APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 1989, p. 2601–2606
0099-2240/89/102601-06$02.00/0

Vol. 55, No. 10

Growth and Luminescence of the Bacterium *Xenorhabdus luminescens* from a Human Wound

PIO COLEPICOLO, KI-WOONG CHO,† GEORGE O. POINAR,‡ AND J. WOODLAND HASTINGS*

Department of Cellular and Developmental Biology, Harvard University, 16 Divinity Avenue, Cambridge, Massachusetts 02138

Received 13 March 1989/Accepted 13 June 1989

***Xenorhabdus luminescens*, a newly isolated luminous bacterium collected from a human wound, was characterized. The effects of ionic strength, temperature, oxygen, and iron on growth and development of the bioluminescent system were studied. The bacteria grew and emitted light best at 33°C in a medium with low salt, and the medium after growth of cells to a high density was found to have antibiotic activity. The emission spectrum peaked at 482 nm in vivo and at 490 nm in vitro. Both growth and the development of luminescence in *X. luminescens* required oxygen and iron. The isolated luciferase itself exhibited a temperature optimum at about 40°C; after purification by affinity chromatography, it showed two bands (52 and 41 kilodaltons) on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, indicative of an alpha and beta subunit structure. Reduced flavin mononucleotide (K_m of 1.4 μM) and tetradecanal (K_m of 2.1 μM) were the best substrates for the luciferase, and the first-order decay constant under these conditions at 37°C was 0.79 s^{-1}.**

It was not until the second half of the 19th century that the bacterial origin of the luminescence of meat was clearly established (15, 22). Earlier explanations were diverse and sometimes amusing. For example, light emission from human cadavers had been reported by some to be associated primarily with the brain, and the intensity of the light was thought to be "proportional to the activity of the nervous system during life" (4). As described by Harvey (15), Puerarius (38) believed that certain parts of the famous luminous mutton of Montpellier were brightest because stimuli of desire arose from those regions, while Cooper and Cooper (5) stated that the "common opinion (concerning the cause of luminosity of dead animals) is that the meat had been struck by lightning."

Human wounds were also known to be sometimes luminous, as especially noted in early medical records from the military (35). As mentioned by Harvey (13, 15), it was generally believed that such wounds were more apt to heal than nonluminous ones. However, the identity of the bacterial species that was responsible for such infections is not apparent. The bacterial luciferase system is commonly found in marine species; indeed, these might have been involved as the infective agent in some cases. A more likely possibility emerged with the discovery (36; see reference 14) that luminous bacteria responsible for the infection of two groups of insects are symbiotic with a parasitic nematode and that the bacteria themselves are not marine and produce a potent antibiotic (1, 34, 37, 42). If luminous bacteria occurring in human wounds also produce an antibiotic, as suggested by this study, this could account for the reports concerning the preferential healing of such wounds.

Such symbiotic bacteria have not been isolated directly from soil or water. Recently, however, luminous bacteria were isolated from a human wound and characterized taxonomically as *Xenorhabdus luminescens* (11), the species responsible for caterpillar luminescence. We undertook a study of the biochemical and physiological properties of one such isolate; while it is similar to other luminous bacteria in many respects, including the luciferase, its temperature optimum of 37°C suggests that it has evolved in association with a warm-blooded host(s).

Bioluminescent strains of the human pathogens *Vibrio vulnificus* (33) and *V. albensis* (12) have also been reported.

MATERIALS AND METHODS

Reagents and solutions. Flavin mononucleotide (FMN), NADH, dithiothreitol, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), molecular weight standards, and DL-α-glycerol phosphate were obtained from Sigma Chemical Co. Glycerol was purchased from Baker, and ferric ammonium citrate (FAC) was purchased from Merck & Co., Inc. Platinized asbestos for the catalytic reduction of FMN with H_2 was obtained from E. H. Sargent and Co., while potassium cyanide and EDTA were purchased from Fisher Scientific Co. Long-chain aldehydes and 2,2-diphenylpropylamine were bought from Aldrich Chemical Co., Inc. Aldehyde stock solutions were prepared by sonication of 0.1% (vol/vol) in distilled water and were freshly diluted to 0.001% for use each day. Superfine Sephadex G-75 and Sepharose 6B were products of Pharmacia. All other chemicals used were of analytical grade.

Cultures and media. A primary form of the luminous terrestrial bacterium *X. luminescens* (2, 3) was isolated from a human wound at the Centers for Disease Control, Atlanta, Ga. (no. 3265-86; 11). Cells were plated on solid low-salt medium at 25°C, and a bright single colony was selected for study.

Low-salt culture medium contains, per liter: distilled water, 750 ml; glycerol, 3.0 ml; tryptone (Difco Laboratories), 5.0 g; yeast extract (Difco), 3.0 g; and seawater, 250 ml (32). A similar medium with NaCl in lieu of the seawater was also used. The cells also grew well in a mammalian cell culture medium (RPMI medium 1640; GIBCO Laboratories) and in LB broth (GIBCO).

