INTRODUCTION

Farming of shrimp became a lucrative commercial avenue globally since the last two decades. During the past few years, the world production of shrimp fluctuated around 712,000 metric tonnes (Garriques and Arevalo, 1995). Moriarity (1998) predicted that the production from commercial cultivation of shrimp might reach to 1.6 million metric tons within the next decade. However this forecast appears as pessimistic one, in the light of the frequent disease problems.

Disease is recognized as the major constraint as well as a limiting factor for sustainable shrimp farming. Estimates of economic bases indicate that developing countries in Asia lost at least US$ 1.4 thousand million due to diseases in 1990 alone. Since then, losses due to diseases have been increasing. According to the 1996 World Bank report, global losses due to shrimp disease are around US$3 thousands million and the Bank recommended investment to the tune of US$275 million in shrimp disease research in the ensuing 15 years (Lundin, 1996).

Environmental factors and poor water quality, resulting from increased effluent discharge, movement of aquatic animals, inadequate farm management, rapid proliferation of farms etc., have been implicated in major disease outbreaks occurring in epizootic proportion. However, the underlying courses of such epizootics are highly complex and difficult to pinpoint. Viral outbreaks have been damaging the shrimp culture in Southeast Asia and South and Central America. At present, over 20 viruses have been identified as important to shrimp, the most threatening being White Spot Syndrome Virus (WSSV) (Wang *et al.*, 1995), which was previously known as Systemic Ectodermal and Mesodermal Baculovirus (SEMBV) (Wongteerasupaya *et al.*, 1995) in Asia and TSV in USA. Diseases caused by bacteria are also considered as equally important in causing mass mortalities wherever shrimp are cultured. Among bacteria, *Vibrio* has been implicated as the causative organism, which may trigger mortalities up to 100% (Nash, 1990). Such bacterial epizootics concomitantly emerge as facultative to shrimp due to primary viral infections or environmental stress (Lightner, 1988; Karunasagar *et al.*, 1996). The short generation time of bacteria ensures massive population, which develops rapidly in the infected host as well as in the host's environment.

According to Costa *et al.*, (1998), bacterial diseases in shrimp may be classified into three forms,

i. Localized spots in the cuticle, which constitute the condition called 'bacterial shell disease'

ii. Localized infections of the gut, or hepatopancreas or localized infections from puncture wounds and limb loss.

iii. Generalized septicemia

In Asia, among the pathogenic *Vibrio* group, 11 species were reported from the shrimp culture systems (Lavilla-Pitogo, 1995). Of these, *Vibrio alginolyticus* and *V. harveyi* are considered as the most important ones in the grow-out ponds of giant black tiger shrimp *Penaeus monodon* in India (Otta, *et al.*, 1999). As several other *Vibrio* species were also reported to cause mass mortalities in shrimp, the present study was initiated with the isolation of *Vibrio* pathogens from the shrimp farms located in Southeast and Southwest coasts of India. Among the bacterial isolates, it was found that *V. alginolyticus* and *V. harveyi* were predominant pathogens, which caused either opportunistic or secondary infections to the shrimp under stress. Therefore, in the present thesis due emphasis was given to characterize them, experimentally transmit them to apparently healthy individuals and to control the disease.

The prevention and control of infectious diseases became a priority globally to make the shrimp aquaculture as an ecologically and economically sustainable venture. Outbreaks of infectious diseases of shrimp can be minimized through careful quarantine, prophylaxis, therapy, and improved farm management practices. The sustainability of production is dependent on the equilibrium between the environmental qualities, the disease prevention by prophylactics and epidemiological surveys of the pathogens, and the health status of the shrimp. Therefore, the prevention and the control of shrimp diseases (management) are emerging as an integrated approach (Bachere, 2000). Disease management strategies can be categorized into two broad aspects:

1. Disease control (once it occurs) defined as the reactive disease management strategy.

2. Disease prevention or prophylaxis, defined as the proactive strategy.

The common tool for reactive management is by antibiotics. Oxytetracycline (OTC) has been used frequently, both in hatcheries and the grow-out period (Chanratchakool *et al.*, 1995) followed by chloramphenicol (CAP). However, the frequent application of antibiotics in the shrimp farming are likely to pose several problems including environmental hazards (Kerry *et al.*, 1995; Capnone *et al.*, 1996), possible development and spread of antibiotic resistant biota (Baticados and Paclibare, 1992; Williams *et al.*, 1992; Karunasagar *et al.*, 1994) and residues in the shrimp tissue at harvest. In addition, antibiotic application seems to be hazardous to human health, due to the development of clinically important resistant bacterial strains and possible failure of antibiotic therapy (Shome and Shome, 1999). As such, no approved antibiotics are available for shrimp farming, barring a few approved for controlling diseases in finfish farming. Therefore, novel antimicrobials with increased potency and least residual accumulation in shrimp tissue are required in lieu of conventional antibiotics for management of bacterial epizootics.

