTGCATTTATAAGTGATTATATTCTTGCAATGCACCCTAATGAAAATTTC SAAAAGAAACTTGAAGAAAATAATCACAGAAAACTCCGTCGGAGATTATTATGGAATGTACA 1
GGAAAGGCGTGCATTTATAAGTGATTATATTCTTGCAATGCACCCTAATGAAAATTTC BAAAAGAAACTTGAAGAAAATTACACAGAAAACTCCGTCGGAGATTATATAGGAATGTACA AGAGACGCGTGCATTTATTAGTGATTATTGTTCTTGAAAATTACAAAAATTTC BAAAAAAAAAATTGCAAGAAAATTACCGAAATTATACGGAGTGTATA 4750 B5750 S800 SCCGCCTAAATTGGCAATGGAGAAATGTGCTGCAAAAAGGTATATTTTTGCCTTTTGAATCATTGATGATGATTTTACACATCAAAATAAACGCAATTGATATTTTAAATTAAA NCCGGCTAAATTGGCAATTGAAAAGTGTGCGGAAAAGTATTATTGCCTTTTGAACCAATGAATG
SCALAGEGGTGCATTTATTAGTGATTATATCTTGCAATGCACCCTAATGAAAATTTC SAAAAGAAACTTGAAGAAAATTACACAGAAAACTCCGTCGGAGATTATATGGAATGTACA 1
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GGAAAGCGGTGCATTTATAAAGTGATTATATTCTTGCAATGCACCCTAATGAAAATTTC BAAAAGAAACTTGAAGAAAATTACACAGAAAACTCCGTCGGAGATTATATGGAATGTACA 1
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GAAACGCGTGCATTTATAAGTGATTATATTCTTGCAATGCACCCTAATGAAAATTTC BAAAAGAAACTTGAAGAAAATTACAGAAAAACTCCGTCGGACATTTATATGGAATGTACA AGAGACGCGTGCATTTATTAGTGATTATTGTTCTTGAAATGCACCCTAATGAAAATTTC BAAAAGAAACTTGAAGAAATTATCAGAAAAACTCCGAAAATTATAGGAATTATATGGAATGTACA 4750 A800 A5750 S800 RGCGGCTAAATTGGCAATGGAGAAAATGTGTGCTGCAATGAAAAATTATTTTCCTTTGAATCAATGAGTGATTTTACACATCAAAATAAAACGCAATTGATAATATTATAAA 1000
SCANACGGGTCCATTTATAAGTGATTATTCTTGCAATGCACCCTAATGAAAATTTC 3AAAAGAAACTTGAAGAAAATTATCAGGAAAACTCCGTCGGAGGATTATATGGAATGTACA 1
SCALAGEGGTECATTTATAAGTGATTATATTCTGCAATGCACCCTAATGAAAATTTC SAAAAGAAACTTGAAGAAATTAATCAAGAAAAACCCGTCGGGGGATTAATATGGAATGTACA 4750 4850 GEGGCTAAATTGGCAATGGAGAAAAGTGTATGTGCTGTCTTTGAATGCAATGAAAATTAAACTTGAAGAAATAATTGCAGAAAAACCCGTGCGGAAAATTAATCGGAGTGTATA 4800 GEGGCTAAATTGGCAATGGAGAAAATGGTGGTGCAAAAAGGTATATTATTGCCTTTGAATCAATGATGATTTTACACATCAAATAAAACGCAATTGATATTGTCAATGATAATATTAAA 1860 1860 1860 1860 1860 1870 1880 1
SGANACCCOTGCATTATANAGTCATTATATTCTCCAATCCACCCTAATGAAAATTTC SAAAAGAAACTTGAAGAAATTAATCACAGAAAACTCCGGAGAATTATATGGAATGACAAAACGACTCAAAAACGACTCCAAAAACGACTTCAAAAAAAA
SCALAGEGETICATTIATATAGGATTATATTCTTGCAATGCACTCTAATGAAAATTTC SAAAAGAAACTTGAAGAAAATTACACAGAAAACTCCGTCGGAGATTATATGGAATGTACA AGAGACGCGTGCATTTATTAGTGATTATTGTTATTATGGAATGTACA AT30 A5750 A5750 A5750 A5800 RCCGGCTAAATTGACGAATGGAAAATTGCTGCAAAAGGATATTATTGTCCTTTGAATCAATGAACGTGATTATACAAAATAAAACGCAATTGATTATTGATCAATGAATAATATTAAAA RCCGGCTAAATTGGCAAATGGAAAATTGATGCAAAAGGTATATTATTGTCCTTTGAATCAATGAATG
SGANAGCGCTGCATTATATAGTCATTATATATCTTGCAATGCACCCTAATGAAAATTTC SAAAAGAAACTTGAAGAAATAATCACAGAAAACTCGTCGGAGATTTATATGGAATGTACA AGAGACGCGTGCATTTATTAGTGATTATTTTTTTTTT
SCHAMAGGCTCATTTATATAGGCATTATATTCTTGCAATGCACCCTAATGCAAATTTCCAAAAATTACAAAAAATTACAAGAAATTACAAGAAATTACAAGAAATTATTATATTATATATA
SCALAGGGCTGCATTTATATAGTGCATTATATTCTTGCAATGCACCCTAATGAAAATTCCAAAAAAAA
SEALAGGGGGGCATTTATATAGTCATTCCATTCCACCCCTATCALANTTTC CALANGALATTCCACACALALACTCCCTCGGGANTCTACACACACACACACACACACACACACACACACACA
SCALAGGGGGGCATTTATAMGTGATTATTTTCCAMCCACCTMATCAMATTTC AMAMGAMACTTCAMGAMATANTCACACAMACTCCCTCGGGANTTTANGGANTTTANGGANTTTANGGANTTTANGGANTTTANGGANTTTANGGANTTTANGGANTTTANGGANTTTANGGANTTANGGANTTANGGANTTTANGGANTTANGGANTTANGGANTTANGGANTTANGGANTTANGGANTTANGGANTTANGGANTTANGGANTTANGGANTTANGGANTTANTGGANTTANGGANTTANTGGANTTANGGANTTANTGGANTTANGGANTTANTGGANTTANAGGANTTANTGGANTTANAGGANTTANTGGANTTANGGANTTANTGGANTTANGGANTTANTGGANTTANAGGANTTANTGGANTTANTGGANTTANTANGGANTTGGANTANTANGGANTTGGANTANTANGGANTTGGANTANTANGGANTTGGANTANTANGGANTTGGANTANTANGGANTTGGANTANGGANTANTANGGANTANTANTANAGGANTTGGANTANGGANTANGGANTANGGANTANTANTANGGANTGGANTANGGANTANGGANTANGGANTANGGANTANGGANTANTANGGANTANTANGGANTANGGANTANGGANTANGGANTANGGANTANGGANTANGGANTANGGANTANGGANTANGGANTANGGANTANGGANTANGGANTANGGANTANGGANTANGGANTANGGANTANGGANTANGGANGG