HEPES culture medium (26) contains, per liter of distilled water: glycerol, 3.0 ml; 50 mM HEPES; $MgSO_4$, 0.1 g; KCl,

* Corresponding author.
† Present address: Department of Biophysical Science, University of Houston, Houston, TX 77004.
‡ Present address: Department of Entomological Sciences, University of California, Berkeley, CA 94720.

0.1 g; NH_4Cl, 1.0 g; NaCl, 10 g; α-glycerol phosphate, 0.21 g; Casamino Acids (Difco), 0.5 g; vitamin stock (thiamine hydrochloride, 0.4 mg; biotin, 2 μg; vitamin B_{12}, 2 μg); and NaOH to adjust the pH to 7.0. For experiments involving iron, we added 5.0 μg/ml of FAC, as indicated, into the HEPES-minimal medium. To study the effect of ionic strength on the growth, the concentration of seawater (or NaCl) was changed. For solid media, 15 g of agar (Difco) per liter was added.

Liquid cultures were grown in 500- to 1,000-ml flasks in a temperature-controlled New Brunswick G-10 shaker at 150 rpm. Samples (3.0 ml) were removed from the flask at the times indicated and used for measurements of in vivo luminescence, luciferase activity, and cell density (optical density at 660 nm [OD_{660}] in a Kontron spectrophotometer).

Light emission. Bioluminescence was measured by using 3.0-ml samples in a scintillation vial placed in a photometer (29), calibrated in quanta per second by using the standard of Hastings and Weber (20) corrected by a factor 3 (6). Cyanide (15 μM) and *n*-decanal (0.1%) were added by injection from a syringe into the vial positioned in front of the phototube, with continuous recording of the bioluminescence of the sample (31). For in vivo and in vitro emission spectra, measurements were made in a spectrofluorimeter (Perkin-Elmer MPF 44) with the excitation lamp off.

For the isolation of luciferase, cells were harvested by centrifugation and frozen. Upon thawing, cells were lysed osmotically with ultrasonic disruption in 30 mM phosphate buffer (pH 7.0)–1.0 mM dithiothreitol–10 mM EDTA (1 g of cells per 5 ml of buffer). For purification, this extract was subjected to ammonium sulfate fractionation, with the precipitate collected between 60 and 75%. After dialysis, the material was passed through a calibrated G-75 Sephadex column, which indicated its molecular mass to be about 85 kilodaltons. The active fractions were pooled and purified by chromatography on 2,2-diphenylpropylamine-Sepharose (23, 24). The purified material gave two principal bands corresponding to molecular masses of about 40 and 50 kilodaltons based on sodium dodecyl sulfate–10% polyacrylamide gel electrophoresis; a minor band was estimated to represent an impurity of about 15%. Luciferase activity was measured by rapidly injecting 1.0 ml of 5 μM $FMNH_2$ (reduced catalytically) with 100 μl of cell extract containing luciferase and 0.001% *n*-decanal in 1.0 ml of the same buffer (44). The pH optimum was between pH 6.5 and 7.0.

Effect of oxygen tension. The technique described by Nealson and Hastings (30) was used to study the effect of oxygen concentration on the development of luminescence. Different volumes of culture were placed in 500-ml flasks and subjected to only moderate shaking, such that the flasks with larger volumes of culture had lower dissolved oxygen concentrations than those with smaller volumes.

RESULTS AND DISCUSSION

Marine luminous bacteria are commonly characterized by a requirement for high osmolarity for both growth and luminescence (which, however, are affected independently). In these cases, both growth and luminescence typically exhibit optima at concentrations in the range of 1 to 4% NaCl (7, 18). With *X. luminescens*, both growth and luciferase synthesis were optimal at about 20% seawater (Fig. 1), close to the ionic concentration found in mammalian tissues and blood. Luminescence in vivo, however, exhibited a very different pattern, being favored by lower ionic concentrations and, surprisingly, with the maximum being at the lowest concentration. This indicates that the cellular luciferase content is not necessarily the determining factor for the level of bioluminescence in vivo in this organism.

