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Nucleotide sequence of the *luxC* gene encoding fatty acid reductase of the *lux* operon from *Photobacterium leiognathi*

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SUMMARY The nucleotide sequence of the *luxC* gene (EMBL Accession No. 65156) encoding fatty acid reductase (FAR) of the *lux* operon from *Photobacterium leiognathi* PL741 was determined and the encoded amino acid sequence deduced. The fatty acid reductase is a component of the fatty acid reductase complex. The complex is responsible for converting fatty acid to aldehyde which serves as the substrate in the luciferase-catalyzed bioluminescent reaction. The protein comprises 478 amino acid residues and has a calculated M_r of 53,858. Alignment and comparison of the fatty acid reductase of *P. leiognathi* with that of *Vibrio harveyi* B392 and *Vibrio fischeri* ATCC 7744 shows that there is 70% and 59% amino acid residues identity, respectively.

Bacterial luciferase is a heterodimeric ($\alpha\beta$) flavin monooxygenase that is responsible for catalyzing the light-emitting reaction in luminous bacteria. The overall reaction is shown below:

$$RCHO + FMNH_2 + O_2 \longrightarrow RCOOH + FMN + H_2O + h\nu$$

The enzyme functions *in vivo* with the aid of several accessory enzymes that provide the substrates $FMNH_2$ and aldehyde through interaction with other cellular components (1-3). The enzymes, fatty acid reductase, acyl-transferase and acyl protein synthetase, formed the fatty acid reductase complex which is responsible for converting the fatty acid to aldehyde as substrate for the reaction. In general, the genes involve in the bioluminescent reaction formed the *lux* operon or regulon. It was defined as that the *luxCDE* encoded fatty acid reductase, acyl-transferase and acyl protein synthetase, and the *luxAB* encoded α and β subunits of luciferase from

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Abbreviations: FAR, fatty acid reductase; *luxC*, gene encoding FAR; *R&R*, regulatory region; SD, Shine-Dalgarno sequence.

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V. harveyi and *V. fischeri* (2-3); in addition, the *lux*R and *lux*I are regulatory genes encoded the regulatory protein LuxR and the enzyme required for autoinducer synthesis (2-3). Nevertheless, the gene expression and regulatory mechanism of the *lux* operon or regulon are not clearly known yet.

The light-emitting reactions in most of bacteria involve the oxidation of reduced riboflavin phosphate ($FMNH_2$) and a long chain fatty aldehyde with emission of blue-green light (λ_{max} close to 490-505nm). However, *P. leiognathi* emits blue bioluminescent light with the wavelength λ_{max} close to 470nm. The *luxA* and *luxB* genes of luciferase and *luxD* from *P. leiognathi* had been determined (4-5). The determination of the nucleotide sequence of the *lux* operon from *P. leiognathi* will provide information to investigate the gene expression and regulation of the *lux* regulon and *lux* operon, and to understand the significance of evolution.

MATERIALS AND METHODS

Materials Restriction enzymes were purchased from New England Biolabs and Promega Corp. USB Sequenase version 2.0 DNA Sequencing Kit was used for DNA sequencing. α-[^{32}P]dATP was obtained from Du Pont-New England Nuclear.

Bacterial Strain and Phage Bacterial strain used in this work were *Escherichia coli* strain JM103y. JM103y (courtesy of Dr. G. Gussin, Masschusetts General Hospital) is a phage P1-free derivative of strain JM103 and which was used as host for phage M13 derivatives, mp18, mp19, and cloning works.

Plasmids Plasmid pL741 carrying the *lux* operon of *P. leiognathi* PL741 was from Dr. K. H. Nealson of the University of Wisconsin at Milwaukee.

Nucleotide Sequencing The nucleotide sequence of the *luxC* was obtained using the dideoxy chain termination method of Sanger *et al.* (6).

Nucleotide Sequence and Amino Acid Sequence Analyses Nucleotide sequence and amino acid sequence data were analyzed by using PC/GENE program from Intelligenetics, Inc. on an IBM/PC AT386 computer.

RESULTS AND DISCUSSION

Nucleotide sequence of the *lux*C gene and the encoded amino acid sequence of fatty acid reductase of *P. leiognathi*.

The plasmid pL741 carrying a 15-kilobase (kb) *Hin*dIII fragment, which was cloned from a partial *Hin*dIII digest of *P. leiognathi* PL741 genomic DNA. The pL741 plasmid contains the *lux* operon and all the information required to confer plasmid-borne, mimic the mode of regulated bioluminescence upon strain of *E. coli*.

