Impact of light, temperature, salinity and glycerol on the intensity of luminescence and growth of marine bioluminescent bacteria *Vibrio campbellii* (strain STF1)

Light is one of the major factors that affect the morphology of any organism, directly or indirectly. This phenomenon has also been observed in microorganisms and light is known to inhibit and delay their growth[1,2]. Different types of light are known to have an impact on the growth and production of toxins[3] and light sources like blue, white, red, green and dark affect the growth of certain microorganisms[1]. Irradiation of certain microorganisms with laser and ultraviolet light (UV) was found to have an effect on their growth, dimerization of thymine bases and ability to form pigmentation[2,4]. Induction of luminescence in four different marine bioluminescent bacteria was observed when exposed to UV light[5]. However, the intensity of irradiating light on essential gene of a bacterium and its resistance to irradiation results in internal mutations or ultimately death of the microorganisms[4].

Light, temperature and salinity are interrelated factors which affect the distribution, growth and luminescence of marine luminescent bacteria. These microorganisms tolerate a wide range of temperature and salinity[6], and their preliminary identification can be easily made by their temperature sensitivity, nutritional versatility[7] and other inorganic salts[8]. These salts do not have any impact on growth, but combine with certain organic molecule complexes that have an important role in controlling luminescence[9]. Factors such as chemical[10–12], mechanical[12] and physical[5] also found to control luminescence.

The present study was carried out to understand the *in vitro* effect of light, temperature, salinity and glycerol on growth and luminescence of *Vibrio campbellii* (strain STF1), to study its requirement and tolerable concentrations of these parameters, and to induce luminescence of dim strains based on our results.

The strain STF1 was isolated from surface swab of *Synanceia verrucosa* (stone fish), using seawater complete agar medium (SWC) following aseptic routine technique. Molecular analysis of 16s rRNA gene of this strain was carried out according to Mohandass *et al.*[13]. The PCR product was partially sequenced by an automated sequencer (Applied Biosystems, USA) at the National Institute of Oceanography, Goa. The obtained nucleotide sequence was uploaded in GenBank (NCBI) and accession number KF961190 was assigned to this sequence.

To understand the effect of different light sources on the growth and luminescence of *V. campbellii* (strain STF1), a light experiment was set up with VIBGYOR (violet, indigo, blue, green, yellow, orange and red colour lights), white, laser, UV and dark (Figure 1). PVC pipes of 110 mm dia and 30 cm height were used, with one end open for placing the bacterial culture plates and the other end fitted with 15 W colour light bulbs (Philips) in each tube and packed properly with insulation tape.

Pure culture of this strain was streaked on sterile nutrient agar (NA) plates with 3% sodium chloride (NaCl). These plates were incubated under VIBGYOR colours and UV light. For laser light a normal pointer laser (4 W) was used following the method of Vescovi *et al.*[2] with slight modifications as follows: overnight grown bacterial strain in nutrient broth was exposed to laser light for 1 min and from this 100 µl of bacterial suspension was spread onto NA plate and incubated at 32°C for 24 h. Some plates were completely covered with black paper (without exposure to any of these lights) and were incubated in complete darkness at 32°C for 24 h. For natural light plates were incubated under open sunlight for 12 h. Control plates that were not exposed to any of these light sources were incubated (at 35°C for 24 h) in an incubator for comparison with the other plates. All the plates, except those under sunlight and in the incubator were exposed (incubation) to various light sources for 24 h at room temperature (32°C).

Figure 1. Schematic representation of the light model with VIBGYOR colours, UV, laser, dark, white and sunlight.

Table 1. Visual grades for intensity of luminescence and growth of strain STF1 using different types of light

Light source	Light wavelength (nm)	Temperature (°C)	Growth	Luminescence
UV light	100–400	32	++*	+++
Dark	Absence of light	32	++	++
Incubator	Absence of light	35	+++*	++
Orange	590	32	++	++
Red	650	32	++	++
Violet	400	32	++	++
Indigo	445	32	++	++
Blue	475	32	++	+*
Yellow	570	32	++	+
White	400–700	32	++	+
Green	510	32	++	+
Laser	630–670	32	++	–
Sunlight	400–700	32	–*	–

*+++, Best growth and luminescence; *++, Good growth and luminescence; *+, Less luminescence; *–, Inhibition of growth and luminescence.

Table 2. Visual grades of the intensity of luminescence and growth of marine *Vibrio campbellii* (strain STF1) with Na, Mg, K, Ca and glycerol.

