Indian Journal of Geo Marine Sciences
Vol. 51 (01), January 2022, pp. 26-32

Bioluminescent bacterial flora of Nigerian coastal fish species

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Received 24 August 2020; revised 06 January 2022

The scarcity of studies on bioluminescent bacteria in fish species along the coastal and marine environment of Nigeria prompted the assessment of abundance of bioluminescent bacteria and possible isolation on three common coastal fish species (*Drepane africana*, *Sardinella maderensis* and *Mugil cephalus*) displaying luminescence. The population of the luminescent bacteria among the three fish species ranged from 2.95×10^5 to 6.68×10^6 cfu ml^{-1} and the corresponding percentage to the heterotrophic bacteria was between 0.01 and 0.34 %. The surface water had 2.00×10^5 cfu ml^{-1} with 0.20 % ratio to heterotrophic bacteria. The predominant luminescent bacteria observed were gram-negative short rod. Further study of these bacteria showed that growth and light emission occurred concurrently without the exhibition of lag phase; however, maximum luminescence was observed at mid-exponential growth phase suggesting quorum sensing phenomenon. The 16S rRNA gene sequence showed 92.81 % homology to *Vibrio campbelli*, *Vibrio harveyi* and *Vibrio rotiferianus*. This study highlights the occurrence of bioluminescent bacteria in skin of tropical coastal fish species in Nigerian coastal waters and also serves as a fundamental basis for future studies on the biotechnological application of this unique characteristics.

[**Keywords**: Bacteria, Bioluminescence, Fish, Lagos coastal water, *Vibrio* spp.]

Introduction

Bioluminescence is a chemical and enzymatic reaction whereby a conspicuous visible light is emitted by living organisms. This natural phenomenon has been observed in wide diverse marine organisms including prokaryotes, eukaryotes, invertebrates, insects, fishes and plants. However, bacteria are the most abundant luminous organisms in the marine environment. These luminous bacteria exist in symbiotic relationship with higher marine organisms mostly in fish and squids, as saprophytes on organic matters, as parasites as well as free-living organisms[1-6].

Most of the bioluminescent bacteria belong to the class Gammaproteobacteria, and are classified into four main genera namely *Vibrio*, *Photobacterium*, *Aliivibrio* and *Photorhabdus*. Apart from *Photorhabdus* which exists in terrestrial habitat, the others are usually found in marine environment[3,5,7,8]. The oxidation reaction that results in the emission of light by these bacteria involves a long chain aliphatic aldehyde and a reduced riboflavin mononucleotide ($FMNH_2$) catalyzed by an enzyme known as luciferase[9-11]. The excess energy is liberated and emitted as visible light at 490 nm. Light production depends on the reducing power of the organism; therefore, this reaction has been attributed to metabolically active cells[9-11].

Bacterial luminescence is controlled by a set of genes known as *lux* operon (*lux*CDABEG). In most studied species, the expression of the *lux* operon genes is regulated by quorum sensing, a phenomenon involving the production of hormone-like chemicals called autoinducers which switch on and off the production of light by the organisms based on cell population density[9,11-15]. However, light production in a few species has been observed to differ from this popular phenomenon[16].

The attractiveness of this visible light emitted by the luminescent organisms has drawn scientists attention to study this phenomenon over the years[17,18]. Despite the vast study of bioluminescent bacteria for decades around the world, little is known about their occurrence and characteristics around the Nigerian coastal and marine environment. Although, Adoki & Odokuma[19] have studied bioluminescent hydrocarbonclastic bacteria of the Niger Delta, the dearth of data necessitates further research to provide adequate information. Hence, the aim of this study is to assess the abundance of this group of fascinating bacteria

from the native bioluminescent coastal fishes. The study attempts isolation and characterization of pure culture and explore their physiological and growth requirements in view for future biotechnological applications.

Materials and Methods

The three different species of marine/brackish water fishes (*Drepane africana*, *Sardinella maderensis* and *Mugil cephalus*), were sampled along Lagos, Oniru Beach, Victoria Island Lagos [6°27'55.5192' N and 3°24'23.2128' E]. Each of the fish samples was transferred directly from the fishermen's net into a separate sterile containers, labelled accordingly and were transported immediately to the microbiology laboratory of the Department of Biological Oceanography, Nigerian Institute for Oceanography and Marine research (NIOMR), Lagos.

