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Effects of chloramphenicol, erythromycin, and furazolidone on growth of *Isochrysis galbana* and *Chaetoceros gracilis*

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Abstract

This study focused on determining the effects of antibiotics on microalgae used as food for scallop larvae. Six different dose levels of chloramphenicol, erythromycin, and furazolidone were added to cultures of *Isochrysis galbana* and *Chaetoceros gracilis*. An *in vivo* experiment was subsequently conducted to determine the effect of chloramphenicol and erythromycin on larval survival of the Pacific calico scallop *Argopecten ventricosus* in tanks and on the population of its associated bacteria. Results showed that growth of *I. galbana* was not significantly affected by chloramphenicol or erythromycin at the test doses of 0.5, 1.0, 3.0, 6.0, 9.0, and 12.0 mg/l. *C. gracilis* was significantly sensitive to erythromycin and chloramphenicol at doses higher than 0.5 and 3.0 mg/l, respectively. Furazolidone inhibited the growth of both *I. galbana* and *C. gracilis* at all test doses. Results showed that exposure of scallop larvae to a dose of 6 mg/l chloramphenicol or erythromycin did not significantly affect growth of *I. galbana*, significantly enhanced survival of the scallop larvae, and inhibited the growth of *Vibrio* spp. in tanks. This study demonstrated the adverse effect of chloramphenicol, erythromycin and furazolidone on *I. galbana* and *C. gracilis* microalgae but the positive effect on survival of the scallop larvae, decreasing associated bacterial population.

Keywords: Antibiotics; *Isochrysis galbana*; *Chaetoceros gracilis*; *Vibrio* spp.; Mollusk larvae

1. Introduction

Marine microalgae play a key role in aquaculture development (Riquelme and Avendaño-Herrera, 2003). Unicellular marine algae are commonly grown as food for commercially valuable marine organisms (Utting, 1985). The microalgal species *Chaetoceros* sp., *Isochrysis* sp., *Skeletonema* sp., and *Tetraselmis* sp. for instance are frequently used in the culture of marine organisms. *Chaetoceros* sp. and *Isochrysis* sp. have good nutritional qualities, particularly highly unsaturated fatty acids and small cell size, so they are widely used in aquaculture as food in the early larval stages of mollusks, fish, and crustaceans (Godínez et al., 2005).

To enhance survival, rigorous cleaning and aseptic conditions are required. Careful control of the water temperature, salinity, pH, optimization of stocking densities, and balanced nutrition are also very important

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(Beiras et al., 1994; Inglis, 1996). Normally, periodic changes of filtered, sterilized seawater, using filters from 10 to 1 μm and ultraviolet radiation, prevent bacterial infections in larval cultures. Antibiotics, used routinely, accompany these preventive measures. The requirement for use of antibiotics in a culture depends mainly on the quality of the water (Toranzo, 1990). Antibiotics commonly used to avoid the adverse effect of pathogens in aquaculture are furazolidone, chloramphenicol, streptomycin, erythromycin, kanamycin, oxytetracycline, neomycin, and oxolinic acid (Benbroock, 2002). There is widespread concern that antibacterial agents in aquaculture have led to the emergence and selection of resistant bacteria. An improved understanding of how resistance emerges and is selected for among bacteria is essential in evaluating their impact in aquaculture, identifying high risk procedures, and designing ways to reduce the potentially dangerous effects. Bacteria acquire resistance by acquisition of foreign DNA or modification of chromosomal DNA (Inglis, 1996). Despite the risk of selecting for antibiotic-resistant strains and toxicity to the marine organisms, antibiotics are increasingly used. Chloramphenicol is the antibiotic most frequently used in hatcheries (Uriarte et al., 2001). This study evaluated the effect of chloramphenicol, erythromycin and furazolidone on *Isochrysis galbana* and *Chaetoceros gracilis* microalgae, *Argopecten ventricosus* larvae and associated bacterial population.

2. Materials and methods

2.1. Microalgal cultures

I. galbana (strain ISX2) and *C. gracilis* (strain CHX1) were donated by Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE) and cultivated in 125 ml glass flasks containing 100 ml liquid medium (F/2 Guillard medium, 20 psu). Seawater was UV-irradiated and 0.2 μm-filtered. Cultures were grown at 24±0.5 °C under constant illumination. *I. galbana* and *C. gracilis* cells were collected in 5 ml glass tubes, fixed with 1% Lugol's Solution, and counted in a hemacytometer using a phase-contrast microscope (Nikon, Optiphot-2). A calibration curve was set up to determine the growth rate of *I. galbana* and *C. gracilis* during cultivation for 15 days based on sample readings at 750 nm in a Beckman DU 600 spectrometer.

2.2. Antibiotics

Furazolidone (Roberts Laboratories, Inc.), chloramphenicol (Mediatech, Inc.), and erythromycin (Abbott Laboratories) were used in microalgal cultures. A stock solution of each antibiotic was prepared by dissolving 100 mg of the selected antibiotic with 100 ml 0.2 μm-filtered seawater. The concentration of antibiotics in microalgal cultures was adjusted from stock solution.

2.3. Growth of microalgae exposed to antibiotics

Cultures of *I. galbana* and *C. gracilis* were exposed to 0.5, 1.0, 3.0, 6.0, 9.0, or 12.0 mg/l final concentrations of the test antibiotics following the method of Uriarte et al. (2001). An untreated control group was cultured under the same conditions as the treated groups. The microalgae were cultured in triplicate and samples were tested every 48 h for 15 days.

2.4. Broodstock

Healthy adult Pacific calico scallop *A. ventricosus* (shell length, 58.9±3.4 mm) were collected from a culture facility in Bahía de La Paz, near La Paz, Baja California Sur, Mexico and transported to the Universidad Autónoma de Baja California Sur hatchery. They were conditioned for spawning for at least 20 days in a 1500 l fiberglass tank with 0.2 μm-filtered seawater containing 10 mg/l EDTA, under constant aeration at 24±1.0 °C and salinity of 36 ppt (parts per thousand). Filtered seawater was maintained at pH 7.8–8.2. Broodstock were fed 1.5×10^5 cells/ml of a mixture of *I. galbana*, *Chaetoceros calcitrans*, and *C. gracilis* (1:1:2) algae. Tank water was changed at the rate of 50% daily.

2.5. Larvae

Individual scallops were placed in 5 l plastic containers with 1 μm-filtered and aerated seawater, and were induced to spawn by thermal shock, a change from 18 to 28 °C for 20–30 min at each temperature. After spawning, the trochophora stage larvae were cultured in 5000 l fiberglass conical tanks (5 larvae/ml), with constant aeration, filtered seawater at 24±1.0 °C and salinity of 36 ppt. At the veliger stage, a complete water exchange was made every 48 h. Larvae were fed 3.0×10^5 cells/ml *I. galbana* and *C. calcitrans* (1:1).

2.6. Survival of larvae exposed to antibiotics

Based on the foregoing results, the effects of chloramphenicol and erythromycin on survival of the scallop larvae, *A. ventricosus* were evaluated in a bioassay experiment. Groups of larvae (15 larvae/ml) were cultured in 60 l fiberglass tanks under constant aeration.