	NaCl		$MgSO_4$		K_2HPO_4		$CaCO_3$		Glycerol	
Concentration (%)	G*	L*	G*	L*	G	L	G	L	G	L
0	–*	–	++	++	++	++	++	++	++	++
0.1/^1	++*	++	++	++	++	++	++	++	++	++
0.2/^2	++	++	++	++	++	++	++	++	++	++
0.3/^3	+++*	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.4/^4	+++	+++	+++	+++	+++	++	+++	+++	+++	+++
0.5/^5	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.6/^6	+++	+++	+++	+++	+++	++	+++	+++	++	+++
0.7/^7	+++	++	+++	+++	+++	++	+++	+++	++	++
0.8/^8	+++	++	+++	++	+++	+	+++	+++	+	++
0.9/^9	++	+	+++	+	++	+	+++	+++	(+)	+
1.0/^10	+*	+	+++	++	(+)	(+)	+++	+++	(+)	++
^11	(+)*	–								
^12	–	–								

*G, Growth; *L, Luminescence; *+++, Best growth and luminescence; *++, Good growth and luminescence; *+, Lesser growth and luminescence; *(+), Weak growth and luminescence; *–, Inhibition of growth and luminescence; ^, Codes for NaCl concentration.

In order to understand the growth rate, NA plates were streaked with strain STF1 and incubated at different temperatures – 10°C, 16°C, 28°C, 35°C, 38°C, 40°C, 45°C, 48°C and 50°C.

Experiments with various concentrations of salts like sodium (NaCl; major salt from 0% to 12%), potassium (K_2HPO_4), magnesium ($MgSO_4$), calcium ($CaCO_3$; minor salt from 0% to 1.0%) and glycerol (as carbon source from 0% to 1.0%) were supplemented into luminescent agar (LA)[14] and luminescent broth[15], and inoculated with this bacterial strain and incubated at 35°C for 24 h. The concentration of minor salts was studied in the presence of essential component, NaCl 3%. Simultaneously, NA plates were also prepared in a similar manner with addition of sodium chloride (from 0 to 12%) and glycerol (from 0% to 1.0%), and were streaked with this strain and incubated at room temperature (32°C) for 24 h. After the incubation period all plates were compared for growth and luminescence, and visual-based grades were given (Tables 1 and 2).

Intensity of luminescence by the strain (STF1) was observed with different types of light (Figure 2). Under natural light growth and luminescence were inhibited and plates exposed to laser light did not show luminescence. Whereas in control plates not exposed to laser and UV showed luminescence. No inhibition of growth was observed in the plates under UV light, but found with high intensity of luminescence. Plates incubated in dark showed better luminescence than those incubated in the incubator, orange, red, violet and indigo colour lights, and blue, yellow, white and green showed less luminescence (Figure 2).

Growth was observed to be slow in the plates incubated at 45–48°C and no growth was seen at 10°C and above 50°C. The optimum temperature for good growth was found to be from 16°C to 40°C.

Sodium chloride was found to be essential for growth; with omission of this salt there was no growth and also no growth was found at higher concentration of 12%. Maximum tolerability to NaCl concentration was found at 11%, whereas good growth and luminescence were observed at 3% and 6% sodium chloride. Calcium, magnesium, potassium and glycerol were not required for growth and luminescence. However, a range of concentrations (0.3–0.5%) of calcium, potassium, magnesium and glycerol were required for best growth and luminescence. Both potassium and glycerol at concentration of 0.6–1% showed weak growth and less luminescence.

Studies showed that luminescence was good in the absence of light compared to the plates incubated in the incubator and under various light sources. Inhibition of growth and luminescence was observed under high intensity of natural light due

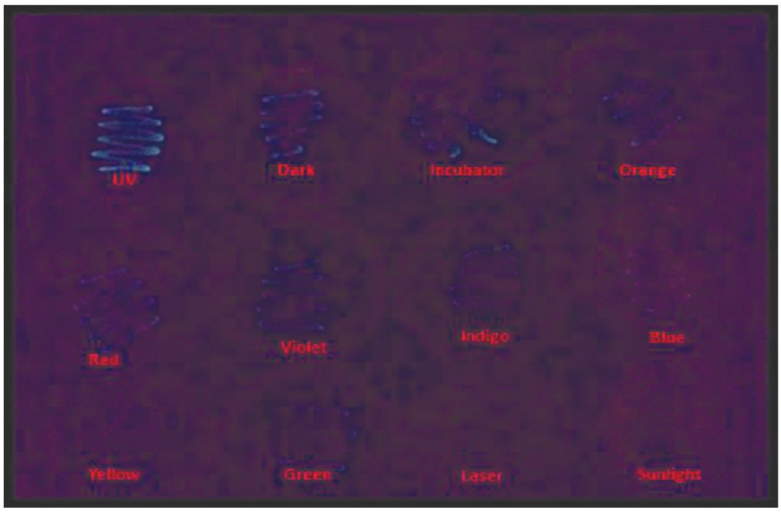

Figure 2. Intensity of luminescence and growth of strain STF1 with different light sources.