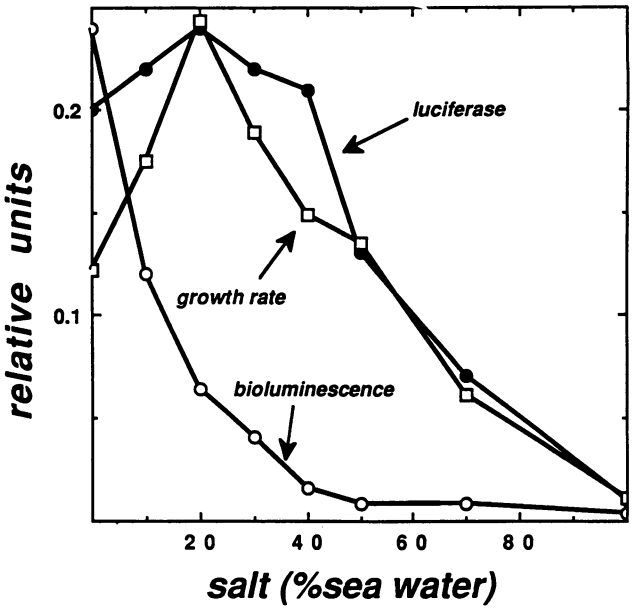

FIG. 1. Effect of ionic concentration on growth, luminescence, and in vitro luciferase activity. Cells were grown at 30°C with different concentrations of seawater; the bioluminescence (○), growth rate (□), and in vitro luciferase activity (●) were measured. The growth rate was measured during log phase and expressed as ΔOD_{660}/h. Bioluminescence and luciferase were measured as described in Materials and Methods and have to be multiplied by 10^9 and 10^8, respectively, to give the experimental data in quanta per second per milliliter.

In recent years, luminescent members of the genus *Xenorhabdus* have been described as symbionts with nematodes, pathogenic for two groups of insects, the *Lepidoptera* and *Coleoptera* (37). Since these bacteria may be present in the soil, their involvement in an infection of a mammalian wound is not unexpected. However, it was interesting to find that the strain exhibits temperature optima above 30°C. As shown in Fig. 2, growth is optimal at about 33°C and luminescence is optimal at 37°C. This property has been stable in cultures maintained in the laboratory at room temperature for 2 years, indicating that its adaptation in this respect is well established.

One of the unusual—indeed unique—features of the bacterial bioluminescent system is that in many different species and strains, it is subject to autoinduction (31, 40, 43). The synthesis of luciferase (as well as other proteins involved in the luminescent reaction) does not occur in growing cultures at low cell densities; during growth, the cells themselves produce a substance which then functions as inducer for the *lux* operon (8, 10, 28). The amount of luciferase per cell thus declines during the eclipse period but then rapidly rises as cell density increases. The luminescent system in *X. luminescens* exhibits these features with regard to both its in vivo luminescence and the luciferase content (Fig. 3).

In some species and strains, this induction is repressed by oxygen, i.e., the development of the luminescent system is favored at low oxygen concentrations (30). This is not so in *X. luminescens* (Fig. 3). At lower oxygen concentrations (obtained by using deep liquid cultures with only moderate shaking), the growth rate of cells is limited by low oxygen

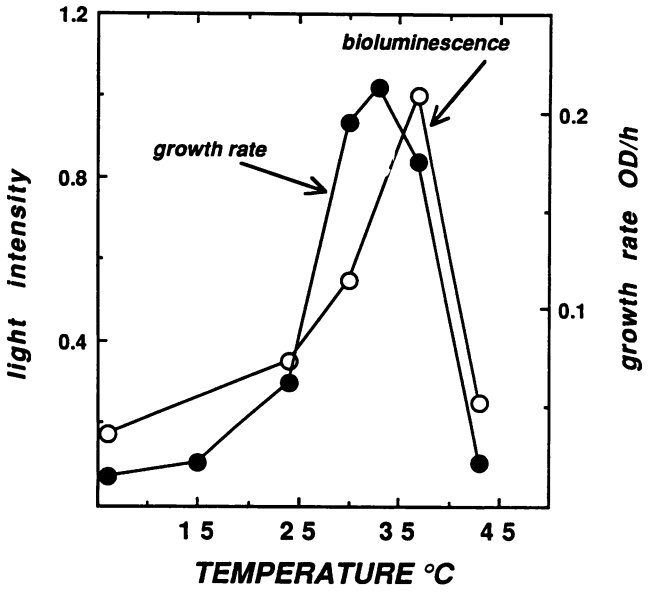

FIG. 2. Temperature dependence of growth and bioluminescence. Cells were grown in low-salt medium at different temperatures. The growth rate (●) was measured during log phase and is expressed as ΔOD_{660}/h. The bioluminescence in vivo (○) was measured at OD_{660} = 0.4.

but the specific luminescence and luciferase are the same. *X. luminescens* will not grow and synthesize luciferase anaerobically with glucose as the substrate, as some species will (9).

Iron has also been found to be involved in the synthesis of the luminescent system in some species, such that its induction is favored at low iron (21, 26). As seen in Fig. 4, low iron limits both growth and luminescence and the addition of iron (FAC) stimulates both, contrary to the situation in the reports cited.

Antibiotic activity was found to be present in extracts of medium after growth of cells for 3 days at 30°C to an OD_{660} of about 6 (K. Nealson, unpublished experiments). The medium was extracted twice with 0.5 volume of ethyl acetate, concentrated by evaporation to 25 ml, and tested for activity against *Bacillus subtilus* and *V. fischeri*. Controls were created with ethyl acetate alone and with 5 mM $FeCl_3$, the latter control to test for the possibility that the growth inhibition might be due to the presence of a siderophore.

The addition of long-chain aldehyde to the growing culture of bacteria increases light emission at all stages of growth by up to 10-fold, depending on the chain length of the aldehyde (Table 1). In *V. harveyi*, by contrast, aldehyde stimulation occurs principally only prior to the early stages of induction (31). Cyanide, which blocks the electron transport pathway, also stimulates luminescence in *X. luminescens* at all stages but only slightly (ca. twofold) and transiently.

