Antibiotic exposure to minimize microbial load in live feed *Isochrysis galbana* used for larval rearing of Indian pearl oyster *Pinctada fucata*

Pearl oyster larvae are reared in static water in dense numbers and fed with large amounts of unicellular algae. The combination of high larval densities, debris from dead larvae and high loads of organic matter stimulates the selection and growth of opportunistic bacteria in larval tanks[1,2]. High microbial load noted in the rearing water, tissue samples and in the micro algal feed, resulted in poor spat production of less than 3.0% in *Pinctada fucata* hatchery[3]. In general, pathogenic microbes invade the hatcheries by three principal routes, viz. sea water, brood stock and algal food[4]. Prophylactic antibiotic usage has been suggested to reduce bacterial load in live feed[5,6]. Here, the exposure time and the minimum dose of chloramphenicol required to reduce the proliferation of bacteria in the mass culture of micro algae, *Isochrysis galbana* is reported.

Experiments were conducted in the Marine Biotechnology Laboratory of Central Marine Fisheries Research Institute, Vizhinjam (South India). *I. galbana* was inoculated in 1 l flasks and maintained under constant illumination for growth. Log phase culture (100 ml) was aseptically dispensed in four 250 ml conical flasks. Chloramphenicol (Hi Media) was added at 10, 100 and 1000 mg/l to each 250 ml conical flask respectively, and one flask was kept as control along with replicates.

The bacterial load in the *I. galbana* culture was determined by the plate count method[7]. The algal samples were collected aseptically at four different time intervals, viz. immediately after the application of antibiotic, and after 3, 6 and 12 h respectively. Each sample was serially diluted, plated in nutrient agar and incubated at 37°C for 24 h. The viability of *I. galbana* was examined using hemocytometer at the respective time interval.

The use of chloramphenicol in *I. galbana* culture resulted in decreased bacterial load with increase in time (Figure 1). After three hours of exposure, 88.7, 90.6 and 94.3% reduction was noted at 10, 100 and 1000 mg/l respectively. The algal cells in the three experimental groups were active. After 6 h of exposure, the reduction in bacterial load was 75, 42 and 93% at 10, 100 and 1000 mg/l respectively. The algal cells at 10 and 100 mg/l were actively moving, while at 1000 mg/l 25% of algae was inactive or dead, indicating the adverse effect of antibiotic (Table 1). In the control group, bacterial population increased with the advancing culture period. The load almost doubled at the end of 12 h, as seen in Figure 1.

Bacterial growth in algal cultures is a complex phenomenon involving quantitative as well as qualitative aspects. Cesar[4] reported high viable counts of bacteria in the phytoplanktonic food for adult oysters and larvae due to their rich source of nutrients. Similar observations of enhanced microbial load in feed and rearing water of *P. fucata* were reported[3,8]. The decomposing cells of micro algae were also reported to be providing a good source for growth of bacteria[9]. Guillard[10] noted that bacteria in micro algal cultures fed to bivalve larvae will adversely affect the larvae.

The results of the present study suggest that 6 h exposure of algal feed, *I. galbana* to chloramphenicol at 10 mg/l is sufficient to reduce the pathogenic bacterial load in rearing medium of pearl oyster larvae without adversely affecting the micro algae.

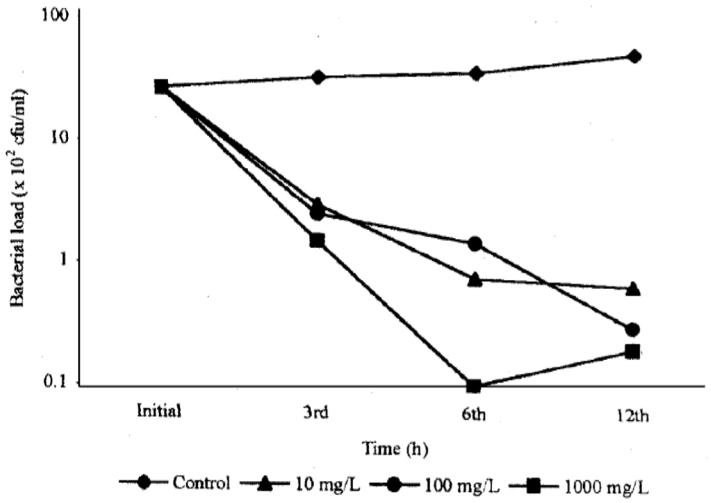

Figure 1. Effect of antibiotic on reduction of bacterial load in *Isochrysis galbana* at different time intervals.

Table 1. Viability of *Isochrysis galbana* in different concentrations of chloramphenicol

Time (h) after exposure	Viability (%) in		
	10 mg/l	100 mg/l	1000 mg/l
3	100	100	100
6	100	100	75
12	100	80	0

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