to irradiation of other sources than visible and UV light rays. Even when these plates were further incubated at room temperature (32°C) for another 24 h, growth was not observed. Good growth and luminescence were observed in plates that were incubated at low intensity of light, in the absence of light and at optimum temperatures (32°C and 35°C). Irradiation of UV and laser lights had an effect on the luminescence. However, bacterial culture grown in the presence of UV light showed highest luminescence compared with all the other light sources (indicates that this *V. campbellii* strain is UV-tolerant) and laser light showed inhibition of luminescence (indicates that laser light strongly inhibited the *lux* genes involved in luminescence expression). Electromagnetic waves other than UV and visible rays might also play a role in inhibition of growth and luminescence on the plates incubated under natural light. UV light was found to be a catalyst that helps in the expression of luminescence reaction faster than under natural environment conditions. An earlier study reported that in the presence of UV light luminescence expression was faster and it could induce luminescence of four species of marine luminescent bacteria as well as dark mutants[5]. This is due to UV-mediated gene (*lux*) that controls luminescence[5] and the same phenomenon has also been observed in the present study. Based on our observations different wavelengths of light also have an impact on the *lux* genes.

Low (10°C) and high (50°C) temperatures inhibited growth completely, whereas maximum tolerable temperature for growth was observed at 45°C and optimum levels of temperature for good growth ranged from 16°C to 40°C. Optimum concentration levels (3–6%) of glycerol showed best luminescence, while inhibition of growth and luminescence was observed at higher concentrations of glycerol, which may be due to acid production[16]. It has been reported that the amount of required nutrients and inorganic contaminants depends on the bacterial strain[8]. It has been observed that potassium, calcium, magnesium and glycerol are not required for growth and luminescence, but at particular concentrations they induced and inhibited both growth and luminescence[9].

In vitro results obtained from this study show that light, temperature, salinity and glycerol are major factors that induce and inhibit the growth and luminescence of *V. campbellii* (strain STF1). These results will also help us to induce luminescence in dark mutant strains. UV light helps in the isolation of luminescent bacteria as it induces luminescence. Comparative studies on UV exposed, laser exposed and non-exposed culture will help us understand the internal changes due to irradiation.

1. Idnurm, A. and Heitman, J., *PLoS Biol.*, 2005, **3**, 95.
2. Vescovi, P. *et al.*, *Lasers Med. Sci.*, e-pub ahead of print 29 September 2012, doi: 10.1007/s10103-012-1197-x.
3. Sivonen, K., *Appl. Environ. Microbiol.*, 1990, **56**, 2658.
4. Claus, W. G., In *Understanding Microbes: A Laboratory Textbook for Microbiology*, W. H. Freeman and Co, New York, 1989, pp. 333–346.
5. Czyz, A., Plata, K. and Wegrzyn, G., *J. Appl. Genet.*, 2002, **43**, 377–389.
6. Farmer, J. J. III and Hickman-Brenner, F. W., *Prokaryotes*, 2006, **6**, 508–563.
7. Nealson, K. H., *Methods Enzymol.*, 1978, **57**, 153–166.
8. Srivastava, V. S. and MacLeod, R. A., *Can. J. Microbiol.*, 1971, **17**, 703–711.
9. Zirpolo, G., Studies on bacteriological bioluminescence, action of magnesium salt. NASA Washington, DC, 1972, p.13.
10. Daniels, R., Vanderleyden, J. and Michiels, J., *FEMS Microbiol. Rev.*, 2004, **28**, 261–289.
11. Fidopiastis, P. M., Sørum, H. and Ruby, E. G., *Arch. Microbiol.*, 1999, **171**, 205–209.
12. Ismail, S. A. and Kaleemurrahman, M., *Curr. Sci.*, 1981, **50**, 555.
13. Mohandass, C., Rajasabapathy, R., Ravindran, C., Colaco, A., Santos, R. S. and Meena, R. M., *Cah. Biol. Mar.*, 2012, **53**, 65–76.
14. West, P. A. and Colwell, R. R., In *Vibrios in the Environment* (ed. Colwell, R. R.), Wiley, New York, 1984, pp. 285–363.
15. Gokhale, T., Wali, A., Parikh, S. and Sood, N., *Eng. Rev.*, 2012, **32**, 17–22.
16. Dunlap, P. V. and Urbanczyk, H., In *Prokaryotes* (eds Rosenberg, E. *et al.*), Springer-Verlag, Berlin, 2013, 4th edn, pp. 495–528.

ACKNOWLEDGEMENTS. We thank Pondicherry University for providing the necessary facilities. First author thanks the Department of Science and Technology, New Delhi for the INSPIRE fellowship. We also thank the anonymous reviewers for their critical comments and suggestions for the improvement of this paper.

Received 15 May 2013; revised accepted 8 January 2014

CHATRAGADDA RAMESH*
RAJU MOHANRAJU
KADA NARAYANA MURTHY
PERUMAL KARTHICK
SUMANTHA NARAYANA

Department of Ocean Studies and Marine Biology,
Pondicherry Central University,
Port Blair 744 112, India
**For correspondence.*
e-mail: chrameshpu@gmail.com