Mariculture of marine sponges for drug development : bioactivity potentials of cultured sponges, *Callyspongia subarmigera* (Ridley) and *Echinodictyum gorgonoides* (Dendy)

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Among all metazoan phyla, marine sponges are considered as the richest source of biologically and pharmacologically active chemicals. More than 5,300 different products are recorded from sponges and their associated microorganisms. Every year, about 200 new metabolites are reported from sponges. Considering the emerging diseases and the rapid development of disease resistance among microbes, the detection of novel metabolites from sponges gains importance and also provides scope for developing new drugs against disease causing bacteria, virus, fungi and parasites. In nature, the chemical interactions in the marine habitat of sponges suggest that products from them function as defense tools to protect them against predators including fish. Sponge product ara-A (vidarabine), the anti-viral drug used against the *Herpes simplex encephalitis* virus has advanced to the late stages of clinical trials. Others such as manzamine A (activity against malaria, tuberculosis, HIV and others), lasonolides (antifungal activity) and psammaplin A (antibacterial activity) are considered as promising leads. However, most sponges contain only trace quantity of the bioactive molecules. The increasing demand for initial experimental trials, possible success and subsequent industrial use for scaling up will lead to severe pressure on the wild population and hence the possible overexploitation and extinction of the target species as such. In view of the limited availability of larger quantities of defined source material (the so-called 'supply problem'), and to cater to the requirements without loss of bioactive potential, mariculture could be considered as one of the best options. Hence, mariculture of two species *viz*., *Callyspongia subarmigera* (Ridley) and *Echinodictyum gorgonoides* (Dendy) was attempted at Vizhinjam Research Centre of CMFRI. The salient findings of the experimental culture of these two species of sponge by two methods *viz*., culture in re-circulatory semi-enclosed aquaria and culture in open sea together with their bioactivity potential during culture conditions are presented.

Sponge culture in the offshore laboratory conditions

All-glass aquarium tanks of 60x45x30 cm fitted with filtration unit and perforated perspex panels served as bioreactors for sponge culture in the laboratory. Compressed air with air–water lift was provided for re-circulating seawater and for development of ammonia oxidising bacteria in the bioreactor.

Explant preparation and culture

Freshly collected sponge *Echinodictyum gorgonoides* from Kanyakumari were washed and explants of about 5 ± 1 g size were prepared by cutting with a sharp surface-sterilised knife. Individual explant was weighed and kept in seawater without allowing them to dry. The explants were fixed to the perspex panel as could be seen from Fig. 1.

Fig. 1. Explants of *Echinodictyum gorgonoides* fixed to perspex panel

Feeding of sponge explants in the laboratory culture system

The sponge explants fixed in perspex sheet in the aquarium were fed with microalga *Nanochloropsis* sp. at the rate of 3.5×10^6 cells initially. The cell density was increased gradually and the algal feed was provided twice per day. The wastes remaining in the culture system were removed every day and water was exchanged on alternate days. At different intervals of time, sponge tissues from the bioreactor were aseptically removed for evaluating the bioactivity using standard microbiological and other bioassay methods.

Sponge culture in the open sea

Experiments were conducted to evaluate the growth and bioactivity performance of sponges cultured in open sea conditions. For this set of experiments, sponges *C. subarmigera, E. gorgonoides* and *C. diffusa* collected off Kanyakumari and Vizhinjam were used. The sponge masses were cleaned with fresh seawater, placed in plastic circular fruit baskets (closed) and held at varying depths of seawater at Vizhinjam Bay, Trivandrum coast. They were tied one above the other at 1 m depth intervals and suspended in the vertical plane and hung in the vicinity of the open sea cage farming site of the Central Marine Fisheries Research Institute, Vizhinjam Bay with proper anchoring. Fouling organisms attached to the baskets were periodically removed. The average pH of the water in the Vizhinjam Bay varied between 7.60 and 8.10. The dissolved oxygen content ranged from 3.6 to 4.17 mg/l; while the salinity was between 29.5 and 35 ppt. Fig. 2 and 3 depict the sponge culture method and growth of *C. subarmigera*.

