

Distribution & Species Composition of Planktonic Luminous Bacteria in the Arabian Sea

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Distribution of the total viable heterotrophic bacteria and the luminous bacteria in the neretic and oceanic waters of the west coast of India was studied. Counts of viable heterotrophs fluctuated widely, generally with a decrease in their number with the depth. Luminous bacterial cells ranged from 0-36 ml⁻¹ and their percent fraction was stable between depths of 60 and 200 m. Significant correlation between total viable count and luminous bacteria colonies in the upper 100 m suggests free-living nature of luminous bacteria. *Vibrio fischeri* and *V. harveyi* dominated the species composition of luminous bacteria. *Photobacterium leiognathi* was only a minor component. A few strains of *P. phosphoreum* were also recovered during the collections from deeper waters.

Marine luminous bacteria comprise Gram-negative and motile rods. Their most unique character is the emission of light. They are widely distributed in the world oceans and can be isolated from a variety of niches¹⁻⁸. Many studies have suggested that the distribution and species composition of luminous microflora are influenced by season, temperature, nutrient concentration, depth and geographical locations⁹⁻¹².

Information on the distribution of luminous bacteria in the Indian seas is largely restricted to a few locations in the nearshore waters and sediments^{4,13,14}. No attempt has so far been made to study their distribution in offshore locations. Hence, the present study has been undertaken to find out the distribution pattern and species composition of luminous bacteria in the nearshore and offshore waters along the west coast of India.

Materials and Methods

Water samples were collected using JZ bacteriological samplers during the *R V Gaveshani* cruises 158 (24 Oct. to 2 Nov. 1985), 160 (22 Nov. to 10 Dec. 1985) and 166 (31 March to 11 April 1986) along the west coast (Fig. 1). All samples were immediately processed onboard. The procedures employed for isolation of luminous bacteria were essentially the same as those suggested by Baumann and Baumann¹⁵. Seawater complete medium (SWC — 750 ml seawater, 250 ml tapwater, 5 g bacto-peptone, 3 g yeast extract, 3 ml glycerol and 15 g agar) and membrane filtration technique were used for enumeration of total viable count (TVC) and luminous bacteria. Aliquots (2-20 ml) of water samples were filtered in duplicate through sterile membrane filters (0.45 µm pore size, Millipore, USA) and placed on SWC

agar plates. All the plates were incubated aerobically at 20-25°C. After 18 h of incubation, plates were observed periodically in dark for the presence of luminous colonies. Final counts of luminous colonies were recorded when there was no further increase in their numbers. This was usually attained at 36 h of incubation. Total counts of all the colony forming units were recorded after 3-4 d of incubation.

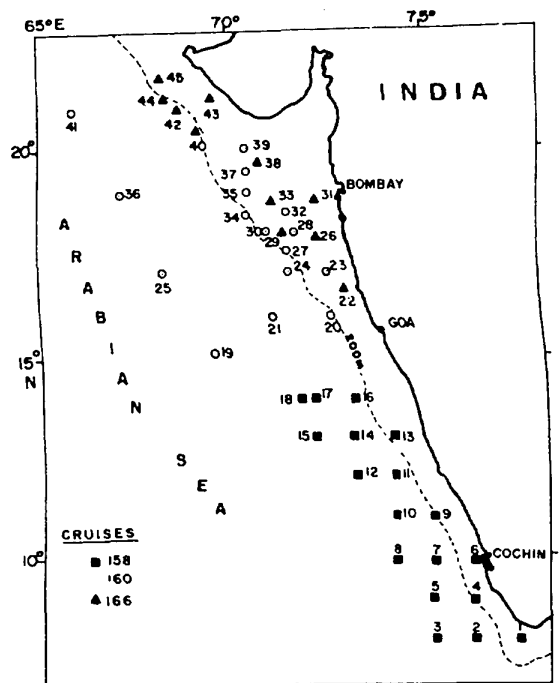


Fig. 1 — Sampling stations

During the cruises 158 and 160, representative colonies of luminous bacteria were isolated from all the positive plates. Colonies selected at random were picked and transferred to agar slants (SWC) with a sterile inoculation needle. Feeble light from the spirit lamp was sufficient to distinguish between luminous and non-luminous colonies. All luminous cultures were further purified and maintained on SWC containing 50 mM Tris buffer.

