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The Immunology Evolutionary Relationship between Signal Transducer and Activator of Transcription Genes from Three Different Shrimp Species in Response to White Spot Syndrome Virus Infection

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Abstract : Unlike the common presence of both innate and adaptive immunity in vertebrates, crustaceans, in particular, shrimps, have been discovered to possess only innate immunity. This further emphasizes the importance of innate immunity within shrimps in pathogenic resistance. Under the study of pathogenic immune challenge, different shrimp species actually exhibit varying degrees of immune resistance towards the same pathogen. Furthermore, even within the same shrimp species, different batches of challenged shrimps can have different strengths of immune defence. Several important pathways are activated within shrimps during pathogenic infection. One of them is JAK-STAT pathway that is activated during bacterial, viral and fungal infections by which STAT(Signal Transducer and Activator of Transcription) gene is the core element of the pathway. Based on theory of Central Dogma, the genomic information is transmitted in the order of DNA, RNA and protein. This study is focused in uncovering the important evolutionary patterns present within the DNA (non-coding region) and RNA (coding region). The three shrimp species involved are Macrobrachium rosenbergii, Penaeus monodon and Litopenaeus vannamei which all possess commercial significance. The shrimp species were challenged with a famous penaeid shrimp virus called white spot syndrome virus (WSSV) which can cause serious lethality. Tissue samples were collected during time intervals of 0h, 3h, 6h, 12h, 24h, 36h and 48h. The DNA and RNA samples were then extracted using conventional kits from the hepatopancreas tissue samples. PCR technique together with designed STAT gene conserved primers were utilized for identification of the STAT coding sequences using RNA-converted cDNA samples and subsequent characterization using various bioinformatics approaches including Ramachandran plot, ProtParam and SWISS-MODEL. The varying levels of immune STAT gene activation for the three shrimp species during WSSV infection were confirmed using qRT-PCR technique. For one sample, three biological replicates with three technical replicates each were used for qRT-PCR. On the other hand, DNA samples were important for uncovering the structural variations within the genomic region of STAT gene which would greatly assist in understanding the STAT protein functional variations. The partially-overlapping primers technique was used for the genomic region sequencing. The evolutionary inferences and event predictions were then conducted through the Bayesian Inference method using all the acquired coding and non-coding sequences. This was supplemented by the construction of conventional phylogenetic trees using Maximum likelihood method. The results showed that adaptive evolution caused STAT gene sequence mutations between different shrimp species which led to evolutionary divergence event. Subsequently, the divergent sites were correlated to the differing expressions of STAT gene. Ultimately, this study assists in knowing the shrimp species innate immune variability and selection of disease resistant shrimps for breeding purpose. The deeper understanding of STAT gene evolution from the perspective of both purifying and adaptive approaches not only can provide better immunological insight among shrimp species, but also can be used as a good reference for immunological studies in humans or other model organisms.

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