Harvesting of sponges cultured in the open sea and testing bioactivity

The initial results indicated that in the open sea culture conditions, among three species, only *C. subarmigera* survived. Portions from the sponge were aseptically removed for bioactivity tests. The excised sponge tissues were macerated with methanol and after requisite period of incubation, the methanol was evaporated and extracts were prepared for different assays. The bioactivity was tested as per the standard microbiological and other bioassay methods. The bioactivity of harvested sponge biomass at each harvested date was evaluated to compare and determine whether repeated harvesting affected the bioactivity of sponge tissue.

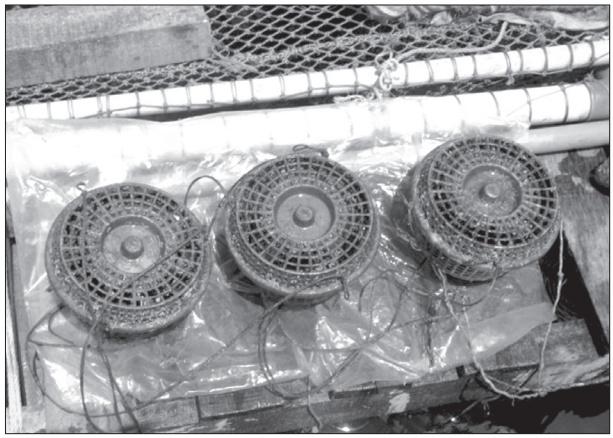

Fig. 2. Baskets containing sponges

Fig. 3. *Callyspongia subarmigera* cultured in Vizhinjam Bay

In laboratory conditions in the aquaria, *E. gorgonoides* survived the experimentation period of 80 days. Though the growth was low, bioactivities such as antibiotic activity as well as the cytotoxicity were retained. The experimental results are furnished in Table 1 and 2.

In the open sea culture conditions, *C. subarmigera* grew at an average rate of 88.94 mg per day. The overall growth was 93.37% compared to the initially stocked sponge tissue. Retention of bioactivity was noticed through the repeated harvests, though there was reduction in the bioactivity. The details of bioactivity of extract before and after culture are presented in Table 3 and 4.

Table 1. Antibacterial activity of fresh and laboratory cultured *Echinodictyum gorgonoides* extract

Test Bacteria (fish pathogenic bacteria)	*Callyspongia* extract (%)	Zone of Inhibition (mm)		
		Fresh extract	Activity after 40 d culture	Activity after 75 d culture
Pseudomonas aeruginosa	0.1	7	7	10
	1.0	8	8	12
Vibrio harveyi	0.1	8	7	7
	1.0	10	8	8
Vibrio alginolyticus	0.1	7	-	9
	1.0	8	0	10
Vibrio pelagius	0.1	-	8	8
	1.0	8	11	9

Table 2. Brine shrimp cytotoxicity of cultured *Echinodictyum gorgonoides*

Extract (%)	Lethality (%) in the extract of sponge before introduction for culture (Fresh)	Lethality (%) in sponge extract after 40 d culture	Lethality (%) in sponge extract after 75 d culture
Control	-	-	-
0.1	-	15	5
1	10	30	20
10	25	40	40

Table 3. Antibacterial activity of extract of *Callyspongia subarmigera* cultured in the open sea

Test bacteria (fish pathogenic bacteria)	*Callyspongia* extract (%)	Zone of Inhibition (mm)		
		Fresh extract	Activity after 40 d culture	Activity after 75 d culture
Pseudomonas aeruginosa	0.1	8	7	-
	1.0	9	-	-
Vibrio harveyi	0.1	7	-	7
	1.0	7	7	7
Vibrio alginolyticus	0.1	7	-	7
	1.0	8	9	8
Vibrio pelagius	0.1	11	8	7
	1.0	19	14	8

Table 4. Brine shrimp cytotoxicity of *Callyspongia subarmigera* cultured in the open sea conditions

Extract (%)	Lethality (%) in sponge extract before introduction for culture (Fresh)	Lethality (%) in sponge extract after 40 d culture	Lethality (%) in sponge extract after 75 d culture
Control	-	-	-
0.1	30	20	20
1	50	35	20
10	70	50	35

The results of sponge culture experiments and the data regarding bioactivity suggest that sponge species that produce bioactive compounds can be cultivated in the laboratory with seawater re-circulatory system as well as in open sea mariculture conditions. In both the systems, the sponges retained their health state to a large extent and also their potency to produce bioactive compounds. This is the first experimental results based report of culture of sponges for bioactivity in tropical conditions prevailing in the Indian subcontinent.

