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ORIGINAL ARTICLE

Diversity of purple nonsulfur bacteria in shrimp ponds with varying mercury levels

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Abstract This research aimed to study the diversity of purple nonsulfur bacteria (PNSB) and to investigate the effect of Hg concentrations in shrimp ponds on PNSB diversity. Amplification of the *pufM* gene was detected in 13 and 10 samples of water and sediment collected from 16 shrimp ponds in Southern Thailand. In addition to PNSB, other anoxygenic phototrophic bacteria (APB) were also observed; purple sulfur bacteria (PSB) and aerobic anoxygenic phototrophic bacteria (AAPB) although most of them could not be identified. Among identified groups; AAPB, PSB and PNSB in the samples of water and sediment were 25.71, 11.43 and 8.57%; and 27.78, 11.11 and 22.22%, respectively. In both sample types, *Roseobacter denitrificans* (AAPB) was the most dominant species followed by *Halorhodospira halophila* (PSB). In addition two genera, observed most frequently in the sediment samples were a group of PNSB (*Rhodovulum kholense*, *Rhodospirillum centenum* and *Rhodobium marinum*). The UPGMA dendrograms showed 7 and 6

Abbreviations: APB, anoxygenic phototrophic bacteria; AAPB, aerobic anoxygenic phototrophic bacteria; PNSB, purple nonsulfur bacteria; PSB, purple sulfur bacteria

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clustered groups in the water and sediment samples, respectively. There was no relationship between the clustered groups and the total Hg (Hg_T) concentrations in the water and sediment samples used ($<$ 0.002–0.03 μg/L and 35.40–391.60 μg/kg dry weight) for studying the biodiversity. It can be concluded that there was no effect of the various Hg levels on the diversity of detected APB species; particularly the PNSB in the shrimp ponds.

1. Introduction

Shrimp farming is one of the most important aquacultural activities in Asia and South America, but farmers face many serious problems like shrimp diseases partly due to problems with the shrimp having to cope with poor water quality during cultivation (Rattanachuay et al., 2011). Only 20–30% of the nitrogen supplied from their feed is converted into shrimp biomass, while the rest accumulates at the bottom of the shrimp ponds (Kutako et al., 2009) and is converted anaerobically by microbes with the production of ammonia and also hydrogen sulfide (H_2S). Both these compounds are toxic for shrimp (Rao et al., 2000; Antony and Philip, 2006); this means that they should be controlled at levels that are safe for shrimp growth. Among the microbes associated with the carbon, nitrogen and sulfur cycles, of shrimp ponds, purple nonsulfur bacteria (PNSB) are probably the most useful to improve the water quality during shrimp cultivation (Antony and Philip, 2006; Zhou et al., 2009). PNSB are versatile organisms able to grow with photoautotrophic or photoheterotrophic or heterotrophic conditions depending on the availability of light, oxygen and a suitable source of carbon. This means that they can consume organic matter under light and dark conditions and some can also remove H_2S (Antony and Philip, 2006; Kornochalert et al., 2014).

Normally, PNSB exist in illuminated anoxic habitats in nature such as in aquatic sediments, of freshwater rivers and lakes, and wastewater treatment systems including shrimp ponds (Panwichian et al., 2010). PNSB are also considered to be beneficial microbes as their cells have a high protein content, they produce essential amino acids, and contain a high content of vitamin B12, ubiquinone and carotenoids (Shapawi et al., 2012; Kornochalert et al., 2014), so they have a great potential for use as probiotics in aquaculture (Shapawi et al., 2012). However, very little work has been published on the diversity of PNSB in shrimp ponds; hence it is worthwhile to study their diversity in shrimp ponds in order to gain knowledge that might be useful for finding a use for them during shrimp cultivation.

The sequencing of the *pufM* gene that codes for the M subunit of a pigment-binding protein in the photosynthetic reaction center has been used to study the diversity and phylogenetic composition of anoxygenic phototrophic bacteria (APB) such as the purple sulfur bacteria (PSB, *γ-Proteobacteria*), PNSB (α-, *β-Proteobacteria*) and aerobic anoxygenic phototrophic bacteria (AAPB) (*α-Proteobacteria*) (Yutin et al., 2007; Asao et al., 2011). This is because this gene is part of the unique *puf* operon that codes for the subunits of the light-harvesting complex (*pufB* and *pufA*), and the reaction center complex (*pufL* and *pufM*) (Beja et al., 2002) present in all purple bacteria.

It is well recognized that shrimp cultivation in Thailand has occurred over at least the last 30 years (Lebel et al., 2010), and shrimp ponds are generally located near coastal areas of the Thai peninsular because natural seawater is required for shrimp cultivation. Use of untreated seawater for shrimp cultivation may cause problems if the seawater is contaminated especially with heavy metals including mercury (Hg) and other pollutants (Thongra-ar and Parkpian, 2002; Cheevaporn and Menasveta, 2003; Panwichian et al., 2010). In some cases the pollutants have accumulated in situations where the shrimp ponds have been used over a long period of time. In addition Hg contamination in the shrimp ponds has been caused by impurities in agricultural fertilizers, lime and chemicals used during shrimp cultivation (Lacerda et al., 2011). The contamination of heavy metals; particularly Hg should be of concern because it is known to have a major effect on the activities of microbes and the biogeochemical processes which they mediate (Harris-Hellal et al., 2009). Hence, Hg contamination in long-term cultivated shrimp ponds could affect the dynamics and the diversity of bacterial communities during shrimp cultivation, in particularly PNSB. As far as we know this is the first report on the effects of Hg concentrations on PNSB in shrimp ponds. With regard to the above information, this work aimed to study the diversity of PNSB in shrimp ponds using the specific *pufM* gene for understanding which species of PNSB were dominant. This might help us to understand their physicochemical properties and roles and propose ways for them to be used optimally to facilitate the cultivation of shrimp in shrimp ponds. This also includes the influence of Hg concentrations on PNSB diversity and their ability to facilitate shrimp growth in the presence of Hg.

2. Materials and methods

2.1. Study areas and samples collecting in shrimp ponds

Water and sediment samples were collected from 16 shrimp ponds used for cultivating white shrimp (*Litopenaeus vannamei*) with different lengths of operation times in the coastal areas of the Thai Peninsular (Gulf of Thailand and Andaman sea). These shrimp ponds were in the districts of Ranot (RN: RN1 and RN2), Tepa (TP: TP1, TP2 and TP3) and Sating Phra (ST: ST1, ST2 and ST3), in Songkhla Province; the districts of Pak Panang (PN: PN1, PN2, PN3 and PN4) in Nakhon Si Thammarat Province; Kantang (KT: KT2), Sikao (SK) and Yan Ta Khao (YT) in Trang Province and Mueang (PT) in Pattani Province. Therefore, the 16 shrimp ponds were in eight districts; and all those in the same district were cultivated by the same owner. Hence, the operation and management systems for shrimp cultivation in the same district were not different. During intensive shrimp cultivation,