The nucleotide sequence of the 4.2-kb region of pL741 containing the *luxC* gene of the *lux* operon has been determined. The nucleotide sequence analysis reveals

```
 -10       -1
TAATGGAGATTGC
      -SD-
1          .          .          .          .         50         .
ATGATTAAAAAGATCCCACTGATTATTGGAGGAGAAGTTCAAGACACGTCAGAACATGAT
METIleLysLysIleProLeuIleIleGlyGlyGluValGlnAspThrSerGluHisAsp
                     .          .          .        100         .
GTCCGTGAACTTACGCTTAACAATAACACCGTCAATGTACCTATCATTACGGACAAAGAT
ValArgGluLeuThrLeuAsnAsnAsnThrValAsnValProIleIleThrAspLysAsp
          .          .        150          .          .          .
GCTGAATCTATCACCTCACTAAAAATAGAAAATAAGTTAAATATCAACCAGATAGTTAAC
AlaGluSerIleThrSerLeuLysIleGluAsnLysLeuAsnIleAsnGlnIleValAsn
          .        200          .          .          .          .
TTCTTGTATACTGTAGGGCAAAAATGGAAGAGTGAGAACTACAGCCGTCGCCTCACTTAT
PheLeuTyrThrValGlyGlnLysTrpLysSerGluAsnTyrSerArgArgLeuThrTyr
     250          .          .          .          .          .       300
ATTCGTGATCTAGTAAAATTCATGGGCTACTCCCCTGAGATGGCAAAACTAGAAGCGAAC
IleArgAspLeuValLysPheMetGlyTyrSerProGluMetAlaLysLeuGluAlaAsn
          .          .          .          .        350          .
TGGATTTCGATGATTCTATGTAGCAAAAGTGCGCTATATGACATTGTTGAAAATGATCTC
TrpIleSerMetIleLeuCysSerLysSerAlaLeuTyrAspIleValGluAsnAspLeu
          .          .          .        400          .          .
AGCTCTCGTCATATTGTTGATGAATGGCTCCCCCAAGGTGATTGCTATGTTAAAGCGCTA
SerSerArgHisIleValAspGluTrpLeuProGlnGlyAspCysTyrValLysAlaLeu
          .          .        450          .          .          .
CCAAAAGGTAAATCTATCCATTTATTAGCGGGTAACGTTCCGCTATCAGGTGTGACATCG
ProLysGlyLysSerIleHisLeuLeuAlaGlyAsnValProLeuSerGlyValThrSer
          .        500          .          .          .          .
ATCCTGCGTGCAATTTTAACTAAAAATGAATGTATCATTAAAACATCATCTGCAGATCCA
IleLeuArgAlaIleLeuThrLysAsnGluCysIleIleLysThrSerSerAlaAspPro
     550          .          .          .          .          .       600
TTTACCGCAACTGCGCTGGCTTCCAGTTTTATTGATACCGATGCCAACCACCCAATTACT
PheThrAlaThrAlaLeuAlaSerSerPheIleAspThrAspAlaAsnHisProIleThr
          .          .          .          .        650          .
CGATCAATGTCAGTTATGTATTGGTCACATAATGAAGATATCACTATCCCACAAAAAATC
ArgSerMetSerValMetTyrTrpSerHisAsnGluAspIleThrIleProGlnLysIle
          .          .          .        700          .          .
ATGAACTGTGCAGATGTGGTTGTAGCTTGGGGTGGCAACGATGCAATCAAATGGGCAACA
MetAsnCysAlaAspValValValAlaTrpGlyGlyAsnAspAlaIleLysTrpAlaThr
          .          .        750          .          .          .
AAACATAGCCCTGCGCATGTCGATATTCTTAAATTTGGTCCAAAGAAAAGTATTTCAATT
LysHisSerProAlaHisValAspIleLeuLysPheGlyProLysLysSerIleSerIle
          .        800          .          .          .          .
GTTGATAACCCAACAGATATTAAGGCCGCTGCCATTGGTGTTGCTCACGACATCTGTTTT
ValAspAsnProThrAspIleLysAlaAlaAlaIleGlyValAlaHisAspIleCysPhe
     850          .          .          .          .          .       900
TATGATCAACAAGCATGTTTCTCTACCCAAGATATCTATTACATGGGTGATAAACTCGAT
TyrAspGlnGlnAlaCysPheSerThrGlnAspIleTyrTyrMetGlyAspLysLeuAsp
          .          .          .          .        950          .
GTATTTTTCGATGAACTGACTAAACAGCTCAACATCTATAAAGTTATTTTGCCTAAAGGT
ValPhePheAspGluLeuThrLysGlnLeuAsnIleTyrLysValIleLeuProLysGly
          .          .          .       1000          .          .
GACCAATCTTTCGATGAAAAGGGTGCGTTTTCTTTAACCGAAAGAGAATGTTTATTTGCC
AspGlnSerPheAspGluLysGlyAlaPheSerLeuThrGluArgGluCysLeuPheAla
          .          .       1050          .          .          .
AAATATAAAGTGCAAAAAGGTGAAGAGCAGGCTTGGTTATTAACGCAATCTCCAGCGGGT
LysTyrLysValGlnLysGlyGluGluGlnAlaTrpLeuLeuThrGlnSerProAlaGly
          .       1100          .          .          .          .
ACTTTTGGTAATCAACCGTTATCACGCTCAGCATATATTCATCATGTTAACGATATTTCA
ThrPheGlyAsnGlnProLeuSerArgSerAlaTyrIleHisHisValAsnAspIleSer
     1150          .          .          .          .          .      1200
GAAATAACACCGTATATACAAAATGATATAACCCAAACGGTTTCAATTACCCCATGGGAA
GluIleThrProTyrIleGlnAsnAspIleThrGlnThrValSerIleThrProTrpGlu
          .          .          .          .       1250          .
GCATCATTTAAATATAGAGATACATTAGCGTCACATGGTGCGGAACGTATTATTGAATCA
AlaSerPheLysTyrArgAspThrLeuAlaSerHisGlyAlaGluArgIleIleGluSer
          .          .          .       1300          .          .
GGTATGAATAATATATTTCGTGTTGGTGGTGCACATGATGGTATGCGCCCTCTTCAGCGC
GlyMetAsnAsnIlePheArgValGlyGlyAlaHisAspGlyMetArgProLeuGlnArg
          .          .       1350          .          .          .
CTTGTTAAATATATTTCACATGAAAGACCATCAACTTATACCACTAAAGATGTCGCTGTA
LeuValLysTyrIleSerHisGluArgProSerThrTyrThrThrLysAspValAlaVal
          .       1400          .          .          .
AAAATAGAACAAACTCGCTACCTAGAAGAAGATAAATTCCTCGTTTTCGTACCATAA
LysIleGluGlnThrArgTyrLeuGluGluAspLysPheLeuValPheValPro .
```