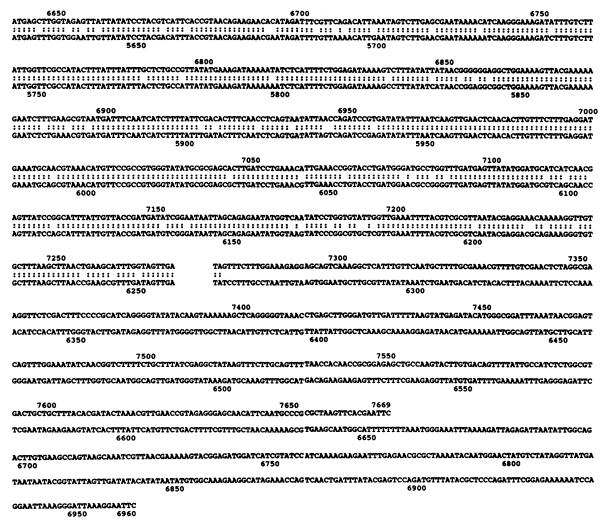


FIG. 2-Continued.

which contains the X. luminescens DNA inserted at the EcoRI site. Cells were grown in Luria-Bertani broth containing 50 μ g of ampicillin per ml and 50 μ g of kanamycin per ml at 37°C. Under these conditions, there is sufficient synthesis of T7 RNA polymerase that the lux DNA under control of a T7 promoter is fully expressed. In vivo luminescence (in light units per milliliter of culture) was determined as a function of cell density (optical density at 660 nm). One light

TABLE 1. Comparison of the locations and nucleotide sequences of the lux genes of two X. luminescens strains

