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Effects of dietary live or heat-inactivated autochthonous *Bacillus pumilus* SE5 on growth performance, immune responses and immune gene expression in grouper *Epinephelus coioides*

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Abstract

To evaluate the possible dietary application of live and heat-inactivated probiotic *Bacillus pumilus* SE5 in grouper *Epinephelus coioides*, juveniles (14.6 ± 0.2 g) were fed either a basal control diet (without probiotic) or the basal diet supplemented with 1.0×10^8 CFU g^{-1} live (T1) and heat-inactivated *B. pumilus* SE5 (T2). The heat-inactivated probiotic significantly improved the final weight, weight gain (WG) and specific growth rate (SGR) at day 60 and significantly decreased the feed conversion ratio (FCR) at day 30 and 60, while the viable probiotic significantly decreased the FCR at day 60 ($P < 0.05$). Phagocytic activity, serum complement C3 and IgM levels as well as SOD activity elevated significantly in fish fed the heat-inactivated probiotic for 60 days ($P < 0.05$). Furthermore, the heat-inactivated probiotic remarkably up-regulated expression of TLR2 and pro-inflammatory cytokines (IL-8 and IL-1β) in head kidney ($P < 0.05$), but the viable probiotic failed to do so. These results indicated that heat-inactivated *B. pumilus* SE5 can effectively improve the growth performance and immune responses of *E. coioides*.

KEY WORDS: *Bacillus pumilus*, *Epinephelus coioides*, growth performance, heat-inactivated probiotic, immune gene expression, immune response

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Introduction

Groupers of the genus *Epinephelus* are economically valued fish in East Asian regions for excellent biological characteristics, such as fast growth and popular taste (Hseu *et al.* 2003; Sun *et al.* 2010). Grouper farming has suffered from several diseases, such as nervous necrosis virus (NNV)-related disease (Kuo *et al.* 2012) as well as vibriosis caused by pathogenic *Vibrio* species (Harikrishnan *et al.* 2010a). During the past few years, antibiotics and chemical prophylaxis have been used to control the diseases, but due to the emergence of bacterial resistance and environmental threat, these are no longer recommended in practice. Therefore, the use of probiotics has been suggested as an alternative method for the prevention and control of various diseases in fish (Merrifield *et al.* 2010; Pérez-Sánchez *et al.* 2011). Currently, several studies have demonstrated the potential application of probiotics in grouper (Chiu *et al.* 2010; Liu *et al.* 2012; Sun *et al.* 2013). Dietary administration of *Saccharomyces cerevisiae* and *Bacillus subtilis* E20 enhanced growth and immune response and resistance against *Streptococcus sp.* and an iridovirus in grouper (*Epinephelus coioides*) (Chiu *et al.* 2010; Liu *et al.* 2012). Sun *et al.* (2013) reported that *Bacillus pumilus* – or *B. clausii*-containing diets contribute to the improvement of health and the development of gastrointestinal (GI) tract in larval *E. coioides*.

Probiotics are usually members of the normal indigenous microbiota, which play an important role in fish health, and their addition can assist in returning a disturbed gut microbiota to normal beneficial composition (Merrifield *et al.* 2010). Probiotics have also been reported to enhance