It could be inferred that the bioactivity potential of the sponge in culture conditions is determined by the survival and growth, which are influenced by the farming environment. This view is supported by the fact that only *C. subarmigera* could survive and produce bioactive metabolites, as the species was collected from the Vizhinjam coast as well as cultured in the nearby vicinity. The other two species could not survive for long as they were collected and transported from elsewhere. Thus the marine environment influences the biosynthesis and yield of target metabolite. However, it may be too early to conclude about the survival of different species of sponges in culture conditions based on the results of present set of experiments as repeated seasonal trials are yet to be made. Hence, further elaborate culture trials in the open sea conditions are essential along with repeated harvests and their impact on bioactivity pattern. For these, sponges having different bioactivity patterns are to be collected from different locations and cultured in different depths of a select marine habitat. The preliminary studies also made it clear that in order to achieve the maximum production of specific metabolites or molecules from marine sponges or any other organism with bioactivity potentials, it is essential to devise and develop novel culture methods with considerable flexibility. It is also important to develop efficient economic farming technologies before sponge metabolites are needed in commercial qualities and quantities for drug production, thereby ensuring sustained supply.

An overview of marine fish landings in India during 2005 - 2006

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The estimated all India total marine fish landings during the year 2006 was 2.71 million t compared to 2.30 million t in 2005 which showed 18.1% increase The sector-wise contributions in 2005 were, 69.5%, 25.9% and 4.6% by the mechanised, motorised and non-motorised sectors respectively and in 2006 it was 71.1%, 24.1% and 4.8% respectively (Table 1). Trawl nets, gillnets, dol/bagnets and seine nets are the important gears operating along the Indian coasts.

During 2005, south-west region comprising of Kerala, Karnataka and Goa contributed 36% of the total landings, north-west region comprising Maharashtra and Gujarat 31%, south-east region comprising of Andhra Pradesh, Tamil Nadu and Puducherry (Pondicherry) 20% and north-east region comprising of West Bengal and Orissa contributed 13%. During 2006, the contributions from the above regions were 35%, 33%, 22% and 10% respectively (Fig. 1).

Table 1. All India marine fish landings during 2005 and 2006

Sector	Catch / Effort	2005	2006
Mechanised	Landings ('000 t)	1595	1928
	Units (x 000)	2389	2664
Motorised	Landings ('000 t)	594	652
	Units (x 000)	4650	5547
Traditional	Landings ('000 t)	106	130
	Units (x 000)	3161	3025
Total	Landings ('000 t)	2295	2711
	Units (x 000)	10199	11236

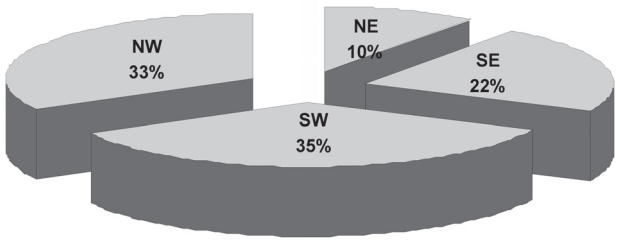

Fig. 1. Region-wise fish production in India during 2006

Oilsardine contributed 14.6% each, ribbonfish 8.7% and 5%, Indian mackerel 5.2% and 5.5%, penaeid prawns 6.4% and 7.5%, non-penaeid prawns 6.3% and 5.3%, Bombayduck 4.4% and 5.3%, croakers 4.4% and 5%, threadfin breams 4.1% and 3.9%, cephalopods 5% and 4.2% respectively