Each of the fishes was immersed in a beaker containing 50 ml of sterile 3 % NaCl solution. Fishes was held in place with the help of a sterile forceps and the entire skin surface was gently scraped and rinsed in the NaCl solution. The resultant solution served as stock solution. The stock solution was serially diluted for subsequent inoculation on the different growth media.

Aliquots (0.1 ml) of appropriate dilutions of the water sample and fish skin stock solution were inoculated onto specific agar plates for isolation and enumeration of the different groups of bacteria. Nutrient Agar (Oxoid) and Luminous medium (30 g of NaCl; 0.88 ml of glycerol; 10 g of Bacteriological peptone and 15 g of Agar in 1 L distilled water) was used for culturing of viable heterotrophic bacteria and luminescent bacterial species, respectively. The plates were incubated at 27±2 °C for 18 to 24 h. Bacterial colonies were counted using Lapiz digital colony counter and expressed in colony forming units per ml (cfu ml^{-1}) for each fish stock solution. Bioluminescent colonies were randomly selected, further purified and stored on Boss medium slants for further analysis. The morphology of the isolates was determined by gram staining followed by viewing under the microscope. Biochemical assays such as: Oxidase, Simmons citrate, Methyl red, Voges-Proskauer test and triple sugar utilization were also carried out to establish some physiological characteristics.

Cells of pure culture of the selected isolate in Luminous broth were harvested after 24 h by centrifugation at 4000 rpm for 7 min, washed with phosphate buffer and re-suspended in phosphate buffer solution up to optical density of 1 unit. Aliquots of this bacterial suspension were subsequently used to inoculate replicate tubes of Luminous broth (5 ml) which was used to monitor the bacterial growth and luminescence production at 6 hourly intervals (0, 6, 12, 18, 24, 30, 36, and 48 h). Growth was estimated by measuring the optical density of the cultures using a spectrophotometer (Lamotte Maryland, USA) at a wavelength of 600 nm; while the intensity of light production was measured in Relative Light Unit (RLU) using a luminometer (Kikkoman Lumitester PD-20).

The bacterial suspension used to assess the effect of glycerol and NaCl concentrations on growth and luminescence production of the selected bacteria was prepared as described above. However, aliquots were used to inoculate replicate tubes of luminous broth (5 ml) with varying concentrations of glycerol (0, 1, 2, 3, 4 and 5 %) and NaCl (2, 3 and 4 %). Extent of growth and light production were measured after 24 hours.

Molecular identification was done by 16S rRNA gene sequencing. The genomic DNA was extracted from 24 h pure culture following manufacturer's instructions on the Qiagen DNA Extraction Kit. The extracted DNA was stored at -20 °C and later was sent to Inquaba Biotechnical Industries Ltd, Pretoria, South Africa for sequencing. There the 16S ribosomal unit was amplified by Polymerase Chain Reaction (PCR) using universal primers for bacteria: forward primer 5'–3' (27F AGAGTTTGATCMTGGCTCAG) and reverse primer 5'–3' (1492R AGAGTT TGATCMTGGCTCAG). The integrity of the PCR amplicons were visualized on a 1 % agarose gel (CSL-AG500, Cleaver Scientific Ltd) stained with EZ-vision® Bluelight DNA Dye. The bacterial nucleotide sequence was determined by Sanger sequencing using Applied Biosystems ABI 3500XL Genetic Analyser, and Sequence chromatogram analysis was performed using FinchTV analysis software. The DNA sequence obtained was further used to identify the isolate by comparing with other rRNA in public database. Subsequently, this partial 16S rRNA was submitted to the GenBank and accession number (MT512030) was obtained. The phylogenetic relationship of this strain with other bioluminescent bacteria based on the partial 16S rRNA was established by constructing a phylogenetic dendogram using Mega-X software.

Graphpad Prism software 5.03 (San Diego, CA. USA) was used for data analysis and graphical representation.

