Quorum Sensing: correlation in the bacterial world

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Abstract

We show that, in particular experimental conditions, the time course of the radiant fluxes, measured from a bioluminescent emission of a *Vibrio harveyi* related strain, collapse after suitable rescaling onto the *Gumbel distribution* of extreme value theory. We argue that the activation times of the strain luminous emission follow the universal behavior described by this statistical law, in spite of the fact that no extremal process is known to occur.

Introduction

Bioluminescence refers to the ability of some living organisms to transform chemical energy into visible light [1]. About 30 different bioluminescent systems are known to exist, all with their own peculiarities. Despite of this, bioluminescence could be briefly described as a light-emitting reaction involving molecular oxygen, occurring on a substrate (*luciferin*, in most cases) and catalyzed by an enzyme (*luciferase*). However, significant differences exist among the organisms subject to these reactions, as well as in the nature of luciferin and luciferase. Except for light emission, it seems that the sole common feature among the known bioluminescent systems is the requirement for molecular oxygen.

Bioluminescent bacteria are the most abundant and widely distributed light-emitting organisms [2,3]. They are found in seawater and terrestrial environments, both free-living and growing in dead fishes or animals and as symbionts with fishes or squids. In these latter cases, the function of light emission relates to the use of photogenic organs by the host, whereas bacteria receive nutrients from living in such environments, in a *do ut des* scheme. In these organisms, the light-emitting reaction involves a luciferase-catalyzed oxidation of *reduced flavin mononucleotide*, with the concomitant oxidation of a long chain aliphatic aldehyde. This leads to the emission of blue-green light from an electronically excited species.

Nowadays, bacterial bioluminescence has emerged as an extremely useful and versatile tracking technology to monitor stressful conditions. It provides a sensitive, non-destructive, and real-time assay that allows for temporal and spatial measurement [4]. For the sake of clarity, it should be noted that bacteria are known to emit light continuously, once this bio-activity has started [5].

It has been shown that in bacteria the bioluminescent systems are both inducible and subject to repression, depending mainly on the bacterial population density. As an example, bioluminescent bacteria growing

unconfined in seawater are generally non-luminous, although the same bacteria, cultured in vitro, emit light. This fact attracted a great scientific attention in the past and led to discover that some bacteria (luminous or not) sense their density and regulate gene expression (or repression) by intercellular chemical communication through some small molecules called autoinducers (AIs). In particular, bacteria detect AIs concentration which is known to increases as the cell population density increases. This fascinating mechanism is now generally called quorum sensing (QS) [6,7].

Now we know that both luminous and non-luminous bacteria sense population density and regulate, in a coordinate fashion, a diverse array of physiological activities that are presumably productive only when groups of cells act in concert. By sensing the presence and the level of the AIs, bioluminescent bacteria could so estimate their density and initiate costly processes as those involved in bioluminescence.

Among luminous organisms, *Vibrio harveyi* is a prototypical example both for bacterial bioluminescence and for QS. In effect, in *V. harveyi* light emission is regulated by QS in a rather complex way. This organism is known to produce and respond to three different AIs [8]: HAI-1, a species- specific signal; CAI-1, a genus-specific signal; AI-2, an interspecies signal. AI-2 confers to *V. harveyi* the ability to communicate with other completely different species. Generally, in natural habitats, *V. harveyi* exists in mixed populations containing other species of bacteria. The ability to recognize and to respond to multiple AIs could allow *V. harveyi* to monitor its own population density together with those of the other bacteria in its neighborhood. This fact could informs the *V. harveyi* when it represents the majority or the minority of a population and, consequently, could be a key information when taking a decision. Moreover, *V. harveyi* could respond differently to the three AIs, switching on or off a particular behavior when it exists alone or together with other species. In this work, we present the results obtained during a series of bioluminescence monitoring experiments on a vibrio strain of the Harveyi Clade.

