Marine microlights: the luminous marine bacteria

Peter Herring

Luminous marine bacteria can cause fish to glow and seas to shine. How do they do it? How can we use this amazing phenomenon in biomedical science?

Luminous bacteria have had a long and distinguished career, albeit much of it cloaked in mystery. Glowing meat, fish and shrimp were well known to our less-illuminated ancestors and in 1667 Robert Boyle discovered that their light was reversibly extinguished in a vacuum. A practical, if unwitting, demonstration of the role of micro-organisms was described in 1825 in a macabre experiment on the eerie light emitted by two dissected bodies in a London anatomy school. The luminous material was scraped off and was then used to induce other corpses to glow.

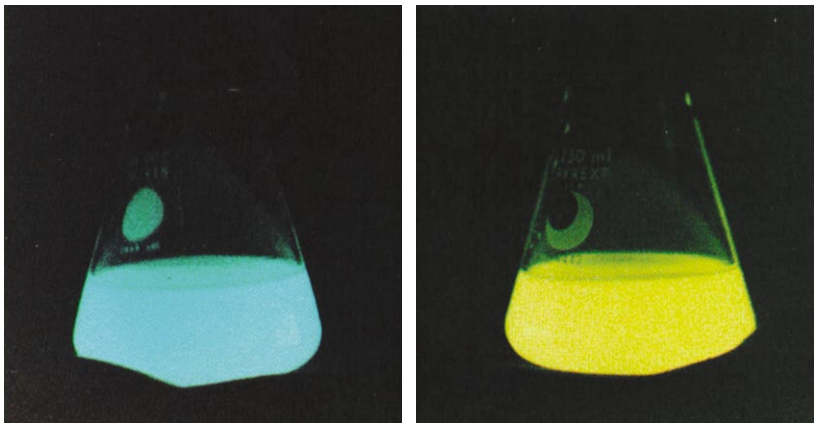
Luminous bacteria were grown in culture in the 1870s and 27 species had been described by 1900. The present tally of culturable marine species is about 10, three of them assigned to the genus *Photobacterium*, five to *Vibrio* and two to *Shewanella*. The taxonomy has been frequently revised and some of the *Vibrio* species previously appeared as *Photobacterium*, *Achromobacter*, *Lucibacterium*, *Neisseria* or *Beneckea*. There are also two non-marine luminous bacteria, *Photorhabdus* (*Xenorhabdus*) *luminescens* and a strain of *Vibrio cholerae*.

Mechanism

How is the light produced? Bioluminescence involves the oxidation of a substrate (generically known as luciferin) in the presence of an enzyme (luciferase). Bacterial luciferase oxidizes $FMNH_2$, in the presence of a long chain aldehyde (tetradecanal). All luminous bacteria share this reaction, but their control systems differ. *In vitro* the reaction emits blue-green light with a maximum at 485–495 nm, yet the light from live *Vibrio fischeri* is bluer (max. at 475 nm) because a blue-fluorescent protein (or lumazine protein) accepts energy from the reaction and emits light at its own characteristic wavelength. A strain of *V. fischeri* (Y-1) also has a yellow fluorescent protein (YFP) and emits yellow light (max. at 540 nm) (Fig. 1). Weakening the bonds between YFP and the reaction system (e. g. by dilution or by raising the temperature) reduces the yellow component of the luminescence. Up to 18 °C the light is yellow; at higher temperatures it is blue.

Single isolated bacteria do not glow, but colonies do. Individual cells produce an 'autoinducer', which accumulates in the medium and at a critical concentration triggers the production of luciferase, thereby switching on the luminescence. Different species have different inducers, but similar systems. Autoinduction of luminescence is an example of quorum sensing, in which population density (signalled chemically) induces metabolic processes, including virulence, in many non-luminous bacteria.