Gene	Initiation in strain:		Termination in strain:		No. of codons ^a	Identity (%)
	Hw	Hb	Hw	Hb	codolis (%)	
luxC	1215	459	2655	1899	480	89.1
luxD	2671	1913	3592	2834	307	90.5
luxA	3776	2885	4856	3965	360	90.2
luxB	4873	3982	5845	4963	324 (327)	86.7
luxE	6160	5144	7270	6254	370 ` ´	91.1

^a lux genes in X. luminescens Hw and Hb have the same number of codons, except for luxB. Hb luxB has three additional codons compared with Hw luxB.

unit corresponds to 2×10^{10} quanta/s on the basis of the light standard of Hastings and Weber (10).

RESULTS

Figure 1 gives the sequencing strategy, restriction maps, and lux gene organization of the EcoRI restriction fragments containing the luxCDABE genes from strains Hb and Hw. Both DNA fragments were sequenced completely in both directions. A comparison of the nucleotide sequences is given in Fig. 2, with the Hw DNA extending about 800 bp farther upstream from the lux genes than the Hb DNA. A high degree of sequence homology between the two DNAs exists, starting at nucleotides 82 of the Hb DNA and 821 of the Hw DNA. Computer analysis revealed no sequence homology between the DNA extending upstream of these points. This homology extends downstream over 350 nucleotides to the start of luxC and continues through the lux genes to just after the termination codon of luxE. The extent of sequence identity in this upstream region is 72%, requiring only four single-nucleotide gaps and one 18-bp gap.

The positions of translation initiation and termination of the *lux* genes are summarized in Table 1. The five *lux* genes

370 ALSLTEAFGS*

luxC PP 50 p kiy ifrr 100 r NMKKISFIIN GRVEIFPESD DLVQSINFGD NSVHLPVLND SQVKNIIDYN ENNELQLHNI INFLYTVGQR WKNEEYSRRR TYIRDLKRYM GYSEEMAKLE ANWISMILCS KGGLYDLVKN V E ELGSRHIMDE WLPQDESYIR AFPKGKSVHL LTGNVPLSGV LSILRAILTK NQCIIKTSST DPFTANALAL SPIDVDPHHP VTRSLSVVYW QHQGDISLAK EIMQHADVVV AWGGEDAINW
V A I N 250
AVKHAPPDID VMKFGPKKSF CIIDNPVDLV SAATGAAHDV CFYDQQACFS TQNIYYMGGH YEEFKLALIE KLNLYAHILP NTKKDFDEKA AYSLVQKECL FAGLKVEVDV HQRWMVIESN 400 450 480
AGVELNQPLG RCVYLHHVDN IEQILPYVRK NKTQTISVFP WEAALKYRDL LALKGAERIV EAGMNNIFRV GGAHDGMRPL QRLVTYISHE RPSHYTAKDV AVEIEQTRFL EEDKFLVFVPluxD 1 50 100 MENKSRYKTI DHVICVEENR KIHVWETLPK ENSPKRKNTL IIASGFARRM DHFAGLAEYL SQNGFHVIRY DSLHHVGLSS GTIDEFTMSI GKQSLLAVVD WLMTRKINNL GMLASSLSAR 150 200
IAYASLSEIN VSFLITAVGV VNLRYTLERA LGFDYLSLPI DELPDNLDFE GHKLGAEVFA RDCFDSGWED LTSTINSMH LDIPFIAFTA NNDDWVKQDE VITLLSSIRS HQCKIYSLLG 250 307 SSHDLGENLV VLRNFYQSVT KAAIAMDNGC LDIDVDIIEP SFEHLTIAAV NERRMKIEIE NQVISLS* luxA 1 50 100 MKFGNFLLTY QPPQFSQTEV MKRLVKLGRI SEECGFDTVW LLEHHFTEFG LLGNPYVAAA YLLGATKKLN VGTAAIVLPT AHPVRQLEEV NLLDQMSKGR FRFGICRGLY NKDFRVFGTD 150
MNNSRALMEC WYKLIRNGMT EGYMEADNEH IKFHKVKVLP TAYSQGGAPI YVVAESASTT EWAAQHGLPM ILSWIINTNE KKAQIELYNE VAQEYGHDIH NIDHCLSYIT SVDHDSMKAK 250 300 4fsy is:
EICRNFLGHW YDSYVNATTI FDDSDKTKGY DFNKGQWRDF VLKGHKNTNR RVDYSYEINP VGTPQECIDI IQTDIDATGI SNICCGFEAN GTVDEIISSM KLFQSDVMPF LKEKQRSLLY* 1 50 p MKFGLFFLNF INSTTIQEQS IARMQEITEY VDKLNFEQIL VCENHFSDNG VVGAPLTVSG FLLGLTEKIK IGSLNHVITT HHPVRIAEEA CLLDQLSEGR FILGFSDCER KDEMHFFNRP 150 m 200