Table 1 — Mean±SD of total viable heterotrophic and luminescent bacteria of three coastal fishes and water sample

Samples	Total heterotrophic bacteria count (cfu ml^{-1})	Total luminescent bacteria count (cfu ml^{-1})	Percentage of luminescent bacteria (%)
Drepane africana	$4.83\times10^{8} \pm 6.52$	$1.65\times10^{6} \pm 1.90$	0.34
Sardinella maderensis	$2.85\times10^{9} \pm 4.89$	$2.95\times10^{5} \pm 2.89$	0.01
Mugil cephalus	$8.76\times10^{9} \pm 1.48$	$6.68\times10^{6} \pm 1.15$	0.08
Water	$9.86\times10^{7} \pm 9.45$	$2.00\times10^{5} \pm 0.0$	0.20

Results

Luminescent bacteria were present both in the water sample and on the skin of all three species of fish sampled in this study (Table 1). The highest number for both the heterotrophic and bioluminescent bacteria was observed in *Mugil cephalus* and the corresponding ratio of the luminescent bacteria to the total heterotrophic bacteria was 0.08 %. *Sardinella maderensis* also showed an elevated count (2.85×10^{9} cfu ml^{-1}) of total heterotrophic bacteria and a total count of $2.95x10^{5}$ cfu ml^{-1} for luminescent bacteria with a resultant ratio of 0.01 %. On the other hand, the highest ratio (0.34 %) of luminescent bacteria was observed in *Drepane africana.* Although it exhibited the least count (4.83×10^{8} cfu ml^{-1}) of heterotrophic bacterial count among the three fish species, the luminescent bacteria total viable count of $1.65x10^{6}$ cfu ml^{-1} was reasonably high. It is noteworthy that although the water sample had the less count of 9.86×10^{7} cfu ml^{-1} and 2.00×10^{5} cfu ml^{-1} for heterotrophic bacteria and luminescent bacteria, respectively the corresponding ratio was 0.2 %.

The selected isolate (ISO A) was a short gram-negative rod. It ferments glucose, fructose, lactose and sucrose but does not utilize citrate. It produced a positive Methyl red test and was negative for the Voges-Proskauer test. The growth curve of this bioluminescent bacterial strain showed steady increase in optical density from 0 h until 24 h (Fig. 1), before it obviously entered the stationary growth phase. Initially, a gradual increase was noted after 6 h as the optical density increased from 0.037 to 0.060 with a corresponding growth rate of 0.081. The maximum rate of growth (0.522 h^{-1}) was observed between 6 h and 12 h as the optical density increased to 1.375. Although the optical density continued to increase up to 1.977 at 24 h, when the peak of growth was exhibited, the growth rate was lower (0.038 and 0.022 h^{-1}). The optical density remained between 1.980 and 1.999 from 30 h till the end of the study at 48 h. The production of light occurred during growth of the bacteria. An average luminescence of 63 RLU (Relative Light Units) was observed after 6 h of culture. A sharp escalation of the luminescence (2344 RLU) was occurred at 12 h, which corresponded with the bacterial growth. Nevertheless, luminescence gradually declined to 2222 and 2084 RLU at 18 and 24 h, respectively, although the bacterial growth was still going on. Further, the luminescence drastically reduced to 742 RLU at 30 h, and was as low as 325 RLU by the end of the experiment at 48 h.

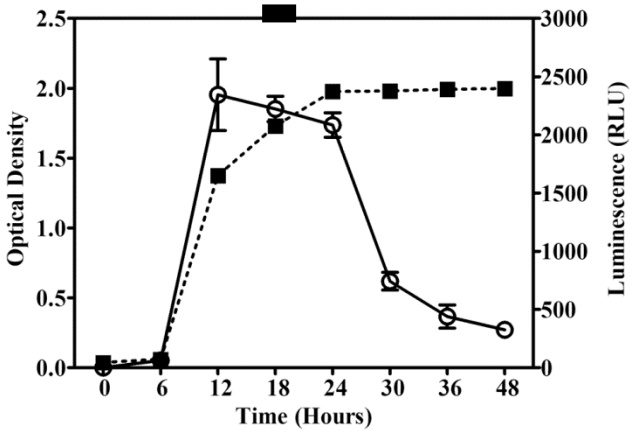

Fig. 1 — Extent of growth ○ and luminescence production of the bacteria isolate (MT512030) during 0 to 48 hour culture period

The effect of concentration of the carbon source (glycerol) and Sodium Chloride (NaCl) on growth and luminescence production was observed after 24 h of culture (Fig. 2a). The highest cell density (2.024) and luminescence (634.3 RLU) was observed in the 1 % glycerol culture. Comparable growth was also occurred at 2 and 0 % glycerol concentrations (1.880 and 1.63, respectively), but the least luminescence production was noted at the culture with 2 % glycerol concentration. Nevertheless, growth and luminescence production occurred in the cultures with higher glycerol concentration (3 and 4 %). The extent of growth and luminescence noted for the three different concentration of NaCl (Fig. 2b) displayed maximum growth performance at 4 % NaCl concentration but optimum luminescence at 3 % NaCl concentration. The least values for both cell density and luminescence production were noted for the lowest (2 %) concentration.

