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# Osmotic control of luminescence and growth in *Photobacterium leiognathi* from ponyfish light organs

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**Abstract.** Osmolarity was found to control the luminescence and growth of *Photobacterium leiognathi* strain LN-1a isolated from the light organ of the ponyfish *Leiognathus nuchalis* (family Leiognathidae). Low osmolarity (ca. 400 mOsm) stimulated luminescence per cell 80 to 100-fold to a level (ca. $2.0 \times 10^4$ quanta $\cdot s^{-1} \cdot cell^{-1}$) equal to that of bacteria taken directly from the light organ and increased the level of luciferase per cell 8 to 10-fold compared to high osmolarity (ca. 800 mOsm). Conversely, high osmolarity stimulated oxygen uptake and growth rate 2 to 4-fold compared to low osmolarity. Of 21 additional tested strains of *P. leiognathi* from light organs of 9 other ponyfish species, all responded similarly. Low osmolarity may be a host control factor that functions to stimulate the luminescence and restrict the growth of ponyfish light organ bacteria in situ.

**Key words:** Luminescence – Luciferase – Osmolarity – Growth – Ponyfish – Light organ symbiosis – *Photobacterium leiognathi* – Salt

Luminous bacteria occur throughout the marine environment as members of planktonic and benthic communities and in saprophytic, commensal and parasitic associations with animals. Certain of these bacteria also establish species-specific luminescent symbioses with the members of several families of marine teleost fishes. In this latter case, the bacteria are harbored in specialized, highly structured, gland-like light organs, and the light they produce is utilized by the host fish (for reviews see Herring and Morin 1978; Nealson and Hastings 1979; Hastings and Nealson 1981; Herring 1982). For one of these associations, the light organ symbiosis between *Photobacterium leiognathi* and ponyfish (family Leiognathidae) (Boisvert et al. 1967; Reichelt et al. 1977), much detailed information has been obtained on the anatomy of the fish's light organ system and on the fish's use of the bacterial light (Bassot 1975; McFall-Ngai 1983a, b; McFall-Ngai and Dunlap 1983, 1984; Dunlap and McFall-Ngai 1984). These studies reveal that ponyfishes have extensive anatomical and physiological adaptations for maintaining the symbiotic bacteria, and that the fish has definite behavioral uses for the bacterial light. Control by the ponyfish to maximize the luminescence and minimize the growth of its light organ bacteria would therefore appear to be adaptive.

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For luminous bacteria in culture, control of the synthesis and expression of the luminescent system can be exerted by a variety of factors, including autoinducer concentration (Nealson 1977), oxygen tension (Nealson and Hastings 1977) and the availability of iron (Makemson and Hastings 1982; Haygood and Nealson 1984). In addition, the luminescence and growth rate of *P. leiognathi* are sensitive to NaCl concentration (Henry and Michelson 1970; Reichelt and Baumann 1974). For ponyfish light organ bacteria in situ, evidence of control has recently been obtained: the luminescence of *P. leiognathi* taken directly from the fish light organ is 10 to 100-fold higher than is typically produced by this bacterium in culture, and the growth rate of bacteria in the light organ appears to be 20 to 30 times slower than in culture (Dunlap 1984b). Furthermore, models of the ponyfish symbiosis have been proposed that postulate control of the bacteria by nutrient limitation (Nealson 1979) and respiratory restriction (Dunlap 1984b). At present, however, the factors that actually control the luminescence and growth of ponyfish symbionts are unknown.

In this study I address the response of *P. leiognathi* to the osmolarity of its growth environment and propose osmolarity as a host control factor in the ponyfish light organ symbiosis. As described here, low osmolarity, at a level similar to that of the tissues and body fluids of marine teleost fishes, increases the levels of luminescence and luciferase of *P. leiognathi*, but restricts oxygen uptake and growth. Conversely, high osmolarity, at a level approaching that of sea water, stimulates oxygen uptake and growth, but sharply limits luminescence. These results suggest that in the ponyfish light organ low osmolarity could serve to stimulate the luminescence and restrict the growth of *P. leiognathi*.