EQYQQQLFEE CYDIINDALT TGYCNPNGDF YNFPKISVNP HAYTQNGPRK YVTATSCHVV EWAAKKGIPL IFKWDDSNEV KHEYAKRYQA IAGEYGVDLA EIDHQLMILV NYSEDSEKAK
UP DN S PG H I D RY E K V DK D S N N 250 EPTRAPISOV ILAMHPNENF EKKLEEIITE NSVGDYMECT TAAKLAMEKC GAKGILLSFE SMSDFTHQIN AIDIVNDNIK KYHMAQ V E N A A N T I I SV P N LMS K V N D E luxE 1 CA 50 100
MTSYVDKQEI TASSEIDDLI FSSDPLVWSY DEQEKIRKKL VLDAFRHHYK HCQEYRHYCQ AHKVDDNITE IDDIPVFPTS VFKFTRLLTS NENEIESWFT SSGTNGLKSQ VPRDRLSIER 150 200
LLGSVSYGMK YIGSWFDHQM ELVNLGPDRF NAHNIWFKYV MSLVELLYPT SFTVTEEHID FVQTLMSLER IKHQGKDICL IGSPYFIYLL CRYMKDKNIS FSGDKSLYII TGGGWKSYEK 250 300
ESLKRNDFNH LLFDTFNLSN INQIRDIFNQ VELNTCFFED EMQRKHVPPW VYARALDPET LKPVPDGMPG LMSYMDASST SYPAFIVTDD IGIISREYGQ YPGVLVEILR RVNTRKQKGC

D D S "T L LFDTFNLSN INQIRDIFNQ VELNTCFFED EMQRKHVPPW VYARALDPET LKPVPDGMPG LMSYMDASST SYPAFIVTDD IGIISREYGG YPGVLVEILR RVNTRKQKGC

FIG. 3. Amino acid sequences encoded by the *luxCDABE* genes of the *X. luminescens* Hw and Hb DNAs. The amino acid sequences are given for the Hw *lux* genes, and any differences for the Hb *lux* genes are recorded below the line. Indicated in lowercase above the sequences are the amino acids previously reported for the proteins encoded by the Hw *lux* genes.

can be readily aligned in the order luxCDABE, providing that gaps of 126 bp are introduced into the Hb intergenic luxDA and luxBE regions (Fig. 2). In contrast, the DNA in the intergenic luxCD and luxAB regions can be aligned with only an additional 2-bp gap between luxC and luxD in the Hb DNA. The sequence identity for the lux genes is between 87 and 91% on comparison of the respective coding regions for the two strains (Table 1).

Indicated also in Fig. 2, between nucleotides 1182 and 6508 in the Hw DNA sequence, are the different nucleotides previously reported by other workers (29). Thirty-six differ-

ences were detected over this range in the present report, including 10 additions or deletions of nucleotides. Corrections in the nucleotide sequence of the Hw DNA are localized to the ends of the DNA with 15 changes in the 5' end of luxC and the remaining corrections after the luxD sequence. Four of these corrections were in coding regions resulting in frameshifts. In the luxC gene, deletion of one nucleotide and addition of another nucleotide further downstream caused a localized frameshift extending over 9 codons, whereas two deletions near the end of the luxA gene caused a shift in the sequence at the carboxyl terminal of the

5378 MEIGHEN AND SZITTNER J. BACTERIOL.





FIG. 4. Alternate alignments of the ERIC sequences between luxB and luxE in the lux operons of X. luminescens Hw and Hb. The upper gene organization corresponds to that for the Hw strain in both sets of alignments. The ERIC sequences (3 to 5) are numbered on the basis of Fig. 1, with the direction of transcription from left to right. The unlabelled region in front of luxE corresponds to an intergenic region of approximately 60 bp with a high degree of homology between the two strains.

