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Light emission miracle in the sea and preeminent applications of bioluminescence in recent new biotechnology

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ABSTRACT

Bioluminescence is referred to the light emission by a living organism due to a specific biochemical reaction. This interesting feature of the organisms could highly influences behavioral and ecosystem dynamics. Luminescence, mostly observed in marine species, is generally higher in deep-living genera than in benthic or shallow organisms. However, among creatures living in land, fireflies, beetles, springtails and fungi have shown some bioluminescent activities. Classically, the emission of light is catalyzed by luciferase from a substrate. Interestingly, light-emitting organisms are more abundant and widespread in marine than terrestrial environments. Novel tools derived from understanding bioluminescent reactions have led to countless valuable applications in modern biotechnology and biochemical engineering. Here, we overview some main properties bioluminescence in marine organism from bacteria to fishes following the latest advances and new discoveries of state-of-the-art bioluminescent tools in molecular biology, bioluminescent bioassays and imaging. The overview showed available and wide biotechnological tools of bioluminescence take advantage of its high detectability, high sensitive, low toxic and quantum efficiency which make wide usage as reporter of many biological functions in different fields, such as studying bacterial pathogens, ecotoxicology, food toxicity, tracking cells of interest in vivo, protein–protein interactions, gene expression and circadian rhythms. With the recent invention of luminescent reporters, future possibilities for the development of additional reporter applications are promising.

1. Introduction

Sparkling lights in the water at night or darkened sea, bioluminescence, natural chemical reaction when the organism emits visible light by itself, is a strange, interesting and natural phenomenon in sea. It is known as the primary source of light in deep sea. In the past several decades, marine bioluminescence has attracted growing interest among different fields including comparative biology [1], biochemistry [2], physiology [3], neuroscience [4], population dynamics [5], and naval applications [6]. Although the researches about bioluminescence began with terrestrial organisms (fire-flies) [7], but later expanded to marine organisms which were readily accessible such as bacteria [8,9], copepods [10], and cnidarians [11]. This is despite the fact that the deep sea is vast potential source of luminous organisms such as almost all major phyla of deep sea zooplankton [12,13]. Of one third of bioluminescent species (700 genera) [14,15] are found the majority of these genera (80%) in the marine habitat [16] particular deep sea where > 90% of organisms are luminous [12,17,18]. There were a variety of marine organisms in the deep sea such as crustaceans, cnidarians, cephalopods, echinoderms, annelids, dinoflagellates and bacteria [16]. In most of these cases, luminescence is produced by the organisms themselves and not by bacterial symbionts. High spectrum of luminescence is in deep-living and planktonic organisms and fewer in benthic or shallow species. While terrestrial fireflies are the most egregious examples of bioluminescence, but the ocean be considered as favorable source for the evolution of bioluminescence. It could be because of comparatively optically clear, stable environmental conditions, the existing of large portions in continuous darkness; and interactions between a varied diversity of taxa in sea. Bioluminescence is supposed to have appeared > 40 times in evolution [12].

Up to date, chemical pathways for the emission of light have been divided to two types in living organisms: 1. classical luciferin–luciferase system, with luciferin as substrate and the light emitting molecule which oxidized by a luciferase as enzyme, in the presence of oxygen and or sometimes cofactors [19,20]. 2. The luciferin, the enzymatic complex and the oxygen system as “pre-charged” compound named photopro-

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tein [21]. The binding with cofactor, such as Ca^{2+} or Mg^{2+} gives the organism a way to precisely control light emission. Light is generated as a result of energy released during a bioluminescent reaction. Moreover, the symbiotic system is another way to emit light as the host (some squids and fishes) [22]. At the moment, there were > 30 luminous systems [19] which various combinations of luciferins, enzymes and cofactors involve in these systems, and it is likely many more to be discovered. Luminous systems are produced in endogenous light-producing cells called photocytes [14] or in bacteria in symbiotic relation with a multicellular organism.

The evolving of bioluminescence during many times from bacteria to fish powerfully influences behavioral and ecosystem dynamics [12]. Haddock et al. (2010) concluded bioluminescence have independently appeared > 40 times in during of evolution. The different functions bioluminescence including defense and reproduction is utilized by marine organisms. Indeed, bioluminescence is clear form of communication in the sea. It can be vertical migration, predator-prey interactions, and the flow of material in the food web [12].

High detectability and rapidity of bioluminescence spectroscopy has made recent developments in molecular biology. These properties have made it suitable for monitoring of biological processes together clinical, diagnostic and drug discovery applications [23]. Because of unique properties, bioluminescence can be used as ultrasensitive and selective bioanalytical tools [23]. Indeed, ultrasensitive binding assays and cell-based assays can be made through bioluminescent proteins [24]. In addition to, the role bioluminescence for environmental assessment should not be ignored. Recombinant luminescent bacteria have been used over the past 20 years for several bioassays because of their specific for the detection of metals and metalloids [25]. The using of bioluminescence imaging (BLI) of bacteria has permitted real-time, sensitive, and noninvasive monitoring of the progression of infection in live animals [26]. The detection of a specific analyte through genetically engineered bacterial whole-cell biosensors is another powerful tool of bioluminescence in biotechnology [27].