## Materials and methods

### *Isolation and maintenance of bacteria*

*Photobacterium leiognathi* strains LN-1a, LEL-2a, and LF-1a were isolated from the light organs of the ponyfish *Leiognathus nuchalis* (Misaki, Japan), *L. elongatus* (Manazuru, Japan) and *L. fasciatus* (Manila Bay, Philippine Islands), respectively. These representative isolates were obtained by aseptic excision of the light organ from the freshly sacrificed fish, followed by homogenization and dilution-plating of the light organ to obtain well isolated colonies, as previously described (Dunlap 1984b). For the capture and handling of fish specimens, see McFall-Ngai and Dunlap (1983, 1984) and Dunlap and McFall-Ngai (1984). The identity of these isolates as *P. leiognathi* (see

Reichelt et al. 1977) was confirmed with standard taxonomic methods (Reichelt and Baumann 1973; Baumann and Baumann 1981). *P. leiognathi* strain ATCC 25521 (isolated from the light organ of *L. splendens* from the Gulf of Thailand; Boisvert et al. 1967) was obtained from P. Baumann (UC Davis). All strains were stored at room temperature (20 to 24° C) and transferred monthly on the sea water complete (SWC) agar medium of Nealson (1978) (5 g peptone, 3 g yeast extract, 3 ml glycerol, 750 ml sea water, 250 ml distilled water, 15 g agar per l) that was modified by the addition of 25 mM Tris-HCl buffer (pH 7.8) and by the reduction of the sea water content to 40% (400 ml sea water, 600 ml distilled water per l).

*Growth media, inoculum, and cultivation conditions*

Experimental cultures of LN-1a and other strains were grown in basal minimal medium (BMM; 50 mM Tris-HCl [pH 7.8], 10 mM $NH_4Cl$, 0.3 mM $K_2HPO_4$, 20 mM glycerol [or 10 mM glucose where indicated]) and 2.8 mg/l ferric ammonium citrate (FAC), plus NaCl, KCl, $CaCl_2$, $MgCl_2$, and $Na_2SO_4$ added in amounts to approximate the salt concentration and ionic composition of different percentages of the sea water described by Lyman and Fleming (1940). For example, BMM-40 (low salt medium, total solute concentration equivalent to that of 40% sea water) contained 120 mM NaCl, 3 mM KCl, 3 mM $CaCl_2$, 16 mM $MgCl_2$ and 8.5 mM $Na_2SO_4$; whereas BMM-80 (high-salt medium, equivalent to 80% sea water) contained 280 mM NaCl, 7 mM KCl, 7 mM $CaCl_2$, 37 mM $MgCl_2$ and 20 mM $Na_2SO_4$. For solid media, 1.5% agar (Difco) was used. Glucose and FAC were added aseptically from filter-sterilized stock solutions to the autoclaved, cooled medium. The osmolarity of BMM-40 was 400 to 420 mOsm and that of BMM-80 was 800 to 820 mOsm, as measured with a Wescor 5130C vapor pressure osmometer. For certain growth experiments, the concentrations of specific salts were varied as described in the text. Mannitol and sucrose, which do not serve as sole carbon and energy sources for *P. leiognathi* (see Baumann and Baumann 1981), were also used to vary the solute content in certain experiments. These solutes were added from filter-sterilized stock solutions to sterile media prepared with proportionally less water.

Experimental cultures were grown on a New Brunswick gyrotory shaker (150 rev · $min^{-1}$) at 26° C in either 100 ml of medium in 300 ml nephelometry flasks (Belco) or in 6 ml of medium in 18 × 150 mm tubes held on the shaker at a 30° angle from the horizontal. Growth was measured as optical density (OD) at 660 nm with a Bausch and Lomb Spectronic 20. An OD of 0.1 was approximately equal to $1 \times 10^8$ cells · $ml^{-1}$ in both BMM-40 and BMM-80. Specific growth rates (μ) were calculated from plots of the linear portion of the growth curves.