 α subunit of luciferase. Both corrections result in a significant improvement in alignment and homology of the amino acid sequences coded for by the Hw luxC and luxA genes with the corresponding sequences of the Hb strain (Fig. 3). The amino acid sequences of the α and β subunits of Hw luciferase differ by 5 and 15%, respectively, from the sequences of the same luciferase subunit encoded by the Hb DNA. The reductase (luxC), transferase (luxD), and synthetase (luxE) polypeptides of the fatty acid reductase differ by 10, 10, and 5%, respectively, on comparison of the amino acid sequences of the respective subunits of the two strains (Fig. 3).

Intergenic regions and ERIC sequences. One of the most striking features of the X. luminescens sequences containing the lux genes is the presence of multiple copies of a 126-bp repetitive element (ERIC or intergenic repetitive unit). A total of five ERIC sequences, labelled 1 to 5 (Fig. 1), were detected in the X. luminescens DNA; four of these repetitive elements are in the Hw DNA, and one is in the Hb DNA. Only one of these ERIC structures (no. 1) is not present in the lux intergenic region but is located about 350 nucleotides upstream of the Hw luxC gene (Fig. 2). This structure occurs immediately after the end of an open reading frame (Fig. 1).

1	о.
c TATACCCAAAATAATTCGAGTTGCAGCAAGGC GGCAAGtGAGTGAATCCCCAGGAGCT	raca?
1TTCCCATTT-TTTGC-TTC-CTCATCA-A-	GT
2A-C-CTA-C-CTA-C-CTA-	G
3GGAGAG	G
4AAAAAA	G
5ACA	G-
	126
c AAGTAAGTGACTGGGGTGAGCGAACGCAGCCAACGCAgctGCAGCtTGAAatATGAcGg	TAT
1 -TCT-CTCATTCGTTCACTTGCAC-ATT	
2C-CT	
3CG-TC-T	

FIG. 5. Alignment of the X. luminescens ERIC sequences with the consensus ERIC sequence for enterobacteria. The consensus sequence (c) comprises 111 sites where the base is statistically significantly different (uppercase) and 15 sites where the base is found in the majority of cases (lowercase) in E. coli and S. typhimurium (23). Horizontal bars in ERIC structures 1 to 5 indicate that the same nucleotide is present as in the consensus sequence. A gap in the consensus sequence has been left so that ERIC 1 can be aligned, as well as single-base gaps in ERICs 1 and 2.

TABLE 2. Differences in X. luminescens ERIC sequences^a

Common	No. of changes in comparison with ERIC:					
Sequence	1	2	3	4	5	
c	54	25	29	21	18	
ERICs		5 0	62			
1		58	62	56	56	
2			21	10	8	
3				19	20	
4					7	

^a Includes deletions and additions as well as substitutions. c, consensus sequence for enterobacteria. Numbers refer to X. luminescens ERIC sequences as given in Fig. 1 and 4.

A comparable ERIC sequence cannot be recognized at this site in the Hb DNA.

A second ERIC structure (no. 2) is present between luxD and luxA in the Hw DNA, whereas this sequence is absent in the intergenic region in the Hb DNA. Indeed, the difference in the luxDA intergenic regions is almost completely accounted for by the presence of the second (no. 2) ERIC sequence in the Hw DNA.

The remaining three ERIC structures are located between the *luxB* and *luxE* genes. In the Hb strain of *X. luminescens*, one ERIC sequence of 126 bp is present (no. 5), whereas in the Hw strain, sequences corresponding to two ERIC sequences (no. 3 and 4) can be recognized (Fig. 1 and 2). In the latter case, it appears that an ERIC sequence (no. 4) has been inserted almost exactly at the middle of a second ERIC structure (no. 3) (Fig. 2).

Two alignments of the intergenic regions between *luxB* and *luxE* for the Hw and Hb strains are possible (Fig. 4). In the first alignment, ERIC sequence no. 5 of the Hb DNA can be split so that it aligns with split ERIC sequence no. 3 of the Hw DNA with a 125-bp gap corresponding to the extra ERIC sequence (no. 4) in the Hw DNA. In the second alignment, the Hb ERIC sequence (no. 5) is aligned with ERIC sequence no. 4 of the Hw DNA. In this case, two gaps must be introduced in the Hb DNA corresponding to the flanking ERIC sequence (no. 3) in the Hw DNA.

