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Changes in microbial communities associated with the conditioning of filter material in recirculating aquaculture systems of the pufferfish *Takifugu rubripes*

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Abstract

We investigated changes in microflora associated with the conditioning of filter material in a recirculating aquaculture system for the culture of the pufferfish *Takifugu rubripes* using a clone library method of partial 16S rRNA gene sequences. Total bacteria on the pebbles used as filter material increased from 8.4×10^{9} cells g^{-1} at peak ammonia concentrations (8 days) to 1.3×10^{10} cells g^{-1} at the end of the study (44 days). As filter material became conditioned, the occurrence of Clostridia, α-Proteobacteria and γ-Proteobacteria on the surface of the pebbles increased, whereas Flavobacteria, Sphingobacteria and Mollicutes decreased. The occurrence of ammonia-oxidizing bacteria related to *Nitrosomonas* decreased from 3.00% at day 8 to 0.95–1.04% at days 15–44. Phylogenetic analysis of the clones related to the genus *Nitrosomonas* using a Bayesian method revealed that two clones obtained in this study formed a cluster with *N. aestuarii* in the *N. marina* sublineage of the *N. oligotropha* lineage, whereas another two clones formed a cluster with *Nitrosomonas* sp. Nm143 of the *Nitrosomonas* sp. Nm143 lineage with high Bayesian posterior probabilities support. Two clones formed a separate cluster from those of the other *Nitrosomonas* lineages. Our results demonstrated the importance of effective utilization of nitrifying bacteria in aquaculture, since number of these bacteria did not vary for the duration of the experiment.

Keywords: Nitrifying bacteria; *Nitrosomonas*; Recirculating aquaculture systems; Filter material; Microflora

1. Introduction

Aquaculture has considerable potential for meeting the increasing demand for aquatic products in many regions of the world. According to the latest Food and Agriculture Organization (FAO) statistics, the contribution of aquaculture to global supplies of fish, crustaceans and mollusks has increased from 3.9% of total production by weight in 1970 to 29.9% in 2002 (FAO Fisheries Department, 2004). Aquaculture is undertaken using various systems including cage culture, pond culture, recirculating water systems, longlines and flow-through systems. Recirculating aquaculture systems have been widely applied to the culture of aquatic animals in various regions of the world, since the systems reuse water with mechanical and biological treatment between each use. This is primarily due to the benefits associated with their operation, including the optimal use of space, lower water requirements

Abbreviations: DAPI, 4′, 6-diamidino-2-phenylindole; PCR, polymerase chain reaction; rRNA, ribosomal RNA.

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compared to conventional aquaculture, capacity for high stocking densities and provision of a predictable and stable environment for the culture species.

However, the mineralization of organic matter associated with the decomposition of unconsumed food, dead aquaculture organisms and their feces is one of the most important parameters in recirculating aquaculture systems. The nitrogen in the proteins of these substances is decomposed to ammonia by proteases and deaminases produced by heterotrophic bacteria in the material of the filter. In addition, the fish also excrete ammonia directly. This toxic ammonia is converted into nitrate via nitrite by nitrifying bacteria that oxidize ammonia and nitrite mainly in the filter material (Kawai et al., 1964; Sugahara et al., 1974; Midlen and Redding, 1998). A diversity of microbial populations is involved in nitrification, ammonification, nitrate reduction, denitrification, proteolysis and sulfate reduction on the filter material of the recirculating aquarium (Kawai et al., 1964; Sugahara et al., 1974). Thus, while it is known that the water quality of recirculating aquaculture systems is maintained by a diversity of microbial communities in the filter material, the difficulties associated with their cultivation (0.001–1%) have meant that the majority of these microbes have not yet been investigated in detail (Amann et al., 1995).

However, the applicability of 16S rRNA gene analysis to bacterial taxonomy and prokaryotic phylogenetics has been demonstrated (Woese, 1987) and the extensive database currently available for 16S rRNA gene sequences has facilitated detailed analyses of the phylogenetic relationships among previously unidentified bacteria. One of the most widely adopted approaches for the examination of bacterial diversity is based on the screening of clone libraries of the 16S rRNA gene, typically collected from naturally occurring bacteria and amplified using the polymerase chain reaction (PCR) with primers that are specific for the 16S rRNA gene (O'Sullivan, 1999; Cottrell and Kirchman, 2000). We previously demonstrated the utility of clone library construction for assessing the bacterial composition of the intestinal microflora, specifically the spirochetes and members of the genus *Vibrio*, in the pufferfish *Takifugu niphobles* (Shiina et al., 2006).

In a study of the microbial communities associated with well-conditioned filter material in recirculating aquaculture systems for carp *Cyprinus carpio* and goldfish *Carassius auratus* using the clone library method, we found that a nitrite-oxidizing bacteria, *Nitrospira*, comprised 8.0–9.8% of the bacterial communities in the filter material (Sugita et al., 2005). Interestingly, no information of the bacterial communities on the filter-materials in recirculating aquaculture systems for marine fish species has been reported to date. We investigated changes in microbial communities associated with the conditioning of filter material in recirculating aquaculture systems of the pufferfish, *Takifugu rubripes*, one of the most important marine aquaculture species in Japan.

2. Materials and methods

2.1. Experimental aquarium and tank

As shown in Fig. 1, a glass aquarium (60 × 35 × 30 cm) with a recirculating system was filled with 50 l of seawater collected at Shimoda, Shizuoka, Japan. A total of 4.0 kg of pebbles (c.a. 5 mm diam.; 0.2 g a pebble) were subjected to heating at 400 °C for 3 h to remove any organic material before being washed with distilled water.

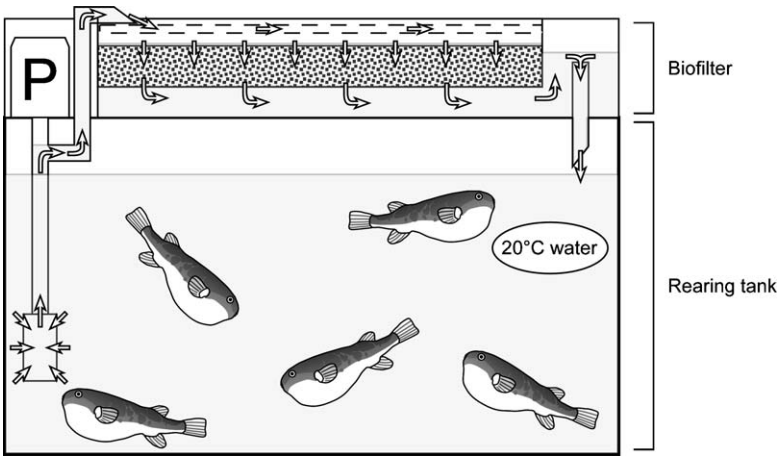

Fig. 1. The recirculation aquaculture system used in this study. A total of 4.0 kg of pebbles were used as the filter material, and the flow rate and temperature of the water were maintained at 8 l min^{-1} and 20 °C, respectively. Arrows represent flows of recirculating water. P, recirculating pump.