During the cruise 166 besides enumerating the viable heterotrophs at 11 stations, samples were collected at close depth intervals at sts 44 and 45 to determine the pattern of vertical distribution of luminous bacteria. Fourteen or more luminous strains from each depth were isolated. Thus 150 strains were collected from these 2 stations.

All the isolates (425) were subjected to 18 different tests. The ability to use different organic compounds as sole source of carbon, production of extracellular enzymes and other characteristics were tested as described by Nealson¹⁶ and Hastings and Nealson⁵. Characteristics of all the luminous bacteria that were reported by Baumann and Baumann¹⁵ were used for species assignment. Nomenclatures employed in the present study were same as those suggested by Hastings and Nealson⁵.

Results and Discussion

A wide fluctuation of a 100 fold in the TVC (0.2 to 596.67 ml⁻¹) was noticed in the present study. Generally, the number decreased with depth. TVC was

very low in the offshore waters compared to near-shore waters. The ranges of luminous bacterial population (cells. ml⁻¹) in positive samples were 0.08 to 9.5; 0.2 to 26.67; 1.11 to 35.56; 0.1 to 36.00; 0.05 to 1.77; 0.03 to 0.59 and 0.05 to 0.16 respectively for surface, 10, 25 to 50, 60 to 90, 100, 200 and 500 m. The mean percentage occurrence of luminous bacteria between 60 and 200 m depth was rather stable indicating uniform vertical distribution (Table 1).

A simple correlation coefficient was calculated to check TVC dependent distribution of luminous bacteria at different sampling depths. No correlation was found between TVC and luminous bacteria at 200 m [df 16; critical r (Cr)=0.47; observed r (Or) = -0.44 and 500 m (df 4, Cr=0.81; Or = -0.42)] at 5% level of significance. This pattern is suggestive of an independent distribution of luminous bacteria. Orndorff and Colwell¹² observed a similar trend in the Sargasso Sea at depths 160 to 320 m and opined that the role of luminous bacteria at this depth is one of the symbionts of fish rather than that of freeliving or planktonic organisms. On the contrary, TVC was highly correlated with luminous bacterial count at 10 m (df 10; Cr = 0.58; Or = 0.65) 25-50 m (df 8; Cr = 0.632; Or = 0.767), 60-90 m (df 18, Cr = 0.444; Or = 0.692) and 100 m (df 19; Cr = 0.433; Or = 0.74) at 5% level of significance. This pattern suggests freeliving nature of luminous microflora in the upper 100 m. All the known species of luminous bacteria are known to exist in this mode. The ecology of a group of microorganisms

Table 1 — Comparative Depthwise Distribution of TVC and Luminous Bacteria (LB)

Depth (m)	No. of samples	Mean bacterial population (x 10 ³ . l ⁻¹) ± SD*			Number of isolates				
		TVC	LB	% of LB	<i>V. harveyi</i>	<i>V. fischeri</i>	<i>P. leiognathi</i>	<i>P. phosphoreum</i>	Total
0	45	55.19 (± 119.96)	0.9 (± 2.06)	1.63	22	30	3	—	55
10	11	187.79 (± 215.44)	8.49 (± 10.45)	4.52	10	5	2	—	17
25-50	9	65.02 (± 77.72)	10.30 (± 13.71)	15.84	37	62	13	—	112
50-90	19	102.77 (± 172.19)	7.01 (± 9.69)	6.82	30	66	14	2	112
100	20	7.99 (± 8.15)	0.56 (± 0.62)	7.01	16	30	10	3	59
200	17	3.12 (± 1.77)	0.19 (± 0.17)	6.08	7	28	1	6	42
300	2	2.53 (± 0.80)	0.14 (± 0.04)	5.53	1	9	—	6	16
500	5	3.14 (± 5.02)	0.07 (± 0.07)	2.23	2	4	—	6	12
Total					125	234	43	23	425
%					29.4	55.1	10.1	5.4	100

*Values given in parentheses.

such as marine luminous bacteria that occupy a number of different niches is likely to be diverse and complex. Relatively high numbers of freeliving or planktonic luminous bacteria in the upper 100 m in the west coast of India may indicate a nutritionally and ecologically congenial habitat. And such occurrence in high numbers of luminous bacteria is thought to be indicative of a higher rate of production and increased availability of nutrients⁹.

