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Silencing tyrosine hydroxylase retards depression of immunocompetence of *Litopenaeus vannamei* under hypothermal stress

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ABSTRACT

Tyrosine hydroxylase (TH), the first and rate-limiting step in the synthesis of catecholamines, is required in catecholamine synthesis of the neuroendocrine regulatory network against stress in shrimp. The immunocompetence, catecholamine biosynthesis, and carbohydrate metabolites were evaluated in *Litopenaeus vannamei* received *L. vannamei* TH (LvTH) double-stranded (ds)RNA, diethyl pyrocarbonate-water, or non-targeted dsRNA for 3 days then transferred from 28 to 20 or 28 °C. The immunocompetence of LvTH-depleted shrimp held at 28 °C was promoted, and those were downregulated under hypothermal stress and revealed higher level than the other two dsRNA treatments. Meanwhile, the decrease of catecholamine biosynthesis was observed in LvTH-depleted shrimp held at 28 °C, and those were elevated under hypothermal stress and revealed lower levels, compared to two dsRNA treatments. The reduced carbohydrate metabolites was observed in LvTH-depleted shrimp held at 28 °C, and those were upregulated under hypothermal stress and showed lower levels than the other two dsRNA treatments. It was therefore concluded that LvTH-depleted shrimp revealed enhanced immunocompetence and reduced carbohydrate metabolites when exposed to a hypothermal stress condition, and in the meantime, even though catecholamine biosynthesis was downregulated, no significant difference was observed in DA or NE levels.

1. Introduction

The Pacific white shrimp, *Litopenaeus vannamei* (Boone), which has become the major cultivated decapod species in the world, is native to Eastern Pacific coasts. Epidemics associated with viruses and vibriosis have caused serious economic losses to commercial white shrimp farms. Cultured shrimp consistently encounter various environmental stressors especially in rapidly degraded environments of intensive culture systems, and those affect the survival, growth, and physiological and immunological responses. Relationship of stress and physiology with immune resistance are of primary concern.

Varying water temperatures are the most-general stressor of aquatic organisms. At temperatures of 20 and 30 °C and salinities above 20‰, *L. vannamei* revealed the best survival [1], and the growth directly increases in the range of 23–30 °C at 33‰ salinity [2]. In crustaceans, the primary response to stress is the release of catecholamines (CAs) to regulate homeostasis, and then induce secondary responses including hyperglycemia and immune suppression [3,4]. In our previous study, hypothermal stress was found to cause increases in dopamine (DA) and norepinephrine (NE) in *L. vannamei* [5]. Weakened immune responses with increased susceptibility to *Vibrio alginolyticus* infection [6,7], and also a transient period of modulations in energy metabolism, osmoregulation, and respiratory responses were noted when *L. vannamei* received DA or NE via an injection [8,9]. Those results indicate that hypothermal stress-induced catecholamine biosynthesis has a massive influence on immunocompetence and physiological responses in *L. vannamei*.

Tyrosine hydroxylases (THs) are highly conserved enzymes that catalyze the conversion of L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA), which is the precursor for the neurotransmitters, DA, NE, and epinephrine (EP, adrenaline) [10–12]. TH messenger (m)RNA expression can be upregulated by lipopolysaccharide (LPS) in rat phagocytes and *Galleria mellonella* hemocytes, and by an immune challenge in *Manduca sexta* hemocytes and fat bodies [13]. In our previous study, TH expression in the brain and hemocytes, and TH activity and glucose level in hemolymph significantly increased in *L. vannamei* subjected to a

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