Materials and methods

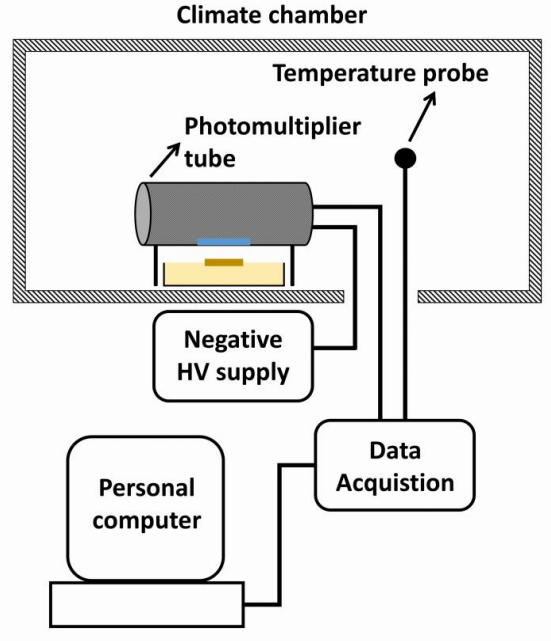


Figure 1. Sketch of the experimental apparatus.

The experiments have been performed using a V. harveyi-related strain (Vibrio sp. PS1) isolated from a marine hydrozoon [9]. The strain samples were cultured on nutrient broth (Difco) containing 3% NaCl at 20 °C to an optical density of 1.0 at 550 nm. Ten $\mu\ell$ of the suspension was spotted on the centre of 3% NaCl nutrient agar plates and incubated at 30 ± 1 °C inside a climate chamber (see Fig. 1) under nearly constant temperature and humidity conditions. Absolute darkness was operated inside the chamber. The experimental setup contained a photomultiplier tube (PMT) Hamamatsu 1P28 able to record the low light emitted by our samples. Its gain factor was 9×10^5, while the nominal PMT spectral sensibility ranged from 185 to 650 nm. Its active window, that we utilized to pick up the light emitted from samples, has an height of 24 mm and a width

of 8 mm; moreover, the spot was positioned directly against the window at a distance of 35 mm. The photomultiplier signals were collected by a workstation interfaced to a personal computer used both as storage and for timing the measurements each 10 min (Δt). A channel of the workstation was utilized to record the temperature. It is worth noticing that we used Petri dishes with- out cover, in order to avoid any filtering effect from the composing plastic material.

The output current given by the PMT is directly proportional to the photons incident on its sensitive window per unit time. In particular, the values obtained from the PMT represent the radiant flux [10] of the colonies, i.e., the rate at which the radiant energy $E(t)$ is delivered over time by the electromagnetic radiation emitted from bacteria. Therefore, multiplying these values by Δt, we obtain an accurate estimation of the variation of the number of the emitted photons.

Results

We performed different measurements that, in the experimental conditions described above, gave consistent results, showing that the number of emitted photons, after an initial lag, had a sudden increase, reached a maximum and then fell down exponentially over the time. It is interesting to note that the data collected have a very good fit with the function that represents the probability density of the Gumbel distribution [11] (represented by a solid line in Fig. 2), given by

$$f(t) = \frac{A}{s}\exp\left[-\frac{t-m}{s} - \exp\left(-\frac{t-m}{s}\right)\right] \quad (1)$$

where *A* is a normalization constant, while *m* and *s* are, respectively, a position and a scale parameter related to the expected value (μ) and the variance (σ^2) by the relations: $\mu = m + \gamma s$ e $\sigma^2 = (\pi s)^2/6$ (with γ being the Euler-Mascheroni constant).

Relation (1) is known to represent one of the three distributions used in extreme-value theory. In particular, it describes the distribution of the largest values of a set of independent and identically distributed (iid) random variables each one characterized by a density function decaying faster than any power (exponential). Normalizing data and using the scaled variable $z = (t-m)/s$, relation (1) reduces to the parameter-free Gumbel distribution $f(z) = e^{-z-e^{-z}}$, with expected value γ and variance $\pi^2/6$, respectively.

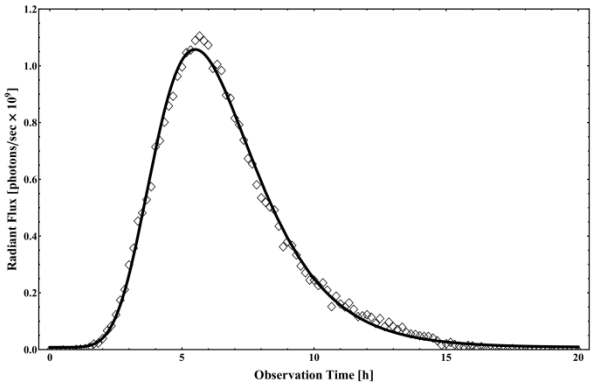


Figure 2. Typical result of the bioluminescence monitoring (points). The solid line represents the fit of the points with function (1).