Luminous bacteria were not detected in 21 out of 45 surface samples (47%) and a few deep samples. Several causes¹² including subsample variance are attributed to such observations.

The species composition of luminous bacteria observed in the present study is similar to that reported for tropical oceans^{11,17,18}. *Vibrio fischeri* contributed to 55.1% of total luminous bacteria followed by *V. harveyi* (30%). Both species were observed throughout the water column. Higher number of *Photobacterium leiognathi* strains were observed in the 100 m

water column during November/December collections. This species is a light organ symbiont of leiognathids^{1,19} that are demersal fishes occurring throughout the shallow neritic waters of the Indian coast²⁰. Shedding of these bacteria by these fish may also contribute to the freeliving populations. However, it is surprising to note that *P. leiognathi* is rather a minor component of the luminous bacterial population. Only 5.4% of total isolates (425) were *P. phosphoreum*. They were recovered mostly during November/December from samples at depths of 85 m and below. This species is reported from depths of 150 m and below both as freeliving and as a symbiont⁵ and can grow²¹ at 4 to 25°C. In none of the samples did temperature exceed 25°C at these depths during the present study. A depth-related species specific symbiotic association is thought to contribute to the occurrence of *P. phosphoreum*²². However, no attempts have been made to identify the fish host symbionts of this species in deep waters. Up-

Table 2 — Vertical Distribution of Luminous Bacteria at Two Stations during April 1986*

Depth (m)	Temperature (°C)	Mean TVC ($\times 10^3 \cdot l^{-1}$)	Luminous bacteria (% of TVC)	Number of isolates			
				<i>V. fischeri</i>	<i>V. harveyi</i>	<i>P. leiognathi</i>	<i>P. phosphoreum</i>
0	26.7	26.06	1.40	5	9	—	—
10	26.7	23.39	2.61	5	10	2	—
25	26.4	26.34	3.68	10	9	6	—
50	25.8	27.03	4.52	13	7	5	—
75	25.6	26.43	3.96	20	9	5	—
100	24.3	27.62	6.68	19	7	8	1

*Data pooled from sts 44 and 45.

Table 3 — Taxonomic Characteristics of Luminous Bacteria

Tests	<i>V. harveyi</i> (n=125)	<i>V. fischeri</i> (n=234)	<i>P. leiognathi</i> (n=43)	<i>P. phosphoreum</i> (n=23)
Growth at 35°C	+	+	+	—
30°C	+	+	+	—
4°C	—	+(39-)	-(2+)	+
Amylase	+	—	—	—
Lipase	+	+	-(2+)	+
Gelatinase	+(10-)	+(43-)	—	—
Growth on				+(2-)
Maltose	+	+	—	—
Cellobiose	+	+	—	—
Gluconate	+	-(43+)	+	+
Glucuronate	+	—	—	—
Mannitol	+	+	—	—
Proline	+	+	+	—
Lactate	+	—	+	—
Acetate	+	+	+	—
Alanine	+	+(5-)	+(3-)	—
Tyrosine	+	—	—	—
Galactose	+	+	+	+
Gas from Glucose	—	—	—	+

welling of deep, cold and nutrient rich waters may also contribute to the presence of *P. phosphoreum* in the shallow regions.

Studies on vertical distribution pattern of the luminous bacteria in the shallow depths are lacking. The observations made during April 1986 from 2 stations (Table 2) indicate uniform distribution of luminous bacteria in the water column. All the 3 luminous species followed similar distribution pattern for other stations. Only one strain of *P. phosphoreum* was recovered from the 100 m sample where the temperature was 24.3°C.

The biochemical responses of a few isolates were different (Table 3) from those of the isolates reported by Reichelt and Baumann²³. Several strains identified as *V. fischeri* in the present study showed a weak growth at 4°C and utilized gluconate as the sole source of carbon. Many strains of *V. fischeri* produced yellow pigment and luminescence was irreversibly lost in these strains. Such discrepancies were also noticed earlier^{9,12,18}.

In the marine nearshore environments wide variations in TVC occur as a result of known and unknown factors. Values ranging from 10^2 to 10^5 . ml⁻¹ for any given day and location are reported and no predictable patterns for these fluctuations have been demonstrated^{24,25}.

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