Due to the residual antibiotics in the shrimp tissue, rejection of the entire consignment in the export market was reported (Srisomboon and Poomchatra, 1995). The exact duration of withdrawal period after the application of antibiotics in the shrimp tissue is needed to ward off such rejections. Although the pharmacokinetics, uptake, distribution and depletion of antibiotics have been documented for fish (Nagata and Oka, 1996; Namdari *et al.*, 1999), a few reports are only available for the penaeid shrimps. Eventhough, the use of CAP was banned in many countries due to its adverse effects to the consumers (Baticados and Paclibare, 1992; Huber, 1986), it was being heavily used in shrimp farms due to its high positive results in the laboratory sensitivity tests (Tonguthai, 1996). Perusal of literature indicated that no systematic study was made on the residual kinetics of CAP in the shrimp tissue, particularly in the tropics. The antibiotic incorporated top-coated medicated feeds are currently prepared with an assumed leaching rate of 50%. Such over-compensation and consequent leaching may also lead to the development of specific resistant bacterial strains. However, very little information is available on this aspect. Considering these lacunae, an attempt was made in the present study to evaluate the level of antibiotic leaching and residual accumulation in the shrimp tissue due to antibiotic incorporated medicated feeds.

The common tool for proactive (disease prevention) is by incorporating compounds that stimulate the host's immune system. The prophylactics are grouped into three categories, viz., nutrients which act indirectly on cell physiology, those that work in a specific manner such as vaccines, and those that are non-specific in nature such as the glucans, alginates and lipopolysaccharide (LPS)-based materials. The immune systems of shrimp are not developed as that of the vertebrates and no specific antibodies or proteins are produced in response to the structural components of a particular pathogen. Though mono and multivalent vaccines have been developed against several bacterial diseases in fish, such vaccine preparations will not be successful in the case of shrimps. In addition, as new diseases and pathogens may emerge from time to time, practically it is impossible to develop proactive strategies using vaccines. But the shrimp immune system could be stimulated in a 'non-specific' manner using a variety of cell wall fragments as well as components from various microorganisms (Devaraja *et al.*, 1998; Itami *et al.*, 1998; Chang *et al.*, 2000). These formulations are highly dose dependent (Raa *et al.*, 1992) and therefore at times they are less successful.

These lacunae, prompted to develop and test novel prophylactic antimicrobials from marine organisms so as to prevent the onset and spread of disease throughout the culture period. Retrospective of work revealed that seaweeds (such as *Ulva* sp.) apart from forming food for herbivorous fish (Shipgel *et al.*, 1998; Zemke and Clements, 1999), also stimulate the defense system of fish by their alginates and polysaccharides (Furuscuva *et al.*, 1991; Nordmo *et al.*, 1995; Okai *et al.*, 1997). The marine based natural products like spray-dried *Tetraselmis suecica* and extract of tunicate *Ecteinescidia turbinata* were experimentally proved of their respective role in managing fish diseases (Austin and Day, 1990; Davis and Hayasaka, 1984). Except of these, the marine secondary metabolites (MSMs) and their role in managing diseases of shrimp are poorly understood, although some land-based products were reported to be effective in stimulating the defense system of shrimp (Aifang *et al.*, 1997; Chamanon *et al.*, 1999).

Apart from seaweeds, the bioactive potential of MSMs derived from marine sponges, were correlated with their wide biosynthetic capabilities (Attaway and Zaborsky, 1993) including successful antimicrobial activities (Ford *et al.*, 1999; Debitus *et al.*, 1998; Schmidt and Faulkner, 1998; Searle and Molinski, 1995; Uriz *et al.*, 1992; Kashman *et al.*, 1990). However, information is lacking on the potentials of MSMs of sponges in managing shrimp diseases.

In order to evaluate potent shrimp therapeutants from MSMs, after their extraction they were subjected to an array of assay systems to explore their biological potency. This inturn formed a systematic drug development programme. Considering the needs arising out of the lacuna persisting in the shrimp disease management strategies and the potentials of MSMs, the present thesis work was initiated with the following objectives:

1. Residual kinetics and leaching rate of commonly used antibiotics such as OTC and CAP in the shrimp tissue and in the environment.
2. Screening of biologically active MSMs from marine organisms using chosen bioassay systems.
3. Isolation, experimental transmission and eco-friendly management of shrimp (*Penaeus monodon*) secondary (*V. alginolyticus*) and opportunistic (*V. harveyi*) bacterial pathogens using marine bioactive secondary metabolites.