In the last 15 years, extreme-value statistics and particularly Gumbel and Gumbel-like distributions attracted a growing interest [12,13]. It has been shown, in effect, that many naturally and laboratory occurring phenomena are distributed according to these statistical laws, although in many cases no underlying extremal process is known to take place. Without any claim of being exhaustive, Gumbel, Gumbel-like, and the other extreme-value distributions have been observed for example in turbulent flow [14-16], *1/f* noise [17], fluctuations of the Danube river level [18], resistance fluctuations near to electrical breakdown [19], fusion plasmas [20], granular gases [21], glassy systems [22], liquid crystals [23], galaxy distributions [24], and recently in protein fluctuations [25].

From a theoretical point of view, it has been understood that the emerging of such extreme-value distributions is often related to the presence of strong correlation among

the random variables composing the system under study [26,27]. In this context, the extreme-value statistics could be mapped into a problem of random sums, whose joint probability leads to non-Gaussian distributions. Recently, Bramwell [28] provided a simple rule based on general scaling arguments and specific to spatially averaged physical properties. His result, for example, teach us that whenever a global observable results from the sum of several variables, defined over a large system, if the mean value depends logarithmically on the system size, then the corresponding distribution should be a (generalized) Gumbel.

Coming back to the samples we used for our experiments, we should note that the light emitted by the colonies arises from the sum over many components of the light radiated by each bacteria. On the other hand, the QS mechanism implies the presence of correlation: bacteria use QS to act as a whole. These observations enable us to explain our experiments within the theoretical frameworks described above and to consider the datasets obtained as representing the distribution of the activation times of the light emission from bacteria. Since our experiments gave us datasets composed of times (t_i) and of the corresponding radiant flux (F_i) measured by the PMT, we used these data to compute the weighted mean (μ) and variance (σ^2) of the activation times for each dataset by means of the formulas

$$\mu = \frac{\sum_i F_i t_i}{\sum_i F_i} \tag{2}$$

$$\sigma^2 = \frac{\sum_i F_i (t_i - \mu)^2}{\sum_i F_i} \tag{3}$$

where the radiant fluxes F_i at time t_i, thus the number of photons per seconds incident on the PMT window, are taken as weights. In this manner, we were able to obtain the corresponding m and s parameters by inverting the relations shown before. Furthermore, normalizing and rescaling the experimental observations according to the z variable, we plotted the resulting data in Fig. 3, which also shows the parameter-free Gumbel distribution. We obtained similar results fitting the data with Eq. (1) and then using the fit parameters to normalize and rescale the dataset. Among the various observations sets, the data convincingly collapse to the parameter-free Gumbel distribution. We note, however, that data in Fig. 3 deviate from the solid line around the maximum. A similar behavior was found in a work by Antal and colleagues [17], where they observed that this should come from the finite size of the data samples under consideration.

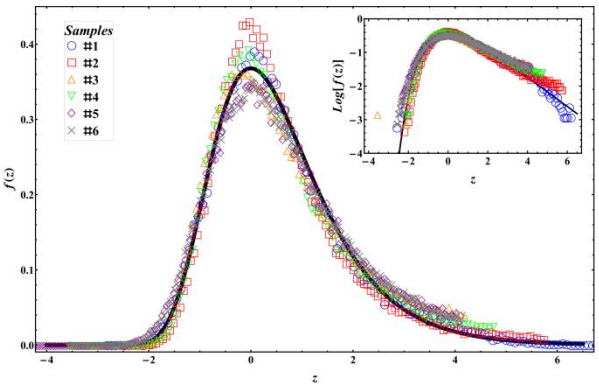


Figure 3. Rescaled experimental data (points) and Gumbel probability density function (solid line). In the inset, the same plot is in log-linear scales.