BELOW:
Fig. 1. Cultures (at 18 °C) of *Photobacterium phosphoreum* (left) and *Vibrio fischeri* strain Y-1 (right) by their own light.
COURTESY TOM BALDWIN



Genetics of luminescence

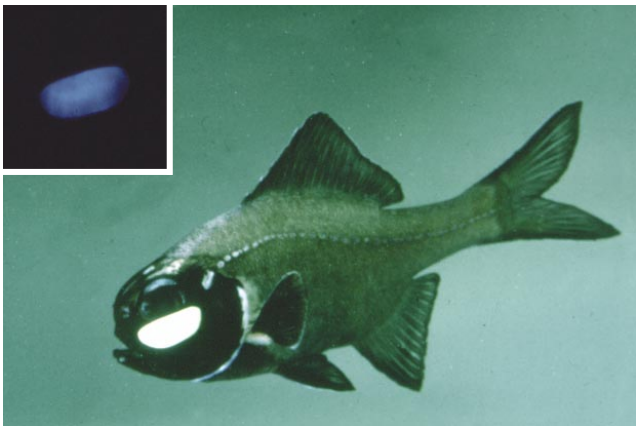
Light emission from marine bacteria is easy to detect. Species such as *V. fischeri* are easy to culture and dim or dark mutants frequently appear. These factors have helped to dissect the genetic basis of luminescence. Bacterial luciferase is composed of two subunits, a and b, encoded by the *luxA* and *luxB* genes. Three other genes (*luxC*, *D* and *E*) control the biosynthesis of tetradecanal. Other genes are essential in particular species. *luxF*, *G*, *H*, *L* and *Y* are involved with flavin metabolism, lumazine proteins and the colour of the light. Of the regulatory genes in *V. fischeri*, *luxI* controls production of autoinducer and *luxR* encodes the autoinducer receptor protein. The interaction of autoinducer and protein stimulates transcription of the *luxICDABEG* operon, producing light (and more inducer). *Vibrio harveyi* has a different regulatory system involving two autoinducers and its genetic alphabet includes *luxL**, *M*, *N*, *Q*, *R**, *S* and *T* (where *luxL** and *luxR** are different in sequence and in function to *luxL* and *luxR* in *V. fischeri*).

Applications

Luciferase genes were cloned in the 1980s and the ease with which these and other genes can be transferred into a wide variety of organisms has provided an almost unparalleled range of tools for studying the regulation of intracellular processes and for following the fate of different bacteria under a wide variety of conditions. The use of these tools is continuously expanding and their applications as reporters of, for example, gene expression, quorum sensing, bacterial detection and cell viability are of huge commercial and research value.

Distribution

Culturable luminous marine bacteria occur in free-living, saprophytic, commensal, symbiotic and parasitic environments. Most species can be found in more than one habitat, though the species composition in seawater varies widely in different seasons and regions and is largely related to water temperature. *Photobacterium phosphoreum* grows at lower temperatures than other species and is more abundant in northern waters, in



TOP LEFT:
Fig. 2. The lure of the deep-sea anglerfish *Melanocetus johnsoni* contains unculturable luminous symbionts.
PHOTO P.J. HERRING

BOTTOM LEFT:
Fig. 3. The large light organ under the eye of the flashlight fish *Photoblepharon palpebratus* (inset: by its own light) contains luminous symbionts.
PHOTO P. J. HERRING

winter and in deep water. Thus in Californian waters *V. fischeri* is found throughout the year while *V. harveyi* predominates in summer and *P. phosphoreum* is occasionally present in winter. The first two species account for 99 % of the isolates and occur at densities of 1–5 per ml. In samples from the Puerto Rico Trench *P. phosphoreum* is dominant from 200 to 1,000 m (at 1–4 cells per ml) but samples from 4,000 to 7,000 m had <1 luminous cell per litre.

