Available online at www.sciencedirect.com

ScienceDirect

Agriculture and Agricultural Science Procedia 11 (2016) 117 – 124

Agriculture and Agricultural Science Procedia

International Conference on Inventions & Innovations for Sustainable Agriculture 2016, ICIISA 2016

Effect of Green Tea Extract on *Vibrio parahaemolyticus* Inhibition in Pacific White Shrimp (*Litopenaeus vannamei*) Postlarvae

Pawapol Kongchum[a,]*, Suphavadee Chimtong[a], Nantanat Chareansak[a], Papimon Subprasert[a]

[a] *Faculty of Animal Sciences and Agricultural Technology, Silpakorn University, Phetchaburi IT Campus, Cha-am, Phetchaburi 76120, Thailand*

Abstract

Inhibitory effect of green tea against *Vibrio parahaemolyticus* was assessed to determine its potential as antibacterial agent for Pacific white shrimp culture. Aqueous extracts obtained by boiling or soaking tea leaves in hot water could inhibit the growth of *V. parahaemolyticus*. Inhibition zone diameters, carried out by agar well diffusion method with $1x10^6$ CFU.mL^{-1} of tested bacteria in agar medium, ranged from 14.4 to 16.4 mm. The minimum inhibitory concentration (MIC) determined by broth dilution method during 24-hour incubation was 10% (v/v). G reen tea extract (GTE) was effective on reducing mortality of shrimp postlarvae challenged with *V. parahaemolyticus* at 10^4 CFU.mL^{-1}. Survival rate of shrimp reared in water treated with 1 mL.L^{-1} GTE (80±5.4%) was higher ($P<0.05$) than that of control (70±2.04%). Total Vibrio counts of whole shrimp, estimated 5 days after the postlarvae were challenged with *V. parahaemolyticus* at 10^6 CFU.mL^{-1}, were $6.4x10^6$ and $2.3x10^6$ CFU.g^{-1} in control and GTE-treated shrimp, respectively. Results of this study suggest that green tea is a promising natural antibacterial agent that can be used for *V. parahaemolyticus* control during the nursery phase of Pacific white shrimp.

Peer-review under responsibility of the Faculty of Animal Sciences and Agricultural Technology, Silpakorn University

Keywords: plant extract; natural antibacterial agent; *Camellia sinensis*; Vibrio infection; shrimp mortality

* Corresponding author. Tel.: +66 32 594037; fax: +66 32 594037.
E-mail address: kongchum_p@silpakorn.edu

Peer-review under responsibility of the Faculty of Animal Sciences and Agricultural Technology, Silpakorn University
doi:10.1016/j.aaspro.2016.12.020

1. Introduction

The Pacific white shrimp, *Litopenaeus vannamei* (Boone, 1931) is a tropical marine species native to the Eastern Pacific coast of Mexico and Central and South America (FAO undated). It is one of the most important farmed species in Latin America and Asia. The world's aquaculture production of Pacific white shrimp has increased steadily in the past years from 1.31 million tons in 2004 to over 3 million tons in 2012 (FAO, 2014).

Intensification in shrimp aquaculture has resulted in disease outbreaks causing massive mortality of cultured penaeid shrimp. These incidents have been reported from many shrimp producing countries and are considered a major constraint to the shrimp production that have caused significant socio-economic losses in the affected areas (Moss et al., 2012; Kumar et al., 2014). Stentiford et al. (2012) estimated that 60% of losses due to diseases in shrimp aquaculture were caused by viral pathogens with a further 20% by bacterial pathogens. However, bacterial diseases have gained more attention in recent years since the causative agent of the early mortality syndrome (EMS) or acute hepatopancreatic necrosis syndrome (AHPNS), an emerging destructive disease that caused an unusual acute mortality in Pacific white shrimp and black tiger shrimp within approximately 35 days after stocking in grow-out ponds, has been identified as *V. parahaemolyticus* (Tran et al., 2013; Joshi et al., 2014). *V. parahaemolyticus* is a Gram-negative, halophilic bacterium that occurs naturally in marine and estuarine environments (Daniels et al., 2000). It is a major *Vibrio* spp. that causes vibriosis in aquaculture species and is usually associated with shrimp diseases. *V. parahaemolyticus* has been reported as the major etiological agent for red disease in black tiger shrimp and had concurrent infections with white spot syndrome virus (Jayasree et al., 2006). The presence of *V. parahaemolyticus* has been associated with necrosis, slow growth, muscle opacity, anorexia, and mortality of shrimp during nursery rearing (Aguirre-Guzmán et al., 2010). Recently, Zhang et al. (2014) reported that *V. parahaemolyticus* is a predominant vibrio species identified in the mass mortality of cultured juvenile Chinese shrimp, *Fenneropenaeus chinensis*.

The heavy use of antibiotics for disease treatment in aquatic animals has resulted in the emergence of antibiotic-resistant pathogens in aquaculture environments making the antibiotic treatment ineffective and this type of incident has been reported from all areas of aquaculture (Immanuel et al., 2004). Moreover, there is a growing concern over the risks of the transfer of resistance determinants to bacteria of land animals and to human pathogens and the presence of antibiotic residues in aquaculture products that constitutes threats to public health (Cabello, 2006; Defoirdt et al., 2007). Thus, alternatives to antibiotic treatment are needed to make aquaculture industry more sustainable and to assure that the produce is safe for human consumption.

Over the past few years, many researchers have focused on the application of natural antibacterial compounds, especially plant extracts for the treatment of aquatic animal diseases (Sudheer et al., 2011; Caruana et al., 2012; Chang et al., 2013; Rattanavichai and Cheng, 2014; Talpur, 2014; Acar et al., 2015; Sivagnanavelmurugan et al., 2015; Thanigaivel et al., 2015; Dhayanithi et al., 2015). Among the plant extracts that were studied, green tea, *Camellia sinensis* has been proved for its antibacterial activity against broad spectrum of Gram-positive and Gram-negative bacteria (Yiannakopoulou, 2012) and *V. parahaemolyticus* (Toda et al., 1989; Xi et al., 2012). However, the effect of GTE on inhibition of *V. parahaemolyticus* and reduction of Pacific white shrimp mortality during the nursery phase has not yet been studied.

The purpose of this study was to investigate the inhibitory effect of green tea against pathogenic *V. parahaemolyticus* isolated from farmed Pacific white shrimp for possible use as a natural antibacterial agent in shrimp aquaculture.

2. Materials and Methods

2.1 Bacterial culture preparation

V. parahaemolyticus used in this study was isolated from the hepatopancreas of EMS/AHPND-diseased *Litopenaeus vannamei* collected from shrimp farm in the inner Gulf of Thailand. A pure culture from agar slant was streaked onto tryptic soy agar supplemented with 1% NaCl (TSA-salt) plate and incubated at 37°C for 24 h. A single colony on TSA-salt plate was inoculated into 5 mL tryptic soy broth supplemented with 1% NaCl (TSB-salt) and incubated at 37°C. After 6 h of incubation, one mL of the medium was transferred to 100 mL of TSB-salt and incubated at 37°C for 18 h. The culture was centrifuged (3,000 rpm, 5 min at 5°C) to collect bacterial cells and the cells were resuspended with sterile 1% NaCl solution. Cell concentration (colony-forming unit; CFU) was estimated