Thesis Title: Development of Nucleic Acid Probe for *Agmasoma penaei* (Microsporidian) Infection in Penaeid Shrimp

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Abstract

The intermediate host required for transmission of a common microsporidian (*Agmasoma)* parasite of the penaeid shrimp, *Penaeus merguiensis* and *P. monodon* is still unknown, so cross-infection studies and studies to develop preventative or therapeutic measures are not possible. The purpose of this study is to develop a specific nucleic-acid probe to aid in finding the intermediate host and to trace the life cycle of the parasite. To prepare the probe, DNA was extracted from purified microsporidian spores derived from infections in *P. merguiensis* and *P. monodon.* The prepared genomic DNAs were then partially-digested with Sau3AI and DNA fragments were ligated to the pGEM7Zf+ vector before being transformed to *E. coli* JM109. Probes were screened by *in situ* colony hybridization, slot blot hybridization and Southern blot hybridization with dATP^{32}P-labelled *Agmasoma* DNA. All the candidate probes from each *Agmasoma* source cross hybridized with whole *Agmasoma* DNA digest from the other source and with roughly the same quality of signal. Finally, clone *3/1-mo* derived from *Agmasoma* of *P. monodon* expressed the best sensitivity because it was found to

be derived from a multi-copy sequence. Since it did not cross-hybridize with other organisms from shrimp ponds (i.e., both shrimp species, *Artemia*, mixed protozoa, *E. coli* and *Vibrio* sp.), clone *3/1mo* was selected as a specific field probe. DNA was extracted from 22 animal species (including fish, crustaceans, molluscs, and mixed plankton). Two kinds of fish, *Priacanthus sp* and *Scatophagus argus*, gave positive reaction with the *Agmasoma* probe and are being further investigated as potential alternative hosts by the bioassay technique.

Since the microsporidian parasites (*Agmasoma* or *Thelohania* sp) of *Penaeus merguiensis* and *P. monodon* have identical morphology by the light and electron microscopes, it was suspected that a single parasite species infected both shrimp. However, cross infection studies to test this hypothesis have not been possible because the intermediate host required for disease transmission is unknown. This study compared DNA extracts from microsporidial spores of infected *P. merguiensis* and *P. monodon* to help answer the question of conspecificity. In these tests, a part of the *Agmasoma* small-subunit rRNA (SSU rRNA) gene from each host was amplified by the PCR reaction using synthesized degenerate primers based on highly conserved sequences of the SSU rRNA gene from another microsporidian (*Variemorpha necatrix*) and from *Escherichia coli* . After amplification, the fragments were inserted into the plasmid pGEM7Zf+ and amplified in *E. coli* JM109. The cloned fragments were verified as *Agmasoma* DNA by signal expression (Digoxigenin labelling) with *Agmasoma* DNA and by lack of expression with DNA from other organisms including the two shrimp hosts, some unidentified aquatic fishes and *E. coli*. The two *Agmasoma* recombinant plasmids were then sequenced using a Tag dye Primer Cycle Sequencing Kit. The amplified regions of the SSU rRNA gene from the two sources were exactly identical for 722 base pairs. The results support the morphological data and strongly suggest that a single microsporidian parasite infects both of these penaeid shrimp .