Comparison of the nucleotide sequences of ERIC structures 1 to 5 to the consensus sequence for enterobacteria shows a very high degree of conservation, particularly for ERIC structures 2 to 5 inside the *X. luminescens lux* operons (Fig. 5). The sequences can be aligned with the consensus sequence (23) over the entire 126-bp region with only one additional nucleotide in ERIC structure 1, a single-nucleotide gap in each of ERIC structures 1 and 2, and a missing nucleotide at the end of ERIC structure 4.

The numbers of nucleotide differences between the con-

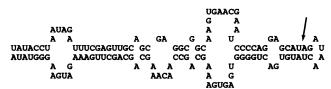


FIG. 6. Potential mRNA stem-loop structure formed from the ERIC sequence (no. 5) between luxB and luxE in X. luminescens Hb. The structure with maximum stability (-48.6 kcal/mol) in this region was calculated by using the DNASIS programs provided by Hitachi Software Engineering Co. Ltd.

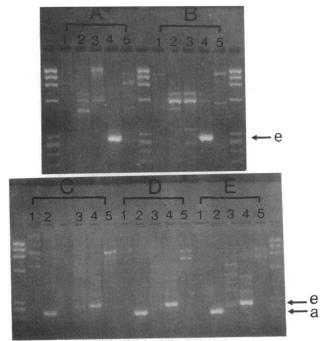


FIG. 7. Agarose gel electrophoresis of PCR products obtained from genomic DNAs from X. luminescens strains amplified with ERIC and lux gene primers. The genomic DNAs from five isolates or strains (A, ATCC 29999, Hb; B, ATCC 29304; C, ATCC 43950, Hw; D, ATCC 43949; E, ATCC 43951) were subjected to PCR. The sequences of the oligonucleotides, referred to as the luxA, ERIC, and luxE primers, are given in Materials and Methods. The primers used were luxA (lanes 1), luxA and ERIC (lanes 2), ERIC (lane 3), luxE and ERIC (lanes 4), and luxE (lanes 5). The arrows indicate the positions of the PCR products for an ERIC structure upstream of luxA (a) and luxE (e). Both products were observed in DNAs from X. luminescens strains isolated from humans (C, D, and E), whereas only the latter product was present in DNAs from X. luminescens strains isolated from insects (A and B). Unlabelled lanes contained molecular weight standards (1.4 and 1.1 kbp and 872, 603, 310, 281, 271, 234, and 194 bp), except for a blank lane between lanes 2 and 3 in sample C.

sensus sequence and the X. luminescens ERIC structures are given in Table 2. The differences from the consensus sequence for the ERIC structures in the lux genes are between 18 and 29 nucleotides out of 126 bp (14 to 23% difference), a relationship as close to the consensus as ERIC sequences from E. coli and S. typhimurium (11). The ERIC sequence upstream of Hw luxC (no. 1) has diverged the furthest, with 54 differences from the consensus sequence. Moreover, all four of the ERIC structures (no. 2 to 5) in the lux DNA have even greater differences from ERIC structure 1 (56 to 62 changes) than from the consensus sequence.

The data in Table 2 also indicate that three of the ERIC sequences (no. 2, 4, and 5) are much more closely related to each other (7 to 10 differences) than to the split ERIC structure (no. 3) in the Hw DNA (19 to 21 changes). Although these data indicate that the alignment of ERIC structures 4 and 5 in the *luxBE* intergenic region may be the preferred alignment (Fig. 4), it should be noted that this alignment requires two insertions or deletions. In contrast, alignment of ERIC structures 3 and 5 could be created by a simple insertion (or deletion) of an ERIC structure. Moreover, the remaining intergenic DNA between the *luxB* and *luxE* genes of the Hb and Hw strains can be aligned without

any additions or deletions. It is possible that once a second ERIC structure (no. 4) was inserted into ERIC structure 3, a much faster divergence of the nucleotide sequence of the first ERIC occurred.

As all ERICs contain a central core with an inverted repeat (23), it is possible to generate a stem-loop structure of very high stability from the transcribed mRNA. Figure 6 shows a potential stem-loop structure for ERIC no. 5 generated by the Hitachi DNASIS program with a stabilization energy of -48.6 kcal/mol. The arrow at the start of the loop indicates the corresponding position in ERIC sequence no. 3 of the Hw DNA where insertion of a second ERIC sequence (no. 4) would have given rise to the double ERIC sequences in the Hw DNA.