Molecular identification using the Megablast of the sequenced 16S rRNA of the isolate (MT512030) from this study showed that ISO A had 92.81 % similarity to *Vibrio campbelli*, *Vibrio harveyi* and *Vibrio rotiferianus*. The phylogenetic tree with other bioluminescent bacteria from GenBank demonstrated closer relationship with the *Vibrio* species (Fig. 3).

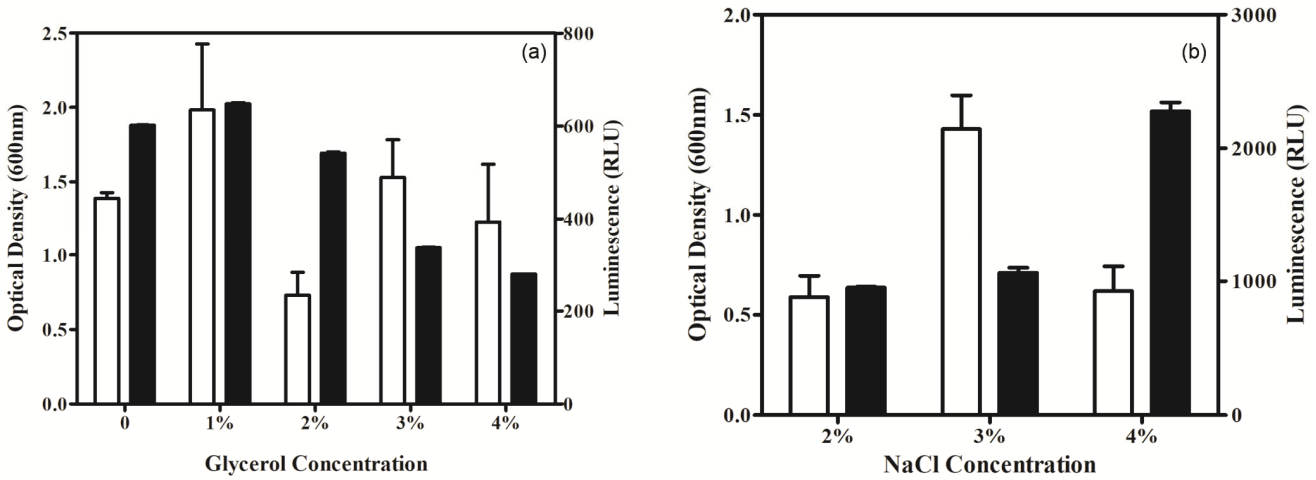

Fig. 2 — The extent of growth ■ and luminescent production of □ the bacteria isolate at different concentrations of glycerol {A} and NaCl {B} concentrations