For consistent results, all strains were first subcultured twice sequentially on solid BMM-40. Cells from a single brightly luminescent colony from the second plate were then transferred into liquid BMM-40 to an OD of 0.01 or less and grown to an OD of 0.2 to 0.3. A 1% inoculum from this culture served to initiate experimental cultures.

*Luminescence, luciferase and oxygen uptake*

Luminescence of 1 ml samples of flask cultures in glass scintillation vials and of the entire 6 ml tube cultures was measured with a photomultiplier photometer similar to that of Mitchell and Hastings (1971). Luminescence is expressed as light units · $ml^{-1}$, where one light unit (LU) equals $5.9 \times 10^8$ quanta · $s^{-1}$ based on light standard no. 231 (provided by J. W. Hastings, Harvard University). Luciferase activity in cell extracts was assayed according to the methods of Rosson and Nealson (1981) and is expressed as arbitrary luciferase units per cell (LU/OD in vitro). Dodecanal and tetradecanal were purchased from Aldrich Biochemicals. To test the effect of aldehyde on luminescence, dodecanal and tetradecanal (as 0.1% sonicated suspensions in water) were added to high-salt cultures grown to an OD of 0.10, 0.40 and 0.80 (10, 20, and 40 μl aldehyde per ml of culture at each OD). Oxygen uptake of cultures in early to mid exponential phase of growth was measured with a polarographic oxygen electrode apparatus (Rank Brothers, Cambridge, England) calibrated to the appropriate air-saturated growth medium. Oxygen uptake is expressed as molecules $O_2$ · $s^{-1}$ · $cell^{-1}$.

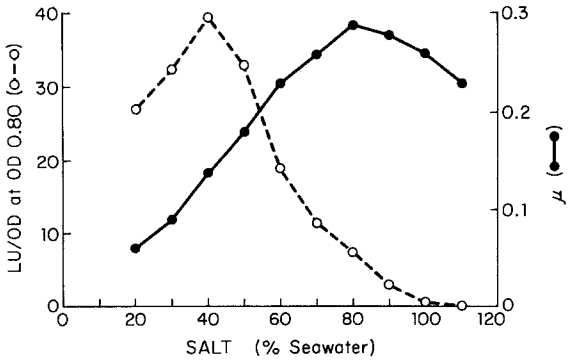


**Fig. 1.** The effect of salt concentration on the luminescence per cell (LU/OD) and growth rate (μ) of *Photobacterium leiognathi* LN-1a. Cells were cultured in basal minimal media with glycerol as the carbon sourcee. Salt concentration, expressed as % sea water, was increased by proportionally increasing the levels of NaCl, KCl, $CaCl_2$, $MgCl_2$, and $Na_2SO_4$ (see Materials and methods). Peak luminescence occurred in each culture at OD ca. 0.80

## Results

The effect of salt concentration on the luminescence and growth rate of *Photobacterium leiognathi* strain LN-1a was examined in minimal media (BMM) that ranged in salt content from 10% to 110% that of sea water (Fig. 1). In general, a lower salt concentration resulted in higher luminescence and slower growth. Greatest luminescence occurred at a salt concentration equivalent to 40% sea water (low-salt medium, BMM-40), a concentration at which the growth rate was markedly depressed. In contrast, growth was fastest at a salt concentration equivalent to 80% sea water (high-salt medium, BMM-80), and light production at this concentration was very low. A similar but much less distinct result was obtained in complete (SWC) medium ranging in sea water content from 10% to 100% (data not shown). Oxygen uptake was lower in BMM-40 compared to BMM-80 (Table 1, with glucose in place of glycerol as the carbon source). As a consequence of the higher level of luminescence and lower oxygen uptake in the low-salt medium, luminescence consumed a 100-fold higher percentage of the total utilized oxygen than in the high-salt medium (Table 1).