Fig. 1. The nucleotide sequence of the *luxC* gene and the encoded amino acid sequence of fatty acid reductase of the *lux* operon from *P. leiognathi* PL741. SD indicates the Shine-Dalgarno sequence of the *luxC* gene. The sequence has been deposited at the EMBL database under No. X65156.

that the *lux* operon regulatory region which included cAMP-CRP binding site, promoter and Shine-Dalgarno (SD) sequence is located before the *luxC* gene. The nucleotide sequence of the *luxC* gene (1437 bp) and the encoded amino acid sequence of the fatty acid reductase is shown in Fig. 1.

Alignment and comparison of the amino acid sequences of the fatty acid reductases from *P. leiognathi*, *V. harveyi* and *V. harveyi*.

The open reading frame was identified as the *luxC* gene by homology of the encoded protein with the fatty acid reductase of *V. fischeri* ATCC 7744 (70%) (4), *V. harveyi* B392 (59%) (7-8) and *Photobacterium phosphoreum* (9). The alignment and comparison of amino acid sequences of fatty acid reductase from different species is shown in Fig. 2. The protein has a calculated M_r of 53,858 and comprises 478 amino acid residues.

```
PlLuxC  MIKKIPLIIGGEVQDTSEHDVRELTLNNNT-VNVPIITDKDAESITSLKI  49
VfLuxC  MNKCIPMIINGMIQDFDNYAYKEVKLNNDNRVKLSVITESSVSKTLNIKD  50
VhLuxC  MEKHLPLIVNGQIISTEENRF-EISFE-EKKVKIDSFNNLHLTQMVNHDY  48
        * * .*.*..* . . ..    *.... .. *..  ...   .   . .

PlLuxC  ENKLNINQIVNFLYTVGQKWKSENYSRRLTYIRDLVKFMGYSPEMAKLEA  99
VfLuxC  RINLNLNQIVNFLYTVGQRWKSEEYNRRRTYIRELKTYLGYSDEMARLEA 100
VhLuxC  LNDLNINNIINFLYTTGQRWKSEEYSRRRAYIRSLITYLGYSPQMAKLEA  98
          .**.*.*.*****.**.****.*.** .***.* ...*** .**.***

PlLuxC  NWISMILCSKSALYDIVENDLSSRHIVDEWLPQGDCYVKALPKGKSIHLL 149
VfLuxC  NWIAMLLCSKSALYDIVNYDLGSIHVLDEWLPRGDCYVKAQPKGVSVHLL 150
VhLuxC  NWIAMILCSKSALYDIIDTELGSTHIQDEWLPQGECYVRAFPKGRTMHLL 148
        ***.*.**********.. .*.* *. *****.*.***.* *** ..***

PlLuxC  AGNVPLSGVTSILRAILTKNECIIKTSSADPFTATALASSFIDTDANHPI 199
VfLuxC  AGNVPLSGVTSILRAILTKNECIIKTSSSDPFTANALVSSFIDVNADHPI 200
VhLuxC  AGNVPLSGVTSILRGILTRNQCIVRMSASDPFTAHALAMSFIDVDPNHPI 198
        **************.***.*.**.. *..***** **. ****....***

PlLuxC  TRSMSVMYWSHNEDITIPQKIMNCADVVVAWGGNDAIKWATKHSPAHVDI 249
VfLuxC  TKSMSVMYWPHDEDMTLSQRIMNHADVVIAWGGDEAIKWAVKYSPPHVDI 250
VhLuxC  SRSISVLYWPHASDTTLAEELLSHMDAVVAWGGRDAIDWAVKHSPSHIDV 248
        ..*.**.**.*..* *... ...  *.*.**** .**.**.*.**.*.*.

PlLuxC  LKFGPKKSISIVDNPTDIKAAAIGVAHDICFYDQQACFSTQDIYYMGDKL 299
VfLuxC  LKFGPKKSLSIIEAPKDIEAAAMGVAHDICFYDQQACFSTQDVYYIGDNL 300