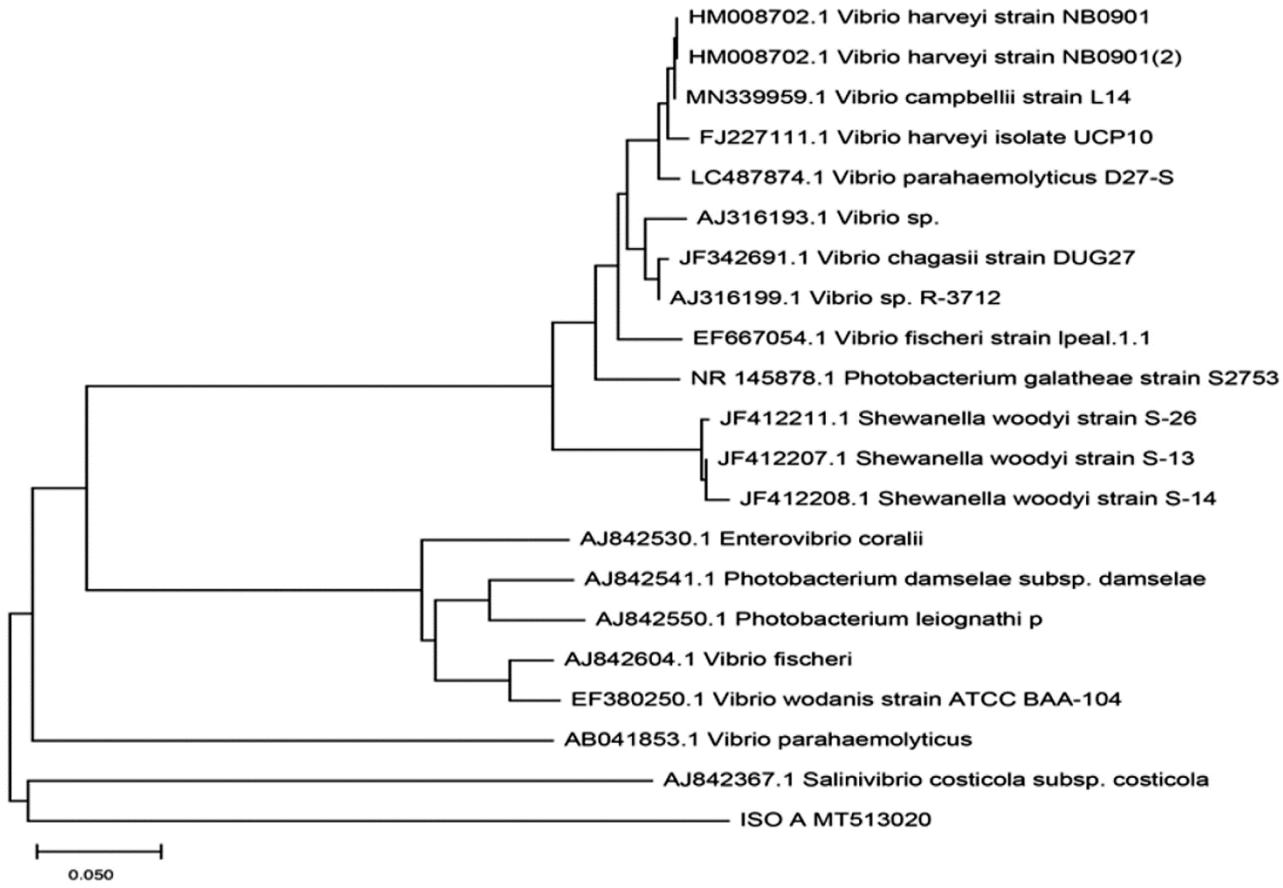

Fig. 3 — Phylogenetic tree comparing the 16S rRNA sequence from the bioluminescent bacteria (MT513020) in this study with other bioluminescent bacteria gene from GenBank

Discussion

The ubiquity of bioluminescent bacteria in the marine environment particularly their association with higher marine animals has been of great interest to scientists around the world. In the present study, the bioluminescent bacteria were found in both the bacterial flora of the fish skin and in the surface water sample (Table 1). This confirmed their ubiquitous nature in marine environment[17,18,20-24]. The population of both the viable total heterotrophic and the luminescent bacteria differed among the three fish species tested with highest number in *Mugil cephalus*. This could be attributed to the fact that types of nutrients consumed by fishes affect the distribution of bioluminescent bacteria[23,24]. Besides, Ramesh & Mohanraju[25] noted that the distribution of bioluminescent organisms which expel these luminous bacteria into the surrounding environment also determines their abundance in a particular area.

The range (%) of the ratio of the luminescent bacteria against the total heterotrophs observed in this study for both the fish skin and water samples was lower than the range reported earlier for temperate and tropical marine waters and fishes[26,20] even in the Niger Delta area of Nigeria[19]. The study of Ramesh & Venugopalan[25] had earlier demonstrated seasonal variation of abundance of luminous bacteria, hence the period of this study possibly have affected the occurrence of luminescent bacteria. Future study will endeavor to assess this population over a period of time for better understanding of seasonal occurrence. It had been known that some species of luminous bacteria are not culturable because they are totally dependent on their fish host for their growth and reproductive needs[27,10]. The successful culture of the bioluminescent bacteria in this study, suggest that they are not completely dependent on the fish for survival and also corroborates the observation of Urbanczyk *et al.*[7], that luminous bacteria usually occur extra-cellularly and are not strictly dependent on their host for reproduction. It was also not surprising that diverse species of the luminescent bacteria were not encountered among the three species of fish tested because it had earlier been noted that specific fish families usually harbor specific single species of bacteria[28].