Are there truly free-living luminous marine bacteria? Possibly not. At best the notionally free-living ones may simply be en route between substrates. The oceans contain flocculent organic aggregates, known as marine snow (see article by Carol Turley on pp. 177–179), on which luminous bacteria abound (in Californian waters 63 % of the aggregates present at night at 60 m were luminous). Saprophytic colonies occur on the surface of fishes and in shell lesions on crustaceans. Lethal (parasitic) infections cause sandhoppers to glow. Commensal populations of *V. fischeri*,*V. harveyi* and *P. phosphoreum* are particularly abundant in the guts of shrimps and fishes, whose faeces contain viable luminous bacteria and often glow visibly, both while sinking and when collected in sediment traps. The deeper (and colder) the water the more likely *P. phosphoreum* will be the only species present. As the commonest habitat for many luminous bacteria appears to be among the gut flora of fishes, the value of bioluminescence to the bacterium may be to attract a fish to ingest a luminous particle. A single bacterium emits only some 10^3–10^4 photons per second and would be invisible to a fish or shrimp, but a population dense enough to trigger autoinduction will be bright enough to see. Glowing colonies on faeces or marine snow are the bacterium's way of getting from one nutritious gut to another.

The dilution effect of seawater should prevent autoinduction occurring in 'free-living' bacteria. Nevertheless, one rare phenomenon, 'Milky Sea', has been attributed to them. Seafarers describe it as like sailing over a sea of milk, with the sky dark and the entire sea surface to the horizon glowing steadily. It occurs most frequently in the Arabian Gulf during the southwest monsoon (July and August). The most likely explanation is luminous bacteria, growing on a surface scum caused by the decay of a 'bloom' of algae, common during this period. The one scientific study within a Milky Sea did indeed find both *V. harveyi* and glowing algae, but the apparent densities of the bacteria from the seawater were too low for autoinduction to occur and several orders of magnitude lower than that necessary for the luminescence to be visible, so the bacterial hypothesis remains likely but unproven.

Symbiosis

Luminescence is an important attribute. Although light energy accounts for <0·01 % of the bacterial cell's total expenditure, >5 % of the bacterial protein may be dedicated to luminescence. The main ecological role for luminous bacteria is probably as light sources for others. Many marine organisms, from dinoflagellates to fishes, are bioluminescent. Most species have their own luciferin/luciferase systems, but some fishes and squids contract out the job of light production to luminous bacteria, culturing them as extracellular symbionts in special light organs. This splendid arrangement nevertheless does have its potential drawbacks. The host needs (1) to maintain the bacteria in good condition (or the light goes out) and to get rid of dead or surplus ones, (2) to restrict the bacteria to the desired site and not let them spread throughout the body, (3) to be able to switch the light on and off at will (induced bacteria glow continuously) and (4) to ensure that the right symbionts are passed to the next generation.

None of this is easy – nor is finding out how they do it. There are about a dozen groups of fishes that employ luminous symbionts, both in the deep sea (e.g. spookfishes, anglerfishes and rattails) and in shallow water (ponyfishes and flashlight fishes). Shallow water fishes and squid use *V. fischeri* or *Photobacterium leiognathi* as their symbionts while deep-sea fishes, not surprisingly, use *P. phosphoreum*. Anglerfishes and flashlight fishes contain bacteria that have not yet been cultured *in vitro* (Figs 2 and 3). The association is very specific – for each host species only one kind of bacterium will do – and populations in the light organs seem to be clonal. The light organs of most of the fishes using *V. fischeri*, *P. leiognathi* or *P. phosphoreum* develop as pouches from different parts of the gut. The symbiont simply has to be 'selected' by the light organ from the normal gut flora. Animals whose light organs do not open into the gut (anglerfishes, flashlight fishes, pinecone fishes and squids) have a more difficult task, but these light organs do have openings to the exterior through which colonization presumably takes place. As we cannot culture the anglerfish and flashlight fish

