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ECOPHYSIOLOGICAL STUDIES ON LUMINOUS BACTERIA ASSOCIATED WITH MARINE GASTROPODS

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ABSTRACT - Information available on the association of luminous bacteria with marine invertebrates is limited. Hence qualitative and quantitative investigations were undertaken with a view to understand the distribution of luminous microflora in relation to the environmental factors and also in the gut of two marine gastropods, *Aplysia benedicti* Eliot and *Bullia tranquebarica* (Roding) from Porto Novo (11°29'N, 79°46'E) waters of the East coast of India.
The gut of the gastropods harboured maximum number of luminous bacteria when compared to the water and sediment. Qualitative analysis revealed the presence of two luminous bacterial components, viz. *Vibrio harveyi* and *V. fischeri*. *V. fischeri* was not found in association with *A. benedicti*.
The isolates of the luminous bacterial species were subjected to different physiological tests such as tolerance to sodium chloride, pH and temperature. Both the species grew well at 3-6 % NaCl and at 7-8 pH. All the isolates registered good growth at 28°C whereas, isolates of *V. fischeri* were found to grow well at 15°C also. Both the strains isolated, exhibited chitinolytic activity.

Key words: luminous bacteria, ecology, physiology, gastropods, *Vibrio harveyi*.

RÉSUMÉ - Les informations disponibles sur les associations entre bactéries luminescentes et invertébrés marins sont limitées. Des études qualitatives et quantitatives sont entreprises pour comprendre la distribution de la microflore luminescente en relation avec les facteurs environnants et pour l'étudier au niveau de l'intestin de deux gastropodes indiens. Il s'agit d'*Aplysia benedicti* (Eliot) et de *Bullia tranquebarica* (Roding) prélevés dans la région de Porto Novo (11°29'N, 79°46'E) sur les côtes Est de l'Inde.
L'intestin des gastropodes est envahi d'un nombre important de bactéries luminescentes comparativement à l'eau de mer et aux sédiments. L'analyse qualitative révèle la présence de 2 bactéries luminescentes, *Vibrio harveyi* et *V. fischeri*. Toutefois, *V. fischeri* n'a pas été observé en association avec *A. benedicti*.
Les souches bactériennes luminescentes isolées étaient soumises à différents tests physiologiques tels que la tolérance à des variations de salinité, pH et température... Les deux espèces se développaient bien à 3-6 % de NaCl et à un pH de 7-8. Les souches isolées présentaient toutes une bonne croissance à 28°C, alors que les isolats de *V. fischeri* étaient aussi capables de croître à 15°C. Les deux souches, inhibaient de plus l'activité chitinolytique.

Mots clés: bactérie luminescente, écologie, physiologie, gastropode, *Vibrio harveyi, Vibrio fischeri*.

INTRODUCTION

Existing information on the ecology of enteric luminous microflora of invertebrates is limited (Nair *et al.*, 1979 ; Venkateswaran *et al.*, 1981 ; Ramesh *et al.*, 1983b). Moreover, very little is known about the physiological characteristics of these procaryotes. Hence, there exists a lacuna in understanding the ecology and physiology of luminous microflora associated with these animals and this was the main incentive to carry out the present study.

MATERIAL AND METHODS

Specimens of *Aplysia benedicti* and *Bullia tranquebarica* were collected from the mouth of the Vellar estuary and the sandy beach of the Bay of Bengal at Porto Novo, from June to August, 1982 when these animals were plentiful.

Live animals were brought to the laboratory in polythene buckets , washed several times with sterile sea water to prevent contamination from shell surface and mantle fluid,and the gut of the animals were aseptically removed. The tissues adhering to the gut were carefully removed using a sterile forceps and the gut homogenized in 100 ml of sterile sea water. Serial dilutions were made from the homogenate and spread into petriplates containing sea water complete (SWC) agar medium (Ruby and Nealson, 1978). The plates were incubated in dark at room temperature (28°C) for 24 hrs following which the total CFU (Colony Forming Units) and luminous CFU were counted. Single, bright luminous colonies were picked randomly from the petriplates and maintained in SWC agar slants at room temperature. The scheme of Nealson (1978) was followed for the identification of the isolates.

The method of Ruby and Nealson (1978) was followed for screening luminous bacteria from the water. The luminous bacteria from the sediment were isolated by the serial dilution-spread-plate technique as in the case of molluscan gut.

The bacterial isolates were subjected to various regimes of temperature, sodium chloride concentration and pH. Sodium chloride concentration and pH tolerance studies were conducted at room temperature (28°C). Growth of the isolates was monitored by measuring the turbidity of the cultures at 550 nm in a Lumetron Colorimeter (Model N° 401, photovolt Inc., New York). The assay of Reissig *et al.* (1955) was employed to determine the chitinolytic activity of the isolates.