Light emission by luminous bacteria has always been attributed to be dependent on cell density invariably demonstrating quorum sensing phenomenon, which means that light is only emitted when the cell density reaches a certain concentration[10,13,14,28-32]. In the present study, the simultaneous measurement of luminescence and cell density did not reveal any lag phase as luminescence was noted even at a very low cell density (Fig. 1). However, the sharp increase in bioluminescence by mid-exponential growth displayed by this bacterial species could be associated with quorum sensing. Recently, Tanet *et al.*[15] observed the absence of lag time between light emission and the growth of *Photobacterium leiognathi* and they suggested that all bioluminescence is not controlled by the quorum sensing. This was further buttressed by the fact that a steady and gradual increase of luminescence with cell density is inconsistent with the quorum sensing[33,34]. The cell density value (1.37) at which the here studied isolate exhibited the maximum luminescence was considerably higher than the range of cell density value (0.6 to 0.8) reported by Grim *et al.*[30].

Salinity is one of the major factors that affect the growth and general performance of bacteria; particularly marine microbes due to the high salinity level of their environments are usually affected by level of salts such as NaCl in growth media[22,24]. Hence, the *in-vitro* effect of NaCl concentration alongside glycerol (Carbon source) on the growth and bioluminescent production of the luminous isolate was performed in this study which showed good performance at 3 to 4 % NaCl concentration and 0 to 2 % glycerol concentration. This was similar to optimum performance at 3 to 6 % NaCl concentration and 0.3 to 0.5 % glycerol concentration displayed by the *Vibrio campbellii* (strain STF1) as reported by Ramesh *et al.*[35]. They noted that NaCl was highly essential for the growth of bacterial species and hence the growth was not observed without NaCl. On the other hand, they showed that glycerol was not necessarily needed for growth and luminescence production although minute concentrations were required for best growth and luminescence production. This was also consistent with the observation made in this study as reasonable growth and luminescence were noted at 0 % glycerol concentration. Further, it's noteworthy that Parmar *et al.*[24] also reported maximum luminescence at 3 and 6 % NaCl concentrations. The high similarity of the bioluminescent bacteria from this study with *Vibrio* species was expected since luminous bacteria usually belong to *Vibrio*, *Photobacterium*, *Aliivibrio*, *Photorhabdus* or *Shewanella*[5,6,10]. The phylogenetic

comparison with other luminous bacteria from GenBank (Fig. 3), also showed closer relationship to *Vibrio* than the other genera.

Conclusion

The results of this study demonstrate the occurrence of bioluminescent bacteria in Nigerian coastal waters and in skin of coastal fishes. Moreover, the 16S RNA sequencing of the bacterial isolate resembles with the *Vibrio* species which are known for their bioluminescence property. Hence, this environment can be explored for the interesting phenomenon of luminescence in bacteria which has great biotechnological potentials as the bioluminescent bacteria were found abundantly in the skin of fish species and in the water samples. The pattern and extent of luminescence production showed by the bacterial species in Nigerian coastal waters is encouraging for further research with reference to the biotechnological applications of bioluminescent bacteria. Further studies on molecular base will be carried out to ascertain the presence of *lux operon* genes as well as to reveal specific features of these luminous bacteria.

Acknowledgments

Authors are grateful to the entire management of Nigeria Institute for Oceanography and Marine Research, Victoria Island Lagos for granting us the permission to carry out this research in the Institute. Special thanks also go to the Director of Biological Oceanography Dr. A. S. Yakub for his support and encouragement.

Conflict of Interest

There is no conflict of interest.

Author Contributions

OAA: Conceptualization, supervision, financial contribution, data analysis, manuscript review; and OJA: Sample collection, laboratory analysis, financial contribution, data collation and manuscript draft writing.

References

1 Czyz A, Wrobel B & Wegrzyn G, *Vibrio harveyi* bioluminescence plays a role in stimulation of DNA repair, *Microbiology*, 146 (2000) 283–288.