To determine whether ERIC structures are present in front of *luxA* and/or *luxE* in other *X. luminescens* strains, the genomic DNAs from two other isolates from humans (ATCC 48949 and ATCC 48951) and one from insects (ATCC 29304), as well as the Hb and Hw DNAs, were analyzed by PCR. An oligonucleotide (28-mer) starting at residue 70 in the *lux* ERIC sequences (Fig. 5) with three places of degeneracy so that it would be identical to the ERIC structures immediately in front of *luxA* and/or *luxE* (no. 2, 4, and 5) was used in conjunction with the *luxA* or *luxE* primer in the reverse direction. The predicted PCR products would be 258 bp if an ERIC structure were located in front of the *luxA* genes, as in the Hw DNA, and 303 bp if an ERIC structure were located in front of the *luxE* gene, as in the Hb and Hw DNAs.

Figure 7 shows the electrophoresis of the PCR products from the five strains when either a single oligonucleotide (luxA, ERIC, or luxE primer; lanes 1, 3, and 5, respectively) was added to the genomic DNA or the ERIC primer was added with the luxA (lane 2) or luxE (lane 4) primer. For X. luminescens strains isolated from insects (samples A and B), one major band corresponding to the predicted 303-bp product (labelled e) for an ERIC structure in front of luxE was detected. For X. luminescens strains isolated from humans (samples C, D, and E), two major bands (labelled a and e) corresponding to the predicted 258- and 303-bp products for ERIC structures in front of luxA and luxE, respectively, were detected. These same PCR products were observed when the cloned lux DNA from the Hw strain was used instead of the genomic DNA. Similarly, only the 303-bp product was observed when the cloned lux DNA from the Hb strain was used in the PCR (data not shown). Although a number of other bands can be detected in the different PCRs with the genomic DNA, these products are significantly smaller in amount and arise primarily when a single oligonucleotide is added to the reaction mixture.

DISCUSSION

The present results have demonstrated that differences exist between the *lux* operons of an *X. luminescens* strain isolated from an insect compared with those of a strain isolated from a human wound. Although a 10 to 15% variation in the nucleotide sequence of the *lux* genes within the same species might not seem that surprising, it was unexpected to find the Hw and Hb sequences diverged upstream of *luxC* and immediately downstream of *luxE*. These results suggest that the *lux* operons are at different positions in the genomes of these two *X. luminescens* strains and demonstrate that the *luxG* gene located immediately downstream of *luxE* in marine luminescent bacteria is absent, at least at this location, in terrestrial bacteria.

The DNA extending upstream of the luxC gene in the Hw

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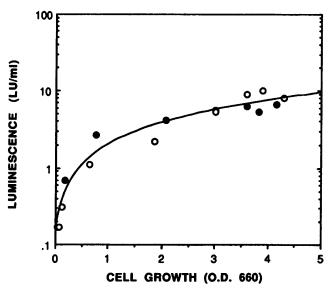


FIG. 8. Expression of the *EcoRI lux* DNA fragments from *X. luminescens* Hb and Hw in *E. coli. lux* DNA was inserted into plasmid pT7 and expressed in *E. coli* K38 with the assistance of plasmid pGp1, which codes for T7 RNA polymerase. Additional details are given in Materials and Methods. Luminescence (in light units per milliliter) is plotted against optical density at 660 nm for *E. coli* containing the Hw (\bullet) and Hb (\bigcirc) DNAs.

and Hb strains could be aligned with 72% nucleotide sequence identity for over 350 bp before the sequences diverged. This conserved DNA would be expected to contain the promoter and operator regions for the X. luminescens lux operon. Within this noncoding region, specific areas were highly conserved, including a 73-bp region with 94% nucleotide sequence identity over 250 bp from the start of luxC. These highly conserved sequences may represent DNA-binding sites for proteins that control the expression of the lux system. No sequence homology exists between the upstream X. luminescens DNA and that from other luminescent bacteria, showing that factors capable of recognizing the regulatory regions in marine and terrestrial bacteria may be different.

