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Effects of antibacterials use in aquaculture on biogeochemical processes in marine sediment

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Abstract

The effects of chloramphenicol on microorganism in marine sediment were studied by spiked experiments in this paper. The results showed that high concentrations of chloramphenicol could inhibit the activities of microorganism in sediment, and that the growth of strains *Pseudomons* and *Acinetobacter* in sediment that can degrade organic matters were inhibited apparently. Furthermore, the resistance of heterotrophic bacteria in sediment had developed due to the use of antibacterials. Based on the above results potential environmental effects of antibacterials on microorganism in marine sediment were analyzed.

Keywords: Chloramphenicol; Biogeochemical processes; Microorganism; Aquaculture

1. Introduction

Antibacterials are applied to prevent diseases in shrimp hatcheries and salmon aquaculture farms throughout the world (GESAMP, 1997). The potential effects of antibacterials use in aquaculture on aquatic environment and human health are now more and more concerned (Redshaw, 1995; FAO, 1997; Bangkok Post, 2000). The primary environmental and human health issues associated with antibacterials used in coastal aquaculture include residues in organisms, resistance to antibacterials, persistence in aquatic environments and effects on sediment biogeochemistry.

There are abundant beneficial microorganisms in the aquatic environment, such as photosynthetic bacteria and nitrifying bacteria, which are of great importance to maintain the ecological stability and the balance of metabolism. These microorganisms are the key components to keep equilibrium between inner and external micro-ecology of aquatic organisms. The sustainable pressure on environment and ecology is of severity in sediment because antibacterials are apt to accumulate in sediment. Besides inhibiting and killing pathogen, antibacterials have opportunities to inhibit the activity of beneficial microbial, which will reduce the rate of organic matter degradation (GESAMP, 1997) and disturb cycling of material and energy flow of ecological system.

Hansen et al. (1992) found 40–50% reduction in microbial density and a >90% decrease in activity (as measured by sulphate reduction rates) with the addition of 100–400 mg/kg of oxytetracycline, oxolinic acid and flumequine. Conversely, the results of the field and lab studies on salmon net-cages aquaculture showed that oxytetracycline residues 1~4 mg/kg in the sediment had

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no effect on the microbial density or activity, ammonia flux, sulphate concentration in interstitial water and oxygen consumption in sediment (Capone et al., 1994; Herwig and Gray, 1997; Herwig et al., 1997). The antibacterials (including oxytetracycline, sulfadimethoxine and ormetoprim in Romet®30) had no apparent effects on the above parameters in sediment when they were employed at certain rate.

Water, sediment and fish from various farms have been evaluated. It has not only been shown that the sediment near some fish farms that have used a great amount of antibiotics harbors a higher frequency of antimicrobial resistance than surrounding farms using a smaller amount of antibiotics but also that some farmed fish carry a larger number of bacteria with individual and multiple antibiotic resistance (Russell et al., 1997).

Although chloramphenicol was banned to be used in the law, it is still used in some developing countries. The environmental issues associated with chloramphenicol, especially the environmental and ecological issues associated with chloramphenicol use, are rather too neglected. The intensity and mechanism of effects of chloramphenicol on microorganism in marine sediment were examined by spiked experiments in this paper. In addition, the resistance status of bacteria in Dalian aquaculture farms to chloramphenicol was analyzed.

2. Material and methods

2.1. Effects of chloramphenicol on bacterial density

Sediment samples were collected from the surface of tidal zone in Dalian, China. They were sieved with stainless steel wire screen (mesh size, 1×1 mm^2) to remove large debris. Chloramphenicol solution was added into sediment samples to final concentrations of 100 mg/kg and 1000 mg/kg, at the same time a blank control treatment with no chloramphenicol was set up. Each treatment had three replications. All sediment samples were incubated at room temperature, and subsamples were collected after 1, 4, 8, 15, 22 and 28 days for the analysis of the bacterial density. For the isolation of bacteria, sediment dilutions were plated on 2216E plates and incubated at 28 °C for 2 days, then the bacterial density was analyzed (GB 17378.7, 1998).

2.2. Effects of chloramphenicol on growth of bacteria

The two species of bacteria used in this experiment were isolated from petroleum-polluted sediment, which grew on benzene as the sole carbon source. They were identified as *Pseudomons* and *Acinetobacter* by biochemical tests respectively. The bacteria strains solutions were added into the 2216E liquid media with 0 mg/L, 10 mg/L, 50 mg/L, 100 mg/L and 200 mg/L of chloramphenicol respectively, and were incubated in thermostatic oscillator at 150 rpm at 28 °C.

The absorbency (OD) values at 550 nm were determined to character the inhibition of chloramphenicol to the growth of two species of bacteria.

2.3. Resistance to chloramphenicol of heterotrophic strains in sediment

Sediment samples were collected from six typical aquaculture farms in coastal areas in Dalian to analyze resistance of strains. Stations 1 and 2 were prawn farms that have used a great amount of chloramphenicol before; station 3 was natural aquaculture farm that have never used antibacterials; stations 4 and 5 were *Paralichthys olivaceus* farms that have used a great amount of other antibacterials such as oxytetracycline regularly; and station 6 was prawn farm that was not known if have used antibacterials before.

For the isolation of chloramphenicol-resistant heterotrophic bacteria, sediment dilutions were plated on 2216E plates supplemented with 12 mg/L of chloramphenicol, 2216E plates with no chloramphenicol as control.

3. Results

3.1. Effects of chloramphenicol on the dynamic change of bacterial density in sediment

The results of the sediment incubation experiment showed (Fig. 1) that the bacterial density of all

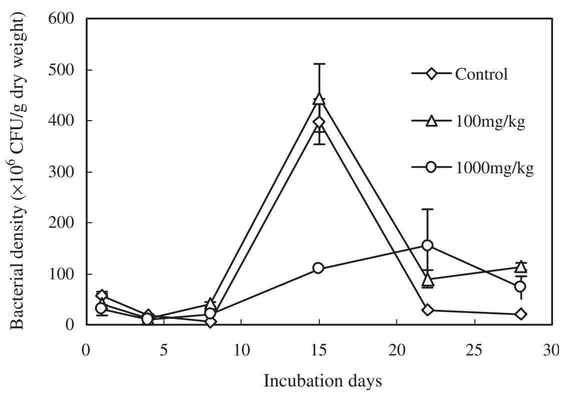

Fig. 1. Dynamic change of bacterial density in sediment.