2 Widder E A, Bioluminescence in the Ocean: Origins of Biological, Chemical and Ecological Diversity, *Science*, 328 (2010) 704–708. https://doi.org/10.1126/science.1174269

3 Prakash V, Rajpurohit D & Suneetha V, Bioluminiscent bacteria as future torch lights-Screening, Characterisation, *Int J Sci Eng Res*, 5 (2014) 949–951. https://doi.org/10.14299/ijser.2014.08.002

4 Martini S & Haddock S H D, Quantification of bioluminescence from the surface to the deep sea demonstrates its predominance as an ecological trait, *Sci Rep*, 7 (2017) 1–11. https://doi.org/10.1038/srep45750

5 Brodl E, Winkler A & Macheroux P, Molecular Mechanisms of Bacterial Bioluminescence, *Comput Struct Biotechnol J*, 16 (2018) 551–564. https://doi.org/10.1016/j.csbj.2018.11.003

6 Yang J, Hu S, Liao A, Weng Y, Liang S, *et al.*, Preparation of freeze-dried bioluminescent bacteria and their application in the detection of acute toxicity of bisphenol A and heavy metals, *Food Sci Nutr*, 00 (2022) 1–13. https://doi.org/10.1002/fsn3.2800

7 Urbanczyk H, Ast J C & Dunlap P V, Phylogeny, genomics, and symbiosis of *Photobacterium*, *FEMS Microbiol Rev*, 35 (2011) 324–342. https://doi.org/10.1111/j.1574-6976.2010.00250.x

8 Yaser N A, Faiz M, Abdullah F, Aris A M & Zainudin I I, Isolation And Identification Of Bioluminescent Bacteria In Squid And Water Of Malaysia, *Int J Adv Agric Environ Eng*, 1 (2014) 225–228.

9 Malave-Orengo J, Rubio-Marrero E N & Rios-Velazquez C, Isolation and characterization of bioluminescent bacteria from marine environments of Puerto Rico, *Curr Res Technol Educ Top Appl Microbiol Microb Biotechnol*, 1 (2) (2010) 103–108.

10 Dunlap P, Biochemistry and Genetics of Bacterial Bioluminescence, In: *Bioluminescence: Fundamentals and Applications in Biotechnology*, edited by Thouand G & Marks R, (Springer-Verlag Berlin Heidelberg), 2014, pp. 37-64. https://doi.org/10.1007/978-3-662-43385-0

11 Nunes-halldorson V S & Duran N L, Bioluminescent Bacteria: Lux Genes as Environmental Biosensors, *Brazilian J Microbiol*, 34 (2003) 91–96.

12 Badar U, Shoeb E, Daredia K, Akhtar J & Ansari M A, Screening and Characterization of Luminescent Bacterial Strain, *J Basic Appl Sci*, 8 (2012) 602–606.

13 Liu H, Srinivas S, He X, Gong G, Dai C, *et al*., Quorum Sensing in Vibrio and its Relevance to Bacterial Virulence, *Bacteriol Parasitol*, 4 (3) (2013) 172-177. https://doi.org/10.4172/2155-9597.1000172

14 Yaser N A, Faiz M, Abdullah F, Aris A M & Zainudin I I, Isolation And Identification Of Bioluminescent Bacteria In Squid And Water Of Malaysia, *Int J Adv Agric Environ Engg*, 1 (2014) 225–228.

15 Fleiss A & Sarkisyan K S, A brief review of bioluminescent systems, *Curr Genet*, 65 (2019) 877–882. https://doi.org/10.1007/s00294-019-00951-5

16 Tanet L, Tamburini C, Baumas C, Garel M, Simon G, *et al*., Bioluminescence: Light Emission in Photobacterium phosphoreum Is Not Under Quorum-Sensing Control, *Front Microbiol*, 10 (2019) 1–9. https://doi.org/10.3389/fmicb.2019.00365

17 Ramesh C, Terrestrial and marine bioluminescent organisms from the Indian subcontinent: a review, *Environl Monit Assess*, 192 (747) (2020) 1–30.

18 Mirjani M, Soleimani M & Salari V, Ecotoxicology and Environmental Safety Toxicity assessment of total petroleum hydrocarbons in aquatic environments using the bioluminescent bacterium *Aliivibrio fischeri*, *Ecotoxicol Environ Saf*, 207 (2021) 1–8.

