Chapter 1

General Introduction
1.1. Introduction

Aquaculture has developed rapidly over the last three decades to become an important activity worldwide. The United Nations (UN) estimates that, by 2037 the world’s population would exceed 10 billion. This rapid growth in humankind will bring with it severe difficulties not only in terms of shelter, education and health care, but also in maintaining current levels of protein intake. The Food and Agricultural Organization (FAO) of the UN acknowledge that global fishery output must be increased by at least 50% to offset projected shortfalls in dietary protein by 2030. At present, production by traditional fisheries and aquaculture is approximately 150 million tonnes. Out of this, commercial and artisanal fisheries account for around 94 million tonnes of seafood. Since most of the world’s fisheries have already exceeded maximum sustainable yields or are being fished at maximum permissible levels, aquaculture, therefore, presents the only method of offsetting predicted fishery shortfalls.

1.2. Crustacean aquaculture

Crustacean aquaculture is considered as a high value activity and tends to have higher monetary value and annual world production is over 8 million metric tonnes (FAO, 2000). Of this figure over half is made up of shrimps and prawns and the proportion of this production coming from farms has increased rapidly since the 1980s. In 2000, more than 85% of the cultured shrimp production was still realised by farmers in the eastern hemisphere, with Thailand as the main farming country, followed by China, Indonesia and India (Rosenberry, 2001). To a lesser extent, shrimps are produced in Latin America, with Ecuador as the leading country. The major species cultured are Marsupenaeus japonicus, Penaeus monodon, P. chinensis, P. merguiensis, Fenneropenaeus indicus and Litopenaeus vannamei. Much of the world shrimp production still comes from extensive culture. However, research on biology and ecology of penaeid shrimp significantly contributed to its aquaculture development and, related to that, its intensification.
1.3. Disease in aquaculture

Outbreak of diseases is being increasingly recognized as a significant constraint on aquaculture production. The most significant diseases of cultured penaeid shrimps have had viral or bacterial aetiologies, but a few have fungal and protozoan agents as their cause.

Many shrimp farms around the world have been badly hit by epidemics of White Spot Syndrome Virus (WSSV) since it emerged first in Asia around 1993 (Lightner, 1996). WSSV infects a wide spectrum of hosts, including shrimps (penaeid and non-penaeid), crabs and aquatic insect larvae (Lightner, 1996; Flegel, 1997). Some species are susceptible enough to become diseased and some are not so highly susceptible so as to succumb to the disease, but the latter are important as carriers, able to spread the pathogen (Wang et al., 1999). The disease is characterized by the appearance of white spots on the carapace and reddish discolouration of the body. WSSV is an enveloped ovoid-virus with a rod-shaped nucleocapsid with flat ends and having 300kb double stranded DNA as genetic material (Wang et al., 1995; Yang et al., 1997). The economic loss due to this single virus was tremendous and in Asia alone the estimated loss was around US$ 4 to 6 billion (Lightner, 2003). It is quite obvious that this problem is very severe, a fact which has been acknowledged by the World Bank who recommended that an investment of US$275 million should be made available for shrimp disease research during the period 1996-2010 (Lundin, 1996).

1.4. Disease control in shrimp aquaculture

Rapid dissemination of viral disease in shrimp aquaculture invites concern over its effective control, rapid diagnosis and treatments. However, control over spreading of pathogen or introduction of new pathogens across borders should be dealt with quarantine protocols including pathogen free ‘certification’ of stock. Diagnostic methods for the rapid detection of aquatic diseases have been improved to a greater extent with the aid of recent
biotechnological tools, but at the same time treatment of the infected stock is still lagging behind, especially viral diseases. In this context the popular quote 'prevention is better than cure' sheds light to the importance of proactive disease management measures to be taken to reduce the risk factors in aquaculture. A proactive disease management strategy, at least in shrimp aquaculture, is a multidisciplinary subject where ecology, environment, nutrition, physiology and genetics of the organism have to be taken care of.

1.5. Use of antibiotic and other chemotherapeutics in aquaculture

The application of antibiotics or other chemicals to culture ponds is expensive and undesirable as it risks contamination of both the environmental and the final product (Capone et al., 1996). A disquieting observation has been the gradual evolution of drug resistance in many bacteria. The use of almost every antimicrobial agents leads, sooner or later, to the selection of resistant strains from previously sensitive bacterial populations. Emergence of a resistant strain at a farm site renders particular antimicrobials useless, and the resistance can easily spread until it is the norm for that species. Antimicrobials do not cause the genetic and biochemical changes that make a bacterium resistant, but they select strains carrying the genetic information that confers resistance (Munn, 2004). The more an antibiotic is used, greater the selection pressure for resistance to evolve.

For most crustacean species, culture still depends on the use of seed produced in hatcheries, mainly from sexually matured females caught from the wild. So selective breeding programmes and the use of genetically modified strains are still having a long way for providing an ethically acceptable and commercially viable means of reducing the problem posed by epidemics. Therefore there has been a growing interest in finding ways to protect stock prophylactically in a manner conceptually equivalent to the use of vaccines, now routine for humans, agricultural livestock and more
recently farmed fish (Smith et al., 2003). Disease prevention in aquaculture by prophylactic use of chemicals emphasise procedures that prevent infections even if pathogens are present in the environment.

1.6. Crustacean immune system

Invertebrates, especially arthropods, are evolutionarily successful groups that have representation in various environments and ecological conditions and survived without the development of antibody-based immunity, relying instead upon relatively non-specific defence mechanisms. Shrimps possess immune system that, although quite complex, is substantially different from that of vertebrates. There is no specific immunity (no true antibodies and substantially less lymphocyte heterogeneity), though few aspects of specific immunity (inducibility) appear to be present in some cases. Shrimps possess both humoral and cellular immune responses, although they are less specialized than vertebrate immune responses. The innate immunity characterized by a diverse array of humoral factors that originate and/or reside in haemocytes and released during the immune response.

1.6.1. Immune system of shrimp.

The immune system of crustaceans is primarily related to their blood or haemolymph and to its circulating cells or haemocytes. Based on the cytochemistry, function and morphology, crustacean haemocytes have been classified into three; viz. hyaline cells, and two kinds of granular cells - semigranular and granular cells (Bauchau, 1981, Hose et al., 1990). It is well established that in arthropods, the defence of the host against invasive or opportunistic microorganisms is effected principally by the phagocytic, encapsulating and agglutinating activity of the circulating haemocytes (Ratcliffe et al., 1985).

1.6.1.1. Haemocytes

Haemocytes play an important role in cellular responses, including clotting, non-self recognition, phagocytosis, melanisation, encapsulation, cytotoxicity
and cell-to-cell communication. Of the three types of haemocytes, hyaline cells in most decapod crustaceans are characterized by the absence of granules, although some cytoplasmic inclusion bodies have been reported by electron microscopic observations (Martin and Graves, 1985) and are capable of phagocytosis (Smith and Soderhall, 1983). The percentage population of hyaline cells varies when different species of crustaceans are compared. In penaeid shrimp P. paulensis it accounts for 41% of total circulating haemocytes whereas, in Macrobrachium rosenbergii it is only 17% (Gargioni and Barracco, 1998).

The semigranular cells, which contain small granules and display some phagocytic activity, are specialized in particle encapsulation (Persson et al., 1987). Semigranular cells can respond to microbial polysaccharides such as lipopolysaccharides and β-1,3-glucan by degranulation process (Johansson and Soderhall, 1985).

The granular haemocytes are filled with large granules. They do not show phagocytic activity and they will not respond to the microbial polysaccharides directly unless they are pre-treated with some haemolymph proteins called pattern recognising proteins (PRP). The main function of these granular haemocytes is to store prophenoloxidase activating system (proPO system), which plays a key role in the defence reaction of crustaceans. The granular cells can be triggered to undergo exocytosis and subsequent release of proPO system from the granules by two endogenous proteins which are associated with the proPO system, a serine protease and the β-1,3-glucan binding protein if previously treated with β-1,3-glucan (Barracco et al., 1991).

1.6.1.2. Haematopoiesis

In decapods, haemocytes are produced within specialised haematopoietic tissue (HPT), the location and architecture vary greatly, even within close taxonomic groups. In lobsters, crabs and crayfish, haematopoietic cells of different morphology are organized and densely packed in small lobules and...
located over the cardiac stomach or the heart (Martin et al., 1993). However, the arrangement is different in penaeid shrimps, where haematopoiesis is believed to occur in paired epigastric nodules, which consists of an extensive network of vessels derived from ophthalmic artery. Morphology of the cells in the haematopoietic tissue of penaeid shrimps was studied by van de Braak et al. (2002) at light and electron microscopic level.

The regulation of haematopoiesis in decapod crustaceans is poorly understood, but is probably influenced by physiological processes such as moulting, reproduction and health status, as well as by environmental conditions like temperature and water quality (Johnson, 1980; Bauchau, 1981; Hose et al., 1992). Production of haemocytes occurs almost exclusively within the HPT, since mitotic haemocytes are rarely observed in the peripheral circulation. Cells released from the lobules appear identical to circulating cells. However, large granular haemocytes are not common in the HPT, suggesting that they can also develop from circulating small granular haemocytes (Martin et al., 1993). The mechanism by which maturing haemocytes are released into circulation is not clear. In shrimps, haemocytes migrate into the lumen of the haematopoietic tubule, which is continuous with the ophthalmic artery (Martin et al., 1987).

1.6.2. Cellular immune responses

1.6.2.1. Phagocytosis

The ability to ingest and kill microorganisms is a key component in the host defence. Phagocytosis is the most common of the cellular defence reactions and together with humoral components constitute the first line of defence. Phagocytic cells are found throughout the animal kingdom, serving nutritive function in lower invertebrates and more specialized functions like defence against microbial infections in higher phyla. Even though phagocytosis is considered as an important cellular defence reaction, little is known about this process in most crustaceans.
Phagocytosis is comparatively inefficient in the absence of opsonins, the co-factors that coat microorganisms and enhance the ability of phagocytes to engulf them (opsonisation). Studies in fresh water crayfish and lobster have revealed the presence of some opsonins in the haemolymph, which enhances phagocytosis (