The unique feature of the *lux* DNA from *X. luminescens* was the presence of multiple ERIC structures. Although a single ERIC sequence is present in the Hb *lux* DNA (no. 5), it is the presence of multiple ERIC sequences in the Hw *lux* DNA that makes analyses of the ERIC structures of particular relevance. These repetitive structures have been recognized in *E. coli*, *S. typhimurium*, and a number of other gram-negative bacteria (23–25). In the *Xenorhabdus lux* systems, four of the ERIC structures are closely related to the consensus sequence with between 76 and 85% identity. A fifth ERIC sequence, upstream of *luxC* by about 400 bp in the Hw strain, is quite different, having only 60% identity with the consensus sequence.

The ERIC structures in X. luminescens differ in several properties from those reported for E. coli and S. typhimurium (23). In particular, ERIC sequences in other bacteria have been found to occur only singly at different genomic locations, whereas there are multiple ERIC sequences within the Hw lux operon with two ERIC sequences at the same site between the luxB and luxE genes. Moreover, the coding region for the last two amino acids and the termination codon of the β subunit of Hb luciferase are part of the

single ERIC structure between *luxB* and *luxE*. Although this result does show that ERIC sequences can occur in coding regions, it should be noted that an ERIC structure is at exactly the same nucleotide position in the Hw DNA. However, since the Hw *luxB* gene is three codons shorter than the Hb *luxB* gene, the ERIC structure starts immediately after the termination codon of *luxB* in this strain. It is likely that a mutation in the *luxB* termination codon in the Hb DNA occurred after introduction of the ERIC sequence, resulting in a slightly larger *luxB* gene. This result provides the first example of translation of an ERIC sequence, as all other ERICs have been found exclusively in the intergenic regions.

The differences in the number of ERIC structures in the X. luminescens strains plus the presence of multiple ERIC structures at one site raise some interesting questions about whether the differences in the number of ERIC sequences in the two X. luminescens strains arose by insertion or deletion. As the start of the ERIC structures at the end of luxB are at identical nucleotide positions in the Hw and Hb DNAs, the first ERIC sequence at this position was likely inserted prior to divergence of the luminescence systems of these two strains. Subsequently, the other ERIC structures would have been added to the Hw DNA or deleted from the Hb DNA to give the present distribution of ERIC structures. It appears likely that the extra ERIC sequence in the Hw DNA after luxB would have been inserted at a later date rather than deletion from the Hb DNA, as the latter mechanism would require exact removal of one ERIC structure.

The PCR experiments also indicate that multiple ERIC structures are present in the lux DNAs from three different isolates of X. luminescens obtained from humans. Two of these strains (ATCC 43950 and ATCC 43951) were isolated from patients in San Antonio, Tex., in 1986 and 1987; the third strain (ATCC 43949) was isolated from a patient in Maryland in 1977 (8). These strains appear to be very similar, particularly if one compares the PCR products by using only the ERIC primer (Fig. 7). Hybridization of the labelled ERIC probe to Southern blots of restricted genomic DNA (data not shown) also suggests that these strains are similar, particularly with respect to the number and distribution of ERIC structures in the X. luminescens strains isolated in San Antonio. By the same criteria (A and B in Fig. 7), the two strains from insects appear to be different in terms of the number and distribution of ERIC structures in the genomic DNA, although both strains lack the ERIC structure in front of luxA and have an ERIC structure in front of luxE. Although these results indicate a clear distinction between X. luminescens strains isolated from humans and insects in terms of the distribution of ERIC structures in the lux operon, additional X. luminescens strains must be analyzed to reach a more definitive conclusion.

This report has not addressed the role of the ERIC structures. Expression of the two different fragments under control of a T7 RNA polymerase promoter demonstrates that the luminescence is the same for both X. luminescens lux DNAs at all stages of growth (Fig. 8). Consequently, it appears that the presence of multiple ERIC structures in the Hw DNA or translation of the initial part of the single ERIC structure in the Hb DNA as part of luxB has caused no significant difference in expression at the transcriptional or translation level under these conditions. These results are consistent with the proposal that the repetitive elements function as "selfish DNA" without any apparent role (11, 22). However, the strong conservation of the ERIC sequences in bacteria from different genera and their high

frequency in the *lux* operon of the *X. luminescens* strain isolated from humans is so remarkable that it is possible that they reflect a function essential for the bacteria. Among the potential functions that have been suggested are structural signals involved in DNA metabolism, organization, or rearrangement (11, 22, 28). If the frequency and function of such signals are related to the environmental, nutritional, and/or physical location of the bacteria, the presence of multiple repetitive elements in the *lux* operons of *X. luminescens* strains isolated from humans will be of particular interest.

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