19 Adoki A A & Odokuma L O, Bioluminescent hydrocarbonclastic bacteria of the Niger Delta, *African J Biotechnol*, 6 (2007) 393–399.

20 Abraham T J, Shanmugam S A, Palaniappan R & Dhevendaran K, Distribution and abundance of luminous bacteria with special reference to shrimp farming activities, *Indian J Geo-Mar Sci*, 32 (2003) 208–213.

21 Chrisolite B, Thiyagarajan S, Alavandi S V, Abhilash E C, Kalaimani N, *et al*., Distribution of luminescent *Vibrio harveyi* and their bacteriophages in a commercial shrimp hatchery in South India, *Aquaculture*, 275 (2008) 13–19. https://doi.org/10.1016/j.aquaculture.2007.12.016

22 Soto W, Gutierrez J, Remmenga M D & Nishiguchi M K, Salinity and Temperature Effects on Physiological Responses of *Vibrio fischeri* from Diverse Ecological Niches, *Microb Ecol*, 57 (2009) 140–150. https://doi.org/10.1007/s00248-008-9412-9

23 Omeroglu E E, Karaboz I & Sudagidan M, Characteristics and genetic diversity of bioluminescent *Shewanella woodyi* strains isolated from the Gulf of Izmir, Turkey, *Folia Microbiol*, 59 (2014) 79–92. https://doi.org/10.1007/s12223-013-0269-z

24 Parmar P, Shukla A, Saraf M & Patel B, Isolation of bioluminescent bacteria from marine organisms Isolation of bioluminescent bacteria from marine organisms, *Indian J Geo-Mar Sci*, 49 (2020) 471–476.

25 Ramesh C H & Mohanraju R, Genetic diversity of bioluminescent bacteria in diverse marine niches, *Indian J Geo-Mar Sci*, 46 (2017) 2054–2062.

26 Ramesh A & Venugopalan V K, Luminous microflora associated with the fishes *Mugil cephalus* and *Tachysurus arius*, *FEMS Microbiol Ecol*, 53 (1988) 27–34.

27 Nealson K H & Hastings J W, Bacterial Bioluminescence: Its Control and Ecological Significance, *Microbiol Rev*, 43 (1979) 496–518.

28 Dunlap P V, Davis K M, Tomiyama S, Fujino M & Fukui A, Developmental and Microbiological Analysis of the Inception of Bioluminescent Symbiosis in the Marine Fish *Nuchequula nuchalis* (Perciformes: Leiognathidae), *Appl Environ Microbiol*, 74 (2008) 7471–7481. https://doi.org/10.1128/AEM.01619-08

29 Hastings J & Greenberg E P, Quorum sensing: The explanation of a curious phenomenon reveals a common characteristic of bacteria, *J Bacteriol*, 181 (1999) 2667–2668. https://doi.org/10.1128/jb.181.9.2667-2668.1999

30 Grim C J, Taviani E, Alam M, Huq A, Sack R B, *et al*., Occurrence and Expression of Luminescence in *Vibrio cholerae*, *Appl Environ Microbiol*, 74 (2008) 708–715. https://doi.org/10.1128/AEM.01537-07

31 Abbas M, Adil M, Ehtisham-ul-haque S, Munir, B, Yameen M, *et al*., *Vibrio fischeri* bioluminescence inhibition assay for ecotoxicity assessment: A review, *Sci Total Environ*, 626 (2018) 1295–1309. https://doi.org/10.1016/j.scitotenv.2018.01.066

32 Ramesh C & Mohanraju R, Isolation and characterization of marine bioluminescent bacteria for toxicity bioassays and biotechnological applications, *Brazilian J Microbiol*, 52 (2021) 1191–1199.

33 Nealson K H, Autoinduction of Bacterial Luciferase occurrence, mechanisms and significance, *Arch Microbiol*, 112 (1977) 73–79.

34 Tanet L, Martini S, Casalot L & Tamburini C, Reviews and syntheses: Bacterial bioluminescence – ecology and impact in the biological carbon pump, *Biogeosciences*, 173757–3778.

35 Ramesh C, Mohanraju R, Narayana Murthy K, Karthick P & Narayana S, Impact of light, temperature, salinity and glycerol on the intensity of luminescence and growth of marine bioluminescent bacteria *Vibrio campbellii* (strain STF1), *Curr Sci*, 106 (2014) 511–513.