While marine organisms are potential source of marine luciferases, the majority used and studied bioluminescent systems are derived from terrestrial organisms and bacteria. For the first time, this review has focused on advancements in understanding luciferase properties derived from different marine organisms instead of focusing on ecology, the natural history of and bioluminescence distribution in the ocean. In this review we summarized briefly current knowledge about properties of light emission by marine organism and finally, possible applications of luminescent marine organisms in aquatic biotechnology were discussed.

2. The Characterization of Bioluminescence in Different Marine Organisms

Bioluminescent organisms occur in a wide variety of marine habitats from oceanic surface waters to abyssal depths. Bioluminescent taxa have been reported from most branches, bacteria to vertebrates. However, some branches such as crustaceans, coleopterans, cnidarians, and teleosts are species-rich. Here we over review some important properties of bioluminescence in different marine organism from marine bacteria to vertebrate. However, there were exceptions among of a few organisms which luminescence may be hard to confirm. Filter-feeding organisms and sponges may be isolated some compounds with bacterial origin [28,29].

3. The Bioluminescence in Marine Bacteria

In summary, bacterial luciferase as a flavin-dependent monooxygenase catalyzes a light-emitting reaction by using substrates including reduced flavin, long chain aldehyde, and oxygen and produces oxidized flavin, carboxylic acid, and water as products with concomitant emission of bluegreen light around 485–490 nm (Eq. (1)). In general, bacterial luciferase is a heterodimer consisting of two homologous subunits, the α- and β-subunits. The α-subunit is as reactive reaction center, whereas the β-subunit is responsible for the active conformation of the α-subunit.

$$FMNH_2 + RCHO + O2 \rightarrow FMN + RCOOH + H_2O + hv \quad (1)$$

Bacterial luciferase is responsible for quantum yield approximately 10–16% [19,30]. Gram-negative bacteria are found as potential and vast sources of these enzymes [31]. Moreover, most prior reports include from well-studied two genera, *Vibrio* and *Photobacterium* from marine environments [32–34].

4. Structure of Marine Bacterial Luciferase

In general, bacterial luciferase as a heterodimeric enzyme composes asymmetric subunits α and β encoded by the luxA and luxB genes, respectively. The average of native molecular masses of α- and β-subunits were estimated (40–42 kDa) and (36–37.5 kDa), respectively. The extracted luciferaes from *Vibrio harveyi* and *Vibrio campbellii* exhibit slow light decay and sequence identities as high as 84–90%. However, the enzymes from *Photobacterium* sp. show only about 60% shared identity among these enzymes and have fast light decay [32,34,35].

Bacterial bioluminescence has been reported as a continuous glow than discrete flashes in other organisms [36]. This property along with its sensitivity and convenience make its vast potential for various fields of environmental toxin monitoring, drug screening, and in vivo imaging. Flavin mononucleotide (FMN) (light-emitting products), and 4a-hydroxy-5-hydro Flavin mononucleotide (HFOH), (an intermediate of the chemical reactions) has been shown to having similar molecular structures and fluorescence wavelengths as candidates for the light emitter [36]. In reports from Luo and Liu [36], it was confirmed HFOH in its first singlet excited state is the bioluminophore of bacterial bioluminescence, because its exhibiting strong bioluminescence in the bacterial luciferase opposite quenched fluorescence of FMN when its bounding to the luciferase. Luo and Liu [36] stated its reason to the structure of the luciferase so that, Tyr110 of luciferase quenches the FMN fluorescence via an electron-transfer mechanism.

The review of Table 1 showed essential element is flavin mononucleotide almost in all of marine bacteria for bioluminescence and its mount can influence reaction. It was shown the role of saturated flavin mononucleotide beside of aliphatic aldehydes with 6–18 total carbons which are active substrates for bacterial luminescence reaction [37]. Aldehyde substrate can markedly affect the emission intensity and kinetics of luminescence reaction through its chain length. In most aldehyde substrates, bioluminescence quantum yields do not change as much as the intensities [37]. Luciferase of bacteria is sensitive to different limiting factors including diphosphopyridine nucleotide (DPN), high concentration of various salts in *Achromobacter fischeri* [38], and cyanide, versene and riboflavin in *Achromobacter fischeri* [39]. The inhibition of bacterial luciferase with versene as well as cyanide indicates the possibility of involving metal in the luminescent reaction. Moreover, the adding of bacterial luciferin can decrease the degree of cyanide inhibition, suggesting the possibility of luciferin as a metal containing compound. The metals such as copper, zinc, and mercury exhibit inhibition effect in *Photobacterium* sp. [40], and bacterium *Beneckea harveyi* showed the sensitivity to trypsin or chymotrypsin [41]. In bacterium *Beneckea harveyi*, the loss of subunit α in luciferase by trypsin and chymotrypsin is accompanied by the appearance of a new species γ. It was shown that the principal proteolytic fragment of α, designated γ, is so nearly the same size for trypsin and chymotrypsin. Moreover, it was implied that there is a protease-susceptible region in the luciferase of *Beneckea harveyi* [41]. The compounds such as naphthalene and phenanthrene were limiting factors for the luciferase of *Vibrio fischeri* [42]. Moreover, chloramphenicol and cyanide plus fluoride had inhibition effect on *Vibrio harveyi* [43]. It was showed the addition of chloramphenicol can stabilized the activity of the luciferase