**Table 1**
Activity of *Photobacterium leiognathi* LN-1a in low- and high-osmolarity media

| Growth medium[a] | Growth rate ($h^{-1}$) | Luminescence[b] (quanta · $s^{-1}$ · $cell^{-1}$) × $10^3$ | Oxygen uptake ($O_2$ · $s^{-1}$ · $cell^{-1}$) × $10^5$ | $O_2$ consumed in luminescence[c] |
|---|---|---|---|---|
| Low-osmolarity (BMM-40) | 0.4 | 8.0 | 4.0 | 2.0 −20%[d] |
| High-osmolarity (BMM-80) | 0.7 | 0.2 | 9.0 | 0.02− 0.2 |

[a] With glucose as the carbon source
[b] At peak luminescence
[c] Percent total $O_2$ consumed due to luminescence
[d] In vivo quantum yield assumed to fall between 0.1 and 1.0

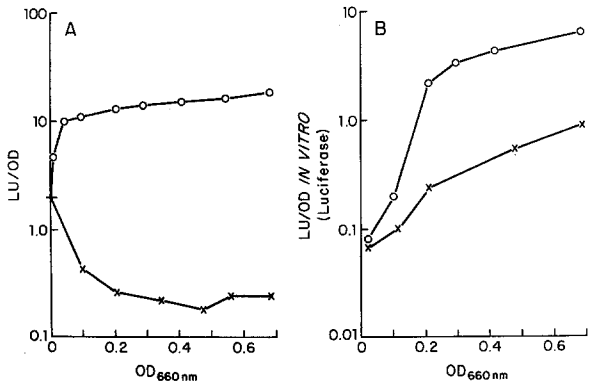


**Fig. 2 A, B.** The effect of salt concentration on the luminescence and luciferase activity of *P. leiognathi* LN-1a. Cells were cultured in BMM-40 (○) or BMM-80 (×) with glycerol as the carbon source. **A** Luminescence per cell (LU/OD); **B** Luciferase (extractable activity in arbitrary units) per cell (in vitro LU/OD) for cultures in **A**

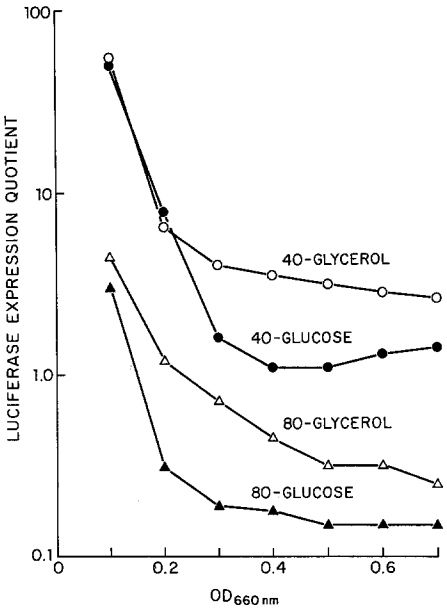


**Fig. 3.** Luciferase expression quotient (in vivo luminescence produced per unit luciferase activity in cell extracts) of *P. leiognathi* LN-1a. Data are from Fig. 2A, B (glycerol-grown cells: BMM-40, ○; BMM-80, △), and from a similar experiment with glucose grown cells (BMM-40, ●; BMM-80, ▲)

In BMM-40, in vivo luminescence per cell (LU/OD) increased rapidly (relative to growth) from the time of inoculation and reached and maintained a high level (generally 20 to 40 LU/OD) (Fig. 2A). The growth rate (μ) of this culture was 0.2 $h^{-1}$. In contrast, in the high-salt medium, luminescence per cell initially declined sharply and then either stabilized (Fig. 2A) or in some cases rose slowly (not shown). Growth rate of the high-salt culture was 0.4 $h^{-1}$. Consequently, luminescence per cell in the low-salt medium was typically 80 to 100 times higher than in the high-salt medium. Similar results were obtained with glucose as the carbon source except that luminescence was generally 2 to 4-fold lower and growth rate was 2-fold higher than with glycerol at both salt concentrations.