Animals	Collection period	Salinity (%)	Oxygen (ml/l)	Temp (°C)	pH
A. benedicti	June, 1982	31.0	3.0	33	7.3
	July	21.5	4.0	30	7.4
	August	30.5	4.0	30	7.6
B. tranquebarica	June, 1982	31.5	3.2	33	7.4
	July	30.5	3.9	29	7.3
	August	31.8	3.8	28	7.4

Table 1 : Physico-chemical parameters of the location from which the animals were collected.

RESULTS AND DISCUSSION

The population size of the luminous microflora (Tab. 2) was more in the gut of the gastropods when compared to that in the water and the sediment. The observations of the present study support the view of Yanagita et al., (1978) that the enteric tract of nekton and benthos are rich in nutrilites when compared to the surrounding environment and hence it is likely that luminous microflora which gained access to the alimentary canal might have multiplied rapidly *de novo*.

The luminous microbial load observed in the gut of the two gastropods was however less than that recorded earlier in bivalves and fish by the authors (Ramesh *et al.*, 1983a, b). The type of food consumed, rate of ingestion of food (Epifanio *et al.*, 1975) and the

digestive ferments (Zhukova, 1963) might have been responsible in controlling the size of the bacterial population in the gut of these animals.

As reported in an earlier study (Ramesh *et al.*, 1983b) no relationship was found between luminous microbiota of the gastropods and the environmental factors (Tab. 1). In this context it may be inferred that generally the bacteria harboured in the gut are insensitive to changes in the environment due to the protective situation inside the gut (Liston, 1957).

Animals	Month	Gut (CFU/Gm dry wt of gut contents)		Water (CFU/ml)		Sediment (CFU/gm dry weight)	
		*TCFU	*LCFU	*TCFU	*LCFU	*TCFU	*LCFU
A. benedicti	June, 1982	1.05×10^5	5.40×10^4	102	24	3.40×10^4	7.60×10^3
	July	7.26×10^4	2.51×10^4	155	18	3.60×10^4	5.00×10^3
	August	8.47×10^4	1.85×10^4	251	36	4.85×10^4	3.00×10^3
A. tranquebarica	June, 1982	4.34×10^5	1.54×10^4	296	74	7.55×10^4	3.57×10^3
	July	5.24×10^4	3.07×10^4	208	39	4.48×10^4	8.20×10^3
	August	2.58×10^4	8.59×10^4	236	28	2.6×10^4	3.60×10^3

Table 2 : Quantitative distribution of luminous bacteria in the gut of gastropods and the environment.

*TCFU = total colony forming units
*LCFU = luminous colony forming units

Vibrio harveyi and *V. fischeri* were the two dominant luminous bacterial species identified in the present study. Of the two, *V. harveyi* was the predominant species. *V. fischeri* showed only a sporadic distribution, inhabiting water, sediment and gut of *B. tranquebarica*. Significantly it was not detected in the gut of *A. benedicti*. Perhaps the conditions prevailing in the gut of *Aplysia* might not have been conducive for the survival of *V. fischeri*. As suggested by Reichelt and Baumann (1973) and McCall and Sizemore (1979) nutritional versatility and production of bacteriocins by *V. harveyi* could be the plausible factors that might account for its relatively wide distribution in the macro (water and sediment) and micro-environments (gut).

Maximum growth of *V. harveyi* was observed at 2-5 % NaCl concentration depending on the organism from which the bacteria was isolated. Shilo and Yetinson (1979) reported maximum growth for *V. harveyi* to occur in 1-3 % NaCl concentration. Lakshmanaperumalsamy *et al.* (1981) observed good growth in 1-5 % NaCl for the same species. However in the case of *V. fischeri*, a concentration of 5-7 % NaCl supported peak growth. The rate of growth was found to be relatively high for *V. harveyi*. Reichelt and Baumann (1974) were of the opinion that variations in relative growth rate and cell yield of the microflora were also due to the type of carbon source provided in the medium. The slackness of growth below pH 7 and above pH 8 (Fig. 2) and peak growth encountered at pH 7 suggests that these luminous bacteria prefer neutral pH. All the luminous isolates favoured an optimum temperature of 28°C (Fig. 3) *V. fischeri* isolates showed considerable decline in growth at 35°C though the same species isolated by Lakshmanaperumalsamy *et al.* (1981) were reported to grow well even at 35°C. Further, the ability of *V. harveyi* to grow at least to some extent even at 40°C suggested that it tolerates higher temperatures as well. In this context our findings are in agreement with those of Yetinson and Shilo (1979) and Lakshmanaperumalsamy *et al.* (1981) who also screened *V. harveyi*

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