Further reading

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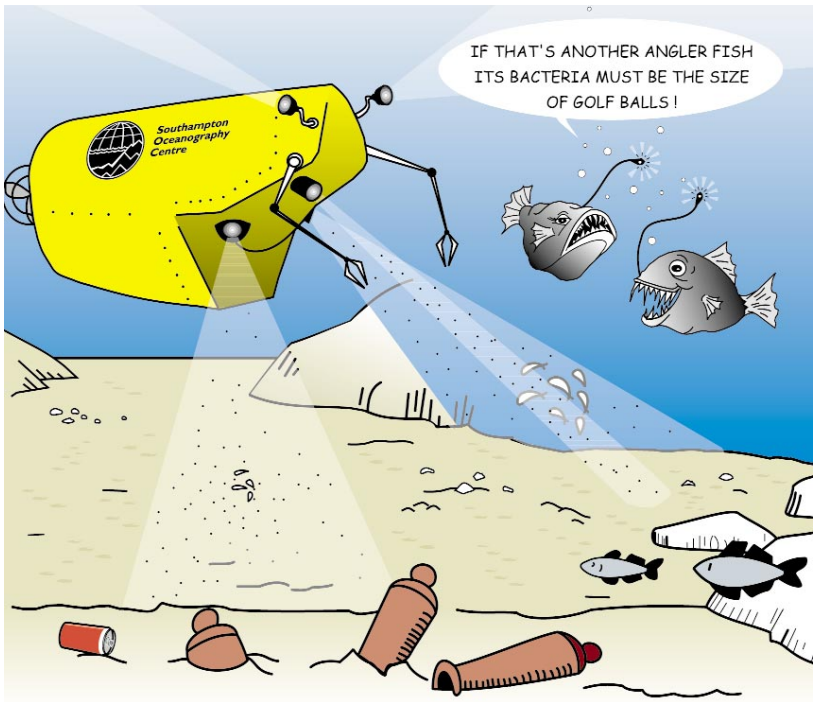
Meighen, E. (1994). Genetics of bacterial luminescence. *Annu Rev Genetics* **28**, 117–139.

McFall-Ngai, M. J. (2000). Negotiations between animals and bacteria: the 'diplomacy' of the squid–*Vibrio* symbiosis. *Comp Biochem Physiol A* **126**, 471–480.

Nealson, K. H. (1993). Bacterial bioluminescence: three decades of enlightenment. *Naval Res Rev* **45**, 13–20.

symbionts, we do not know how abundant they are in seawater. Genetic data suggest that they are related to *Vibrio* species and, remarkably, that each anglerfish species has a different symbiont. (Fig. 4)

The light organs of fishes and squids leak bacteria into the surrounding seawater, in a steady dribble in the case of the fishes (10^7–10^8 cells per hour from organs containing 10^8–5×10^9 bacteria at local densities of about 10^{11} cells per ml) and as a daily expulsion of 95 % of the bacteria in the squid. The doubling time of the bacteria in the light organ is much lower (8 hours to >5 days) than when cultured *in vitro*, but it is not certain how the host controls the growth rate. The leakage of symbionts both provides an environmental reservoir for reinfection of juveniles and offers scope for wider genetic exchange.



TOP RIGHT:
Fig. 4.
CARTOON KATE DAVIS, SOUTHAMPTON OCEANOGRAPHY CENTRE

The larval light organs of squid and ponyfishes acquire their bacteria only when exposed to water recently occupied by leaking adults. Both the squid *Euprymna scolopes* and its symbiont *V. fischeri* can be cultured separately and manipulations of the infection process have clarified how the right bacterium is recognized and acquired (Fig. 5). At the time of hatching the larval light organ contains no bacteria, but the local seawater contains about 500 *V. fischeri* cells per ml. Ciliated lobes on the light organ harvest these bacteria, which then swim into crypts within the organ. Here they grow very rapidly (doubling time 20 minutes) until the crypts are filled (totalling about 10^9 cells). *V. fischeri* is specifically recognized by the cells in the crypt, initiating local morphological changes and sending a signal to the ciliated lobes, which then wither irreversibly in a process of programmed cell death. The specificity of the association is enhanced by the role of *V. fischeri* luciferase in countering the oxidative stress in the crypt environment. Mutants that are deficient in the expression of luminescence are also defective colonizers.

BELOW:
Fig. 5. The little squid *Euprymna scolopes* cultures *Vibrio fischeri* in its two ventral light organs.
PHOTO M. MCFALL-NGAI

Insight into the very complex association between squid and bacterium has huge potential for interpreting the factors involved in the colonization of host tissues by pathogenic vibrios or other bacteria. Luminous marine bacteria are no longer just objects of curiosity but resources for a multitude of biomedical applications – as well as for their hosts.

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