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The Thesis Submitted for the Degree of M.Sc
(in the field of Genetic)

Title

The effect of temperature changes on expression of *HSP20* gene and detection of different *Vibrio* species in *Pinctada radiata*

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ABSTRACT:

Edible oysters are one of the most important marine farming organisms that their consumption in raw or semi-cooked form by humans causes disease, one of the causes of which is the presence of pathogenic *Vibrio* bacteria in oysters. Physiological parameters of bivalve molluscs such as reproduction, metabolism, safety parameters, etc. in mollusks are very sensitive to temperature changes. The *HSP*¹ gene consists of several families that are affected when aquatic organisms are exposed to stressful conditions (salinity, temperature, etc.). The aim of this study was to investigate the expression of *HSP20*, *HSP70* and *HSP90* genes in the gill tissue of oyster shells under temperature treatments of 22, 25, 27 and 29 °C and the development of *Vibrio* bacteria. Gene expression was studied by real time PCR technique and *GADPH* control gene were used in data normalization. Data analysis was performed using SAS software to investigate the presence or absence of significant differences between means (at a significant level of 5%). The results of the analysis showed that the expression of *HSP20*, *HSP70* and *HSP90* genes at 25 °C was significantly increased compared to the control and the highest expression was observed in ocular gill tissue at 25 °C. Also for *HSP20* and *HSP70* genes the lowest expression was observed at 29 °C, while for *HSP90* gene the lowest expression was observed at 27 °C. According to this study, the incidence of *Vibrio* bacteria in temperature treatments of 22, 25, 27 and 29 °C in oysters was investigated using *16S rRNA* gene amplification. *16S rRNA* gene sequencing was performed and sequence alignment analysis was performed using sequence BLAST in NCBI database. The alignment results of *16S rRNA* gene sequence showed 100, 100, 99.92, 99.99 similarity of PM1, PM3, PM5, PM10 and PM19 strains with *Vibrio anguillarum*, *Vibrio parahaemolyticus*, *Vibrio harveyi* NBRC 15634 *Vibrio harveyi* NCIMB1280 and *Vibrio alginolyticus*. Isolation and counting of *Vibrio* bacteria was performed in each temperature treatment. According to the results, with decreasing

¹ Heat Shock Protein

temperature from 29 to 22 ° C, the frequency of isolates belonging to *V. parahaemolyticus* decreased from 30% to 12% and the frequency of isolates belonging to *V. alginolyticus* decreased from 25% to 10%. The prevalence of other non-pathogenic species of *Vibrio* increased from 15% at 29 ° C to 40% at 22 ° C. Therefore, isolates of *V. harveyi* had the highest frequency at 22 ° C and the lowest frequency at 29 ° C. According to the results of this study, temperature was effective in the expression of *HSP20*, *HSP70*, *HSP90* and *Vibrio* bacteria.

Keywords: Oyster, Temperature, *HSP20* genes, *HSP70*, *HSP90*, *Vibrio* bacteria, Real Time PCR