We performed also a preliminary investigation in order to evaluate the effect of the system size on the measurements. However, we did not clearly identify any particular dependence. Despite of this, let us briefly discuss the physical meaning of our experimental observations. We have already mentioned that the PMT give a measure of the rate of change of the total electromagnetic energy radiated over time by bacteria. Consequently, integrating from -1 to t, we obtain the energy $E(t)$ radiated by the whole system as a function of the time. The object that in the system under consideration would represent such a

quantity is clearly Gumbel's cumulative distribution function. At this point, we should note that this object is functionally identical to the Gompertz function used in microbiology to model the logarithm of the bacterial growth curve [29]

$$\log\left(\frac{N(t)}{N(0)}\right) = K \exp\left\{-\exp\left[e\,\frac{\mu_m}{K}(\lambda - t) + 1\right]\right\} \quad (4)$$

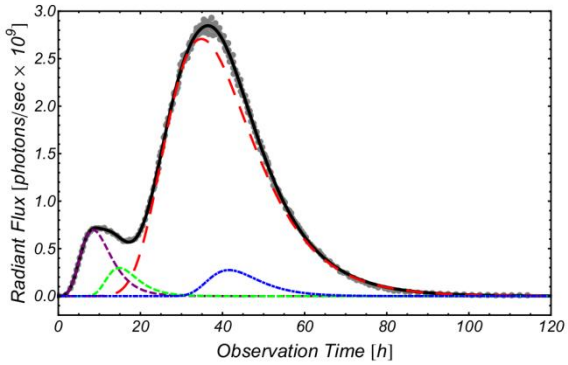
where $N(t)$ represents the number of organisms, while λ is the lag time, μ the maximum specific growth rate, and K the asymptote reached for $t \to +\infty$.

As previously pointed out, in bacteria the light emission is related to presence of the AIs, whose concentration increases with population density. Consequently, we could suppose that $E(t)$ is directly related to the bacterial growth curve,

$$E(t) \propto \log\left(\frac{N(t)}{N(0)}\right) \quad (5)$$

obtaining a dependence on system size as that described by Bramwell [28] for the occurrence of the Gumbel distribution.

It is worth pointing out that 4 of the total 12 datasets did not fit well with function (1). Nevertheless, we were able to obtain a good accordance between these datapoints and a superimposition of two Gumbel contributions. This should come from the occurrence of a second cluster of bacteria that reach the QS threshold with a different and unrelated kinetics with respect to the first. In our experience, such behaviors could be ascribed at least to three different possibilities. In the laboratory practice, the deposition of the bacteria spot could occasionally give rise to satellite colonies that grow independently. Consequently, they also reach the QS threshold differently from the main spot. Alternatively, in case of slightly wetter agar, swarming [30], a multicellular surface movement, could lead bacteria to reorganize continuously on the surface of the growth medium, showing a rich bioluminescence pattern (as shown in Figure 4). Another interesting option concerns a recent finding about AIs, according to which they can act also as biological timers, at least in V. harveyi [31]. The second contribution of Figure 3A could be then interpreted as the switching into action of an autoinducer, consistently with previous observations in different, although similar, experimental conditions [32]. In any case, it seems that the spatial distribution of bacteria plays an important role in the final outcome.



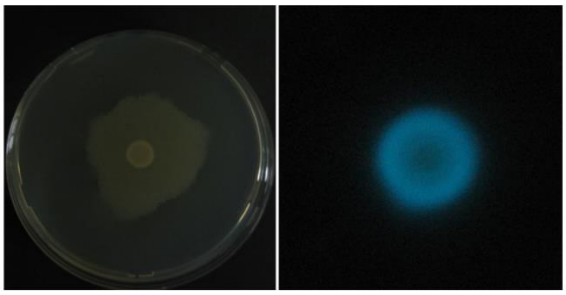


Figure 4. Example of a sample underwent swarming. The radiant flux (on the top) show a richer behavior, resulting from the superimposition of four different contribution, shown with different colors and dashing. The fit is in black solid line. On the bottom, two pictures of the sample surface after 36 hours of observation. Right image, in particular, is representative of the light radiated by the colony. It is important to note that a wetter agar lead also to bioluminescent emissions that last longer in time.

Discussion and conclusions

We showed that in the above-described experimental conditions, the activation

times of light emission in *V. harveyi* follow, after suitable normalization, the universal behavior corresponding to the Gumbel distribution, although no underlying extremal process is known to take place [33]. In particular, it seems that this behavior models the light emission of a single bacterial cluster. This finding may help to elucidate the complex inter-relationships between bacterial luminescence, QS, and growth rates. Due to the "physical" nature of the problem, we expect that in a near future QS could be fruitfully reinterpreted within the theoretical frameworks of critical phenomena on complex networks developed in statistical physics. Indeed, it seems reasonable to look at bacteria colonies as networks of (possibly) cooperating agents, obtaining insights that would be hidden while considering the single microbial entities. As QS regulates many aspects of microbial life in nature, including virulence, this would open the way to a deeper understanding of this fascinating phenomena together with its implications.

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