Bioluminescence emission spectra, illustrated in Fig. 5, are generally similar to those recorded for marine luminous

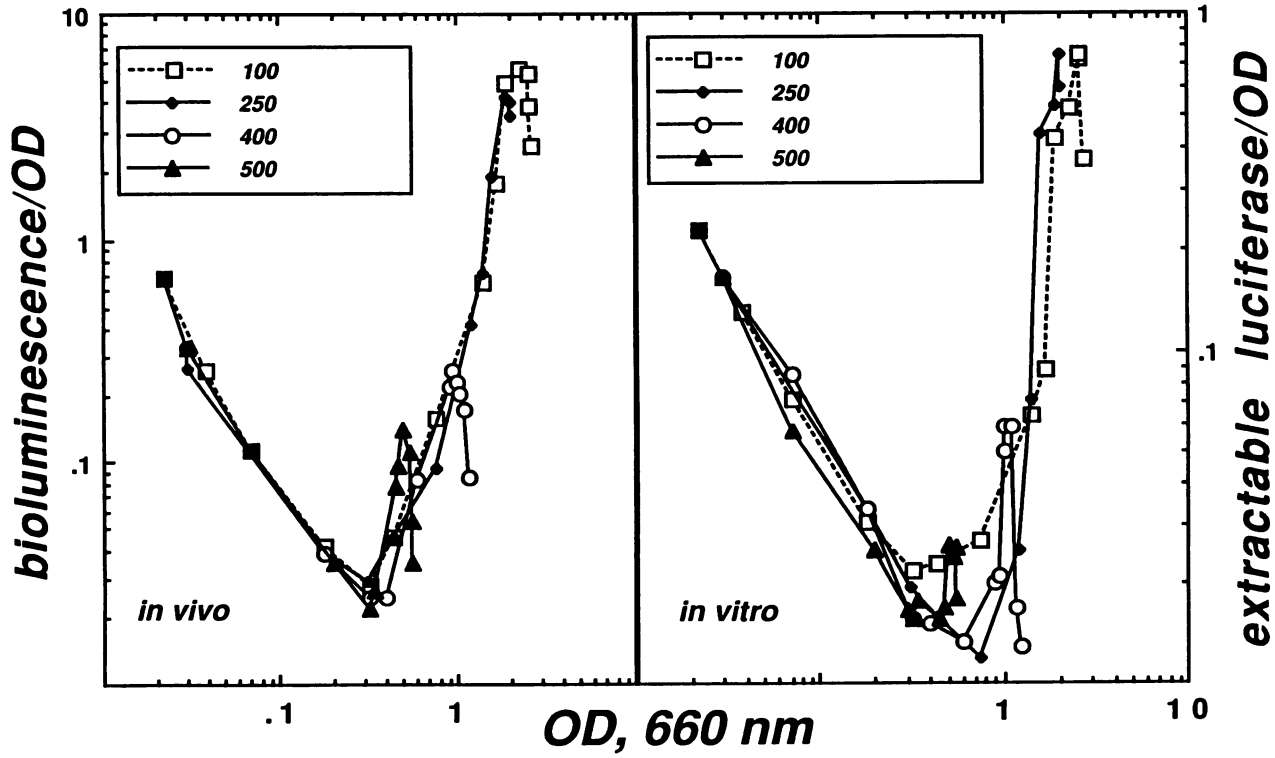

FIG. 3. Effect of oxygen tension on luminescence and luciferase content as related to cell density. Cells were grown in low-salt medium with gentle shaking in 500-ml culture flasks with different volumes of culture (given in milliliters in insets). Samples (3 ml) were collected during growth for measurements of the optical density, bioluminescence, and in vitro luciferase and plotted to express specific luminescence as related to cell density. Bioluminescence and luciferase activity should be multiplied by 10^8 to give values in quanta per second per milliliter.

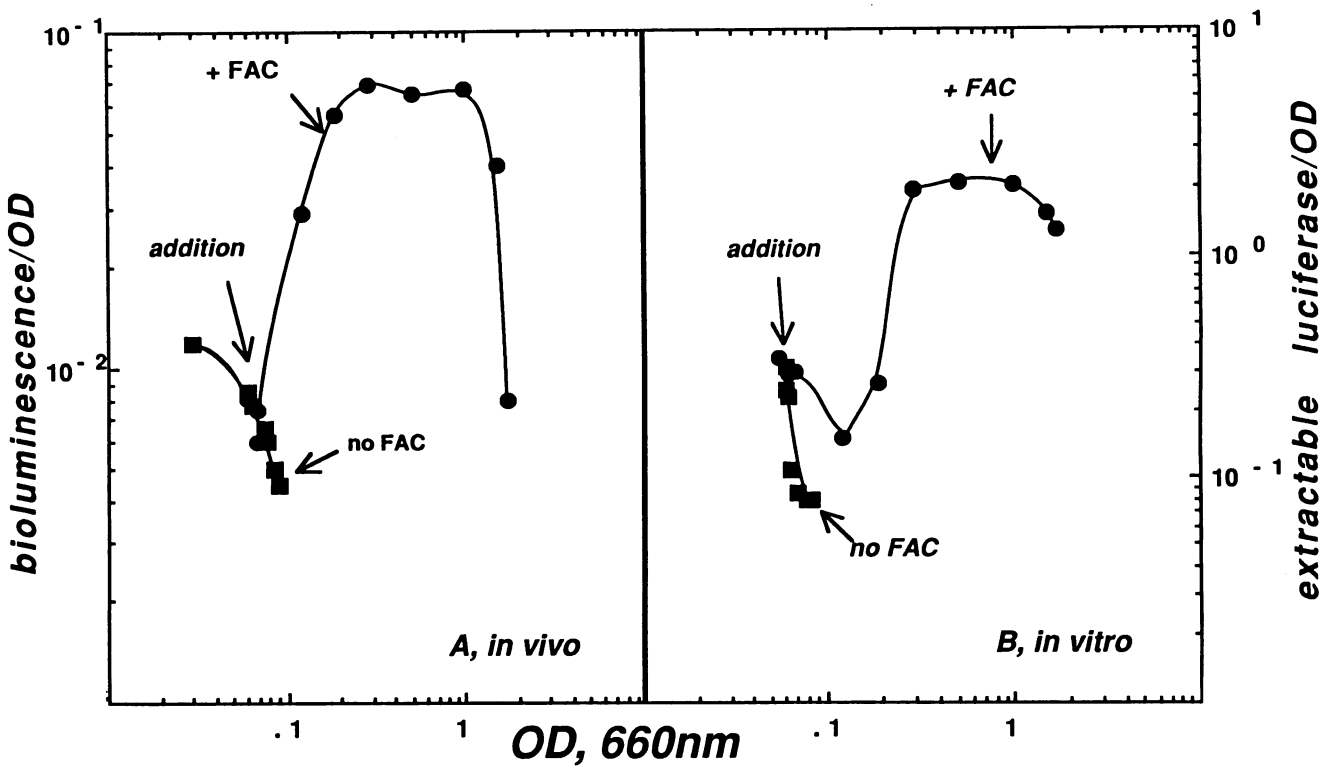

FIG. 4. Effect of iron on cell growth in a HEPES-minimal medium. Addition of FAC (5 μg/ml), at the times indicated by arrows (●), stimulated both growth and bioluminescence, as well as the luciferase synthesis. The control (■) shows (A) very little growth in absence of FAC. The values for bioluminescence and extractable luciferase (B) (in quanta per second per milliliter) should be multiplied by 10^9 and 10^7, respectively.

bacteria. In particular, the blue shift of about 8 nm of the in vivo luminescence as compared to that of the isolated luciferase reaction is similar to that observed in *Photobacterium phosphoreum*, a species generally occurring in deeper water and at lower temperatures (41). The color shift in *P. phosphoreum* has been shown by Lee and colleagues to be due to the presence of and emission from an adventitious protein in the cell carrying an additional chromophore (25).

TABLE 1. Effect of aldehyde chain length on *X. luminescens* luminescence and the luciferase reaction

Aldehyde	Luminescence in vivo[a]	Reaction with in vitro luciferase[b]		
		I_o	K_m (μM)	K_d (s^{-1})
None	1.0	1.0		
Propanal	1.3			
Heptanal	1.5	1.4	12.5	0.02
Octanal	2.5	2.1	6.5	0.03
Nonanal	4.8	200	4.9	0.27
Decanal	5.3	220	4.7	0.26
Undecanal	1.7	45	5.1	0.10
Tridecanal	1.2	36	5.0	0.10
Tetradecanal	9.5	550	2.1	0.79
Octadecanal	1.2	17		0.09

[a] Stimulation of in vivo luminescence by addition of aldehyde, determined by injection of 300 μl of 0.01% aldehyde solution into 3.0 ml of culture medium containing 10^9 cells per ml.

[b] Reactions initiated by injection of $FMNH_2$ (see Materials and Methods) containing luciferase and aldehyde in 50 mM phosphate buffer (pH 7.0) at 40°C.

Whether this or some other explanation pertains for the color shift in *X. luminescens* was not determined.

The optimum temperature of the luciferase reaction is at about 40°C (Fig. 6). This is strikingly different from results for the bacterial luciferases studied previously, which generally have optima below 30°C. Figure 6 also gives data for the effect of temperature on the quantum yield and the rate constant for the decay of the luciferase reaction, with *n*-decanal and $FMNH_2$ as substrates.

The strain of *X. luminescens* studied is not a brightly emitting organism under the conditions we employed. At peak luminescence, it emits about 1.2 quanta/s per cell at 37°C, about 1,000 times less than *V. harveyi* at 25°C. However, the difference in luciferase activity in vitro is only about 30-fold, suggesting that other factors must be involved in vivo, as had already been indicated in the experiments presented in Fig. 1.

Table 1 also gives the in vitro relative maximum light intensities (I_o), the first-order rate constants (K_d) of light decay, and the Michaelis-Menten constants (K_m) of the reaction with each of the different aldehydes assayed at 40°C. Tetradecanal is the aldehyde that results in the highest initial intensity and highest first-order constant for the decay of luminescence. The rate constant for the decay of luminescence at 40°C with *n*-decanal was determined to be 0.26 s^{-1}. It is faster than that for *V. harveyi* luciferase (K_d = 0.12 s^{-1}) but much slower than that for *V. fischeri* (1.03 s^{-1}) or the *Photobacterium* strains (*P. phosphoreum*, 0.78 s^{-1}; *P. leognathi*, 0.64 s^{-1}), all determined at 30°C (32). Naturally, it is

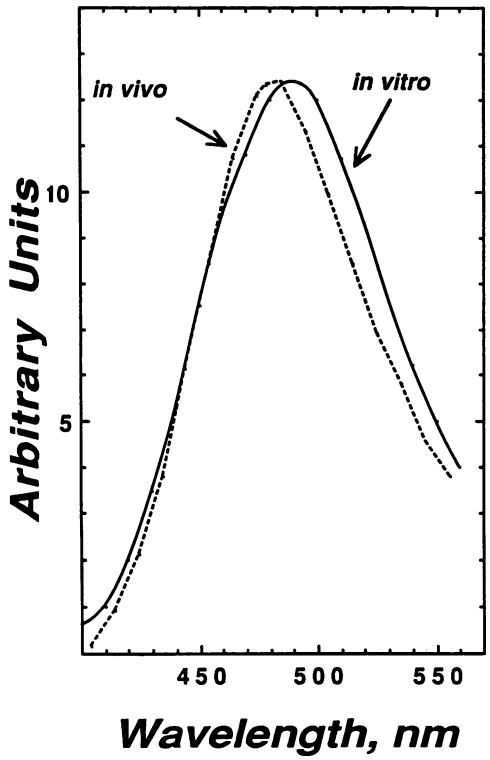

FIG. 5. In vivo and in vitro emission spectra. Cell cultures at the plateau phase were placed in front of the spectrofluorimeter photomultiplier for measurements of the in vivo luminescence spectrum. Aeration was provided by mechanical agitation. A crude extract containing both luciferase and FMN reductase was incubated with decanal and NADH and the in vitro spectrum was recorded. The spectra were corrected for the spectrofluorimeter photomultiplier response and normalized for plotting.

hard to compare reaction kinetics of enzymes that have different optimal temperatures for activity.

We had previously thought that the occurrence of luminous bacteria in infections of human wounds could be accounted for by assuming the involvement of bacterial strains such as those that occur in the nematode-caterpillar system, which could be ubiquitous in the soil. But the results obtained concerning the temperature optimum for luminescence of this isolate from a human wound suggest that this bacterial strain has actually evolved in association with a mammalian host. There are, however, no known symbioses involving these groups (19).

In the nematode-caterpillar system, the light emission could be functional in attracting other animals at night to feed on the caterpillars, as they may be attracted by day by a red pigment (39). Such a function does not seem likely in the case of luminous wounds in mammals.

The luciferase system might have some function or selective advantage not associated with the light emitted and its perception by another organism (16, 17). One suggestion is that the luciferase pathway can serve as a terminal oxidase to allow continued aerobic metabolism under conditions in which the normal cytochrome pathway is blocked, as may occur under conditions of low oxygen or low iron (27). With the isolate from the human wound, neither low oxygen or low iron favors luminescence, so a respiratory-type role for the luminescent system is not indicated.

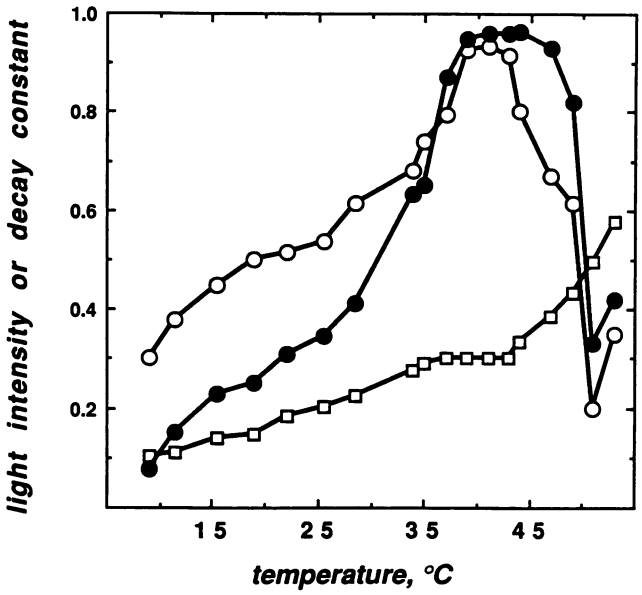

FIG. 6. Effect of temperature on the luciferase reaction. The luciferase reaction was tested at different temperatures, and the intensity with time was recorded graphically. The maximum intensity (I_o [●]), total light (○), and first-order rate constant (s^{-1} [□]) are plotted. I_o and total light values (in quanta per second per milliliter) should be multiplied by 10^9 and 10^{10}, respectively, to give the values obtained.

ACKNOWLEDGMENTS

This work was supported in part by a grant to J.W.H. from the National Science Foundation (DMB-86-16522). P.C. was supported by a Brazilian National Council Fellowship (CNPq), and K.-W.C. was the recipient of a fellowship award from the Korean Science and Engineering Foundation.

We thank J. J. Farmer III for providing the bacterial strain, K. H. Nealson for testing it for antibiotic production, and Thérèse Wilson and David Morse for their helpful suggestions and criticisms.

LITERATURE CITED

1. **Akhurst, R. J.** 1982. Antibiotic activity of *Xenorhabdus* spp. bacteria symbiotically associated with insect pathogenic nematodes of the families *Heterorhadidae* and *Steinernematidae*. J. Gen. Microbiol. **128**:3061–3066.
2. **Akhurst, R. J., and N. E. Boemare.** 1988. A numerical taxonomic study of the genus *Xenorhabdus* (*Enterobacteriaceae*) and proposed elevation of the subspecies of *X. nematophilus* to species. J. Gen. Microbiol. **134**:1835–1845.
3. **Boemare, N. E., and R. J. Ahhurst.** 1988. Biochemical and physiological characterization of colony form variants in *Xenorhabdus* spp. (*Enterobacteriaceae*). J. Gen. Microbiol. **134**: 751–761.
4. **Cabanis, P. J. G.** 1802. Rapports du physique et du moral de l'homme. 6th Mem. De l'influence des témperaments sur la formation des idees et des affections morales (Sec.III) OEuvres **3**:379–381, 1824.
5. **Cooper, D., and R. Cooper.** 1838. On the luminosity of the human subject after death, etc. Phil. Mag. **12(3)**:420–426.
6. **Dunlap, J., and J. W. Hastings.** 1981. The biological clock in *Gonyaulax* controls luciferase activity by regulating turnover. J. Biol. Chem. **256**:10509–10518.
7. **Dunlap, P. V.** 1985. Osmotic control of luminescence and growth in *Photobacterium leiognathi* from ponyfish light organs. Arch. Microbiol. **141**:44–50.
8. **Eberhard, A., A. L. Burlingame, C. Eberhard, G. L. Kenyon, K. H. Nealson, and N. J. Oppenheimer.** 1981. Structural identification of autoinducer of *Photobacterium fischeri* luciferase.

Biochemistry **20**:2444–2449.
9. **Eberhard, A., J. P. Hinton, and R. M. Zunck.** 1979. Luminous bacteria synthesize luciferase anaerobically. Arch. Microbiol. **121**:277–282.
10. **Engebrecht, J., and M. Silverman.** 1984. Identification of genes and gene products necessary for bacterial bioluminescence. Proc. Natl. Acad. Sci. USA **81**:31–44.
11. **Farmer, J. J., III, J. H. Jorgensen, P. A. D. Grimont, R. J. Akhurst, G. O. Poinar, Jr., G. V. Pierce, J. A. Smith, G. P. Carter, K. Wilson, and F. W. Hickman-Brenner.** 1989. *Xenorhabdus luminescens* (DNA hybridization group 5) from human clinical specimens. J. Clin. Microbiol. **27**:1594–1600.
12. **Hada, H. S., J. Stemmler, M. L. Grossbard, P. A. West, C. J. Potrikus, J. W. Hastings, and R. R. Colwell.** 1985. Characterization of non-O1 serovar *Vibrio cholerae* (*Vibrio albensis*). Syst. Appl. Microbiol. **6**:203–209.
13. **Harvey, E. N.** 1940. Living light. Princeton University Press, Princeton, N.J.
14. **Harvey, E. N.** 1952. Bioluminescence. Academic Press, Inc., New York.
15. **Harvey, E. N.** 1957. A history of luminescence. American Philosophical Society, Philadelphia.
16. **Hastings, J. W.** 1968. Bioluminescence. Annu. Rev. Biochem. **37**:597–630.
17. **Hastings, J. W.** 1983. Biological diversity, chemical mechanisms and evolutionary origins of bioluminescent systems. J. Mol. Evol. **19**:309–321.
18. **Hastings, J. W., J. Makemson, and P. V. Dunlap.** 1987. How are growth and luminescence regulated independently in exosymbionts? Symbiosis **4**:3–24.
19. **Hastings, J. W., and K. H. Nealson.** 1981. The symbiotic luminous bacteria, p. 1332–1345. *In* M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (ed.), The procaryotes. Springer-Verlag, Berlin.
20. **Hastings, J. W., and G. Weber.** 1963. Total quantum flux of isotropic sources. J. Opt. Soc. Am. **53**:1410–1415.
21. **Haygood, M. G., and K. H. Nealson.** 1984. Effects of iron on bacterial growth and bioluminescence: ecological implications, p. 56–61. *In* M. Klug and A. Reddy (ed.) Current perspectives in microbial ecology. American Society for Microbiology, Washington, D.C.
22. **Heller, J. F.** 1843. Leuchten gefaulter Hölzer. Deutscher Naturforsche Versammlung Berlin.
23. **Holzman, T. F., and T. O. Baldwin.** 1981. Binding of 2,2-diphenylpropylamine at the aldehyde site of bacterial luciferase increases the affinity of the reduced riboflavin 5'-phosphate site. Biochemistry **20**:5524–5528.
24. **Holzman, T. F., and T. O. Baldwin.** 1982. Isolation of bacterial luciferase by affinity chromatography on 2,2-diphenylpropylamine-sepharose: phosphate-mediated binding to an immobilized substrate analogue. Biochemistry **21**:6194–6201.
25. **Koka, P., and J. Lee.** 1979. Separation and structure of the prosthetic group of the blue fluorescence protein from the bioluminescent bacterium *Photobacterium phosphoreum*. Proc. Natl. Acad. Sci. USA **76**:3068–3072.
26. **Makemson, J. C., and J. W. Hastings.** 1982. Iron represses bioluminescence and affects catabolite repression in *Vibrio harveyi*. Curr. Microbiol. **7**:181–186.
27. **Makemson, J. C., and J. W. Hastings.** 1986. Luciferase-dependent growth of cytochrome-deficient *Vibrio harveyi*. FEMS Micro. Ecol. **38**:79–85.
28. **Meighen, E.** 1988. Enzymes and genes from the *lux* operons of bioluminescent bacteria. Annu. Rev. Microbiol. **42**:151–76.
29. **Mitchell, G., and J. W. Hastings.** 1971. A stable, inexpensive solid state photomultiplier photometer. Anal. Biochem. **39**: 243–250.
30. **Nealson, K., and J. W. Hastings.** 1977. Low oxygen is optimal for luciferase synthesis in some bacteria: ecological implications. Arch. Microbiol. **112**:9–16.
31. **Nealson, K., T. Platt, and J. W. Hastings.** 1970. The cellular control of the synthesis and activity of the bacterial luminescent system. J. Bacteriol. **104**:313–322.
32. **Nealson, K. H.** 1978. Isolation, identification, and manipulation of luminous bacteria. Methods Enzymol. **58**:153–166.
33. **Oliver, J. D., D. M. Roberts, V. K. White, M. A. Dry, and L. M. Simpson.** 1986. Bioluminescence in a strain of the human pathogenic bacterium *Vibrio vulnificus*. Appl. Environ. Microbiol. **52**:1209–1211.
34. **Paul, V. J., S. Frautschy, W. Fenical, and K. H. Nealson.** 1981. Antibiotics in microbial ecology. Isolation and structure assignment of several new antibacterial compounds from the insect-symbiotic bacteria *Xenorhabdus* spp. J. Chem. Ecol. **7**:589–597.
35. **Percy, B.** 1822. Phosphorescence of wounds. Quart. Jour. Sci. Lit. Arts **12**:180–181.
36. **Pfeiffer, H., and H. J. Stammer.** 1930. Pathogenes Leuchten bei Insecten. Z. Morphol. Oekol. Tiere **20**:136–171.
37. **Poinar, G. O., Jr., G. Thomas, M. Haygood, and K. H. Nealson.** 1980. Growth and luminescence of the symbiotic bacteria associated with the terrestrial nematode, *Heterorhabditis bacteriophora*. Soil. Biol. Biochem. **12**:5–10.
38. **Puerarius, D.** 1667. De carnibus lucentibus. Hafnie.
39. **Richardson, W. H., T. M. Schmidt, and K. H. Nealson.** 1988. Identification of an anthraquinone pigment and a hydroxystilbene antibiotic from *Xenorhabdus luminescens*. Appl. Environ. Microbiol. **54**:1602–1605.
40. **Rosson, R. A., and K. H. Nealson.** 1981. Autoinduction of bacterial bioluminescence in a carbon limited chemostat. Arch. Microbiol. **129**:299–304.
41. **Ruby, E. G., E. P. Greenberg, and J. W. Hastings.** 1980. Planktonic marine luminous bacteria: species distribution in the water column. Appl. Environ. Microbiol. **39**:302–306.
42. **Thomas, G. M., and G. O. Poinar, Jr.** 1979. *Xenorhabdus* gen. nov., a genus of entomopathogenic, nematophilic bacteria of the family *Enterobacteriaceae*. Int. J. Syst. Bacteriol. **29**:352–360.
43. **Ulitzur, S., and J. W. Hastings.** 1979. Autoinduction in luminous bacteria: a confirmation of the hypothesis. Curr. Microbiol. **2**:345–348.
44. **Waters, C. A., and J. W. Hastings.** 1977. Mutants of luminous bacteria with an altered control of luciferase synthesis. J. Bacteriol. **131**:519–525.