VhLuxC  LKFGPKKSFTVLDHPADLEEAASGVAHDICFYDQNACFSTQNIYFSGDKY 298
        ********..... * *...** **********.******..*. **.

PlLuxC  DVFFDELTKQLNIYKVILPKGDQSFDEKGAFSLTERECLFAKYKVQKGEE 349
VfLuxC  PLFLNELEKQLDRYAKILPKGSNGFDEKAAFTLTEKESLFAGYEVRKGDK 350
VhLuxC  EEFKLKLVEKLNLYQEVLPKSKQSFDDEALFSMTRLECQFSGLKVISEPE 348
          *  .* ..*. *  .***....**... *..*  *. *.  .* .. .

PlLuxC  QAWLLTQSPAGTFGNQPLSRSAYIHHVNDISEITPYIQNDITQTVSITPW 399
VfLuxC  QAWLIVVSPTNSFGNQPLSRSVYVHQVSDINGVIPFINKNRTQTVSIYPW 400
VhLuxC  NNWMIIESEPGVEYNHPLSRCVYVHKINKVDDVVQYIEKHQTQTISFYPW 398
        ..*... * ..   *.****..*.*...........*... ***.*. **

PlLuxC  EASFKYRDTLASHGAERIIESGMNNIFRVGGAHDGMRPLQRLVKYISHER 449
VfLuxC  EASLKYRDKLARSGVERIVESGMNNIFRVGGAHDSLSPLQYLVRFVSHER 450
VhLuxC  ESSKKYRDAFAAKGVERIVESGMNNIFRAGGAHDAMRPLQRLVRFVSHER 448
        *.* **** .*  *.***.*********.*****...*** **...****

PlLuxC  PSTYTTKDVAVKIEQTRYLEEDKFLVFVP 478
VfLuxC  PFNYTTKDVAVEIEQTRYLEEDKFLVFVP 479
VhLuxC  PYNFTTKDVSVEIEQTRFLEEDKFLVFVP 477
        * ..*****.*.*****.***********
```

Fig. 2. Alignment and comparison of amino acid sequences of fatty acid reductase from *P. leiognathi* (*Pl*), *V. fischeri* (*Vf*) and *V. harveyi* (*Vh*). Asterisks (*) indicate that the amino acids are perfectly conserved, and dots (.) indicate that the amino acids are well conserved.

Gene order of the *lux* operon in *P. leiognathi*.

The nucleotide sequence of the flanking region elicits that there are two open reading frames to run in the opposite direction from the *luxC* gene. One gene is homologous to the *lumP* gene of *P. phosphoreum* (10-11) and another is unknown yet. The sequence between the *luxC* and *lumP* genes shows to be regulatory region of these two operons. Apparently, the bioluminescence genes formed the *lux* operon, not the *lux* regulon, in *P. leiognathi*. The gene order of the *lux* operon and *lumP* of *P. leiognathi* is <*-lumP-R&R-luxC-luxD-luxA-luxB-luxN-luxE-*> (*R&R*: Regulatory region) (5,11), and similar to that of *V. fischeri* and *V. harveyi* (4,8), except for the *luxN* gene; however, not like the *lux* regulon of *V. fischeri* (4), the regulatory genes, *luxI* and *luxR*, are not linked together with the structure genes of the *lux* operon in *P. leiognathi* (4,12). In fact, the LuxR and inducer encoded or synthesized by the regulatory genes, *luxR* and *luxI*, indeed involve in regulation of the gene expression of the *lux* operon; nevertheless, the regulation mechanism is not clearly known yet.

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