Since LN-1a grew exponentially from the time of inoculation into both BMM-40 and BMM-80, these differences in luminescence and growth rate were not due to a lag in growth in the low-salt medium. In addition, results very similar to those shown in Fig. 2A were obtained regardless of whether the inoculum was pre-grown in the low- or high-salt medium. These differences also were apparently not due to aldehyde limitation in the high-salt cultures, since the addition of dodecanal and tetradecanal had no stimulatory effect on luminescence.

For the cultures shown in Fig. 2A, luciferase activity per cell (in vitro LU/OD) increased from the time of inoculation in both BMM-40 and BMM-80, but increased more rapidly in the low-salt medium, reaching a level about 8 to 10 times higher than in the high-salt medium (Fig. 2B). Similar results were obtained with glucose as the carbon source, except that luciferase activity, as found for luminescence, was 2 to 4-fold lower at each salt concentration that with glycerol.

By inspection (Fig. 2A, B) it can be seen that the greater luciferase activity of cells from the low-compared to the high-salt medium accounted for approximately 50% of the higher level of luminescence. The remainder of the difference (50%) can be attributed to a higher level of quanta produced per unit luciferase activity in cell extracts for the low-salt culture (Fig. 3), or a higher luciferase expression quotient (LEQ; see Ulitzur et al. 1981). Above an OD of 0.2 or 0.3, the LEQ values stabilized and those of the low-salt and glycerol-grown cultures exceeded those of the high-salt and glucose-grown cultures, respectively, by 8 to 10-fold.

The lower concentrations of individual ions or salts in the low-salt medium were found not to account for the observed differences in luminescence and growth rate. Increasing the $Na^+$ concentration of the low-salt medium while maintaining its total solute concentration should have caused a sharply decreasing "high-salt" pattern of luminescence if $Na^+$ concentration alone accounted for the observed differences. However, equimolar replacement of up to 85% of the $Na^+$ content of BMM-40 with $K^+$ (KCl for NaCl)

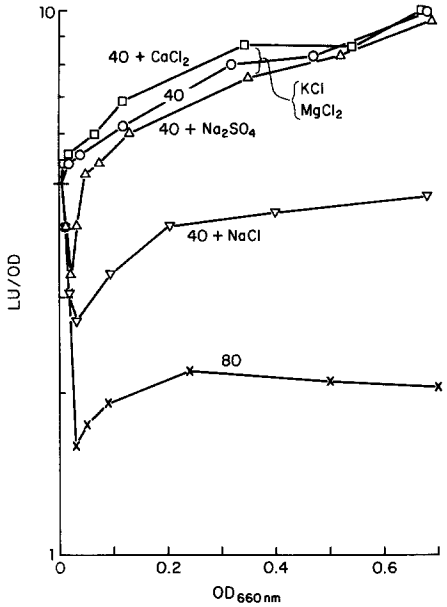


**Fig. 4.** The effect of increased concentrations of specific salts on the luminescence of *P. leiognathi* LN-1a. Cells were cultured in BMM-40, BMM-80, or BMM-40 plus a specific salt (with glucose as the carbon source). Additions to BMM-40 were: KCl, 4 mM; $CaCl_2$, 4 mM; $MgCl_2$, 21 mM; $Na_2SO_4$, 11 mM; or NaCl, 160 mM. Data points for KCl and $MgCl_2$ fell between those of $CaCl_2$ and $Na_2SO_4$ and for clarity are indicated only by the bracket

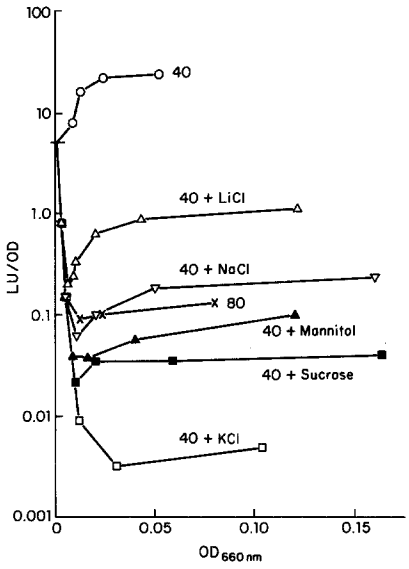


**Fig. 5.** The effect of osmolarity on the luminescence of *P. leiognathi* LN-1a. 225 mM LiCl, NaCl or KCl; or 450 mM mannitol or sucrose were added to BMM-40 (with glucose as the carbon source) as indicated

had essentially no effect on luminescence per cell or growth rate (i.e., results essentially identical to Fig. 2A, curve for BMM-40). Separately increasing the levels of the individual salts in BMM-40 to their respective levels in BMM-80 (without adjusting the total solute content of the medium) also had essentially no effect, except in the case of NaCl, the addition of which caused a luminescence pattern intermediate between the low- and high-salt controls (Fig. 4). However, because NaCl was added at 160 mM, a specific $Na^+$ effect cannot be distinguished from an effect due to an increase in total solute concentration. Additionally, separate deletions of individual salts from BMM-80 (KCl, $CaCl_2$, $NaSO_4$) (without addition of other salts to retain the total solute concentration), or partial replacement of $Cl^-$ with $Br^-$ or $NO_3^-$, had essentially no effect on luminescence or growth (i.e., results essentially identical to Fig. 2A, curve for BMM-80) (in the absence of $MgCl_2$ luminescence and growth were very poor). These results suggest that total solute concentration (osmolarity), rather than the concentration of a specific ion or salt, was responsible for the higher luminescence and slower growth of LN-1a in BMM-40 compared to BMM-80.

The effect of osmolarity was examined by using a variety of solutes to increase the total solute concentration of BMM-40 to a level similar to that of BMM-80 (Fig. 5). Regardless of whether the solute was a salt or a carbohydrate, the addition of each solute (ca. 400 to 450 mOsm increase) caused a sharply decreasing pattern of luminescence characteristic of high-salt cultures. Furthermore, in each case the addition of solute stimulated growth rate ($\mu$ [$h^{-1}$]: BMM-40 = 0.3; + 225 mM LiCl or + 450 mM sucrose = 0.5; + 225 mM KCl or + 225 mM NaCl = 0.6; + 450 mM mannitol or BMM-80 = 0.8). Thus, although the type of solute has some influence over the extent of the decrease in luminescence per cell and the increase in growth rate, the major differences in luminescence and growth rate (and apparently extractable luciferase activity, Fig. 2A) between the low- and high-salt cultures are due to the difference in osmolarity between these two media.

The bright-luminescence, slow-growth response of LN-1a to low osmolarity was found to be characteristic of *P. leiognathi* from light organs of ponyfish. Strains of *P. leiognathi* (LEL-2a, LF-1a, and ATCC 25521) isolated from light organs of other species of ponyfish (see Materials and methods) produced luminescence and growth rate optima in BMM essentially identical to those of LN-1a (Fig. 1), except that for ATCC 25521 the luminescence optimum occurred at a slightly lower salt concentration (ca. 30 to 35% sea water) than for LN-1a. Furthermore, 18 additional strains of *P. leiognathi* (each isolated from a different specimen of the following ponyfish: *Gazza achlamys*, *G. minuta*, *Leiognatus bindus*, *L. edwardsi*, *L. equulus*, *L. splendens* and *Secutor insidiator*) were tested and all exhibited the characteristically higher luminescence and slower growth on low- compared to high-salt minimal medium. In addition, *P. leiognathi* taken from the light organs of freshly sacrificed ponyfish and inoculated directly into BMM-40 and BMM-80 produced low- and high-salt luminescence and growth patterns essentially identical to those of LN-1a (10 trials using bacteria from light organs of 10 specimens of *L. equulus*). Moreover, LN-1a stored and transferred on SWC prepared with 40% sea water retained this response to osmolarity, whereas LN-1a stored and transferred on SWC with 75% sea water tended to lose it. Thus, the response of *P. leiognathi* to osmolarity is not a trait acquired during laboratory culture, but it might be lost under prolonged culture in high-osmolarity media.

In contrast, other species of luminous bacteria (several strains of *P. phosphoreum*, *Vibrio fischeri* and *V. harveyi* examined) respond to salt concentration (osmolarity) in a

manner distinct from that of light organ symbiotic *P. leiognathi* but characteristic of each species (Dunlap 1984a). In addition, *P. leiognathi* strain PL-721, which was isolated from the skin of the mesopelagic evermannelid fish, *Coccorella* sp. (Nealson and Hastings 1977), produces optima for luminescence and growth at much higher salt concentrations, approximately 95 to 100% sea water in BMM (Dunlap 1984a). Thus, the response to osmolarity may distinguish *P. leiognathi* strains of ponyfish light organs from other species of luminous bacteria and from *P. leiognathi* strains that occur in habitats other than the ponyfish light organ.

## Discussion

This study demonstrates that osmolarity is a factor that controls the luminescence and the growth of *Photobacterium leiognathi* from the light organs of ponyfish. Low osmolarity (ca. 400 mOsm), as opposed to high osmolarity (ca. 800 mOsm), leads to greater luminescence and higher luciferase but to lower rates of oxygen uptake and growth (Figs. 1, 2, 5; Table 1). The bright-luminescence, slow-growth response to low osmolarity of *P. leiognathi* from ponyfish light organs appears to distinguish it from other luminous bacteria. Although the mechanism of this osmotic control is unclear, the opposite effect of osmolarity on luminescence and growth suggests that the osmotic response may have importance for host control of bacterial luminescence and growth in the ponyfish light organ.

Consistent with the findings of this study, Henry and Michelson (1970) showed that a high salt concentration (3% NaCl) can extinguish the luminescence of *P. leiognathi*, whereas a lower salt concentration (1% NaCl) results in a high level of luminescence. In addition, Reichelt and Baumann (1974) demonstrated that *P. leiognathi* responded to increased NaCl concentration with faster growth. The 80 to 100-fold increase in luminescence of *P. leiognathi* LN-1a growing in low- compared to high-osmolarity media (Fig. 2A) results from both a higher level of luminescence per unit luciferase activity in cell extracts (Fig. 3) and a higher level of extractable luciferase (Fig. 2B). The difference in luminescence could result from a difference in the availability of substrates for the luminescent reaction. For cells grown in high-osmolarity medium, oxygen and aldehyde do not appear to be limiting, but the availability of reducing power may be low. This could occur through faster utilization of reducing power by the respiratory system, which would account not only for the lower level of luminescence but also the higher rate of oxygen uptake and faster growth of the high-osmolarity culture. Conversely, at low osmolarity more reducing power might be available for luminescence as a result of a lower rate of utilization by the respiratory system. This interpretation would account for the higher luminescence of cells grown at low osmolarity, as well as their lower rate of oxygen uptake, their higher percentage of total utilized oxygen consumed in luminescence and their slower growth rate (Table 1). These interpretations are consistent with information that suggests a possible inverse relationship between the levels of respiration and luminescence in other species of luminous bacteria (Eymers and van Schouwenberg 1937; Watanabe et al. 1975; Ulitzur et al. 1981; Makemson and Hastings 1982).

This proposed re-direction of reducing power to the luminescent system by low osmolarity might be effected by a correspondingly lower level of internal osmoregulatory solutes (e.g., $Na^+$, $K^+$ and glutamate; see Measures 1975; Makemson and Hastings 1979), since the activity of certain respiratory enzymes appears to be influenced by internal solute concentrations (Watanabe et al. 1977; Unemoto et al. 1977; Unemoto and Hayashi 1979). Alternatively, the observed responses at low osmolarity (higher luminescence, slower growth) might result from limitation in the uptake or metabolism of carbon substrate (see Nealson 1979) or iron (see Makemson and Hastings 1982; Haygood and Nealson 1984; Dunlap 1984a).

The second factor involved in the higher level of luminescence of *P. leiognathi* at low compared to high osmolarity, an 8 to 10-fold increase in extractable luciferase activity, may relate to differences in autoinduction (see Nealson 1977). At low osmolarity, synthesis of autoinducer may be more rapid (relative to growth rate), or autoinducer activity (i.e., transport, binding, etc.) may be better. At present, however, although luciferase appears to be an inducible enzyme in *P. leiognathi* LN-1a (Fig. 2B), and while evidence suggestive of autoinduction in this strain has been obtained (Dunlap 1984a), definitive evidence of autoinduction control of luminescence in *P. leiognathi* from ponyfish light organs is not available. Other factors, such as an increased rate of luciferase degradation under high osmolarity, might also account for the observed difference in levels of luciferase.

In regard to ponyfish light organ symbiosis, the response of *P. leiognathi* to osmolarity may be important for the control of bacterial luminescence and growth in situ. Firstly, the osmolarity of the light organ is likely to be low relative to that of sea water. In general, marine teleost fishes osmoregulate and maintain their tissues and body fluids at a solute concentration roughly 30 to 40% that of sea water (ca. 300 to 400 mOsm: Prosser 1973; Fange et al. 1976; Gordon 1977). Although no information is available on osmoregulation specifically in ponyfish, there is no reason a priori to suspect that they differ from those teleosts that have been studied. Secondly, the salt concentration in culture at which LN-1a cells produce highest luminescence is equivalent to 40% sea water (BMM-40, ca. 400 mOsm). Furthermore, the level of luminescence produced by LN-1a at this salt concentration ($2.0 \times 10^4$ quanta $\cdot s^{-1} \cdot cell^{-1}$) essentially equals that of *P. leiognathi* taken directly from the ponyfish light organ ($2.4 \times 10^4$ quanta $\cdot s^{-1} \cdot cell^{-1}$; Dunlap 1984b). In addition, LN-1a grown in BMM-40 and bacteria taken directly from the ponyfish light organ consume in luminescence a similar percentage of the total utilized oxygen (2.0 to 20% [Table 1] compared to 1.7 to 17% [Dunlap 1984b], respectively). These percentages are roughly similar to those reported for other species at maximal luminescence (Eymers and van Schouwenberg 1937; Harvey 1952; Watanabe et al. 1975; Karl and Nealson 1980). The characteristic response to low and high osmolarity of bacteria taken directly from the light organ, and the possible trend for LN-1a stored on high-salt media to lose the distinctiveness of this response are consistent with these correlations between low osmolarity in culture and in the light organ. The bright-luminescence, slow-growth response of *P. leiognathi* to low osmolarity may thereby provide the fish with a means of physiologically stimulating the light production and restricting the growth of its symbiotic light organ bacteria. Based on the data presented here, *P. leiognathi* can be envisioned as inhabiting two osmotically distinct environments: 1. the

ponyfish light organ (low osmolarity), where high levels of luminescence may be physiologically adaptive for survival and growth (Dunlap 1984b), and 2. the gut tract of fish and sea water (high osmolarity), where luminescence may be less important for survival and growth.

This low osmolarity hypothesis is consistent with a slow-growth, nutrient-limitation model for ponyfish bacteria (Nealson 1979), and the data presented here closely fit the predictions of a respiratory-restriction model proposed for this symbiosis (Dunlap 1984b). However, the estimated much slower growth rate of ponyfish bacteria in situ ($\mu = 0.03\ h^{-1}$; Dunlap 1984b) compared to in culture at low osmolarity ($\mu = 0.2$ to $0.4\ h^{-1}$) suggests that, while osmolarity exerts a major control over luminescence, other factors, such as nutrient limitation (Nealson 1977) and iron limitation (Makemson and Hastings 1982; Haygood and Nealson 1984; Dunlap 1984a) may play an important role in restricting the growth rate of ponyfish light organ bacteria in situ. Definitive support for the low osmolarity hypothesis awaits the examination of ponyfish light organ fluid and analysis of the effect of these other possible control factors on the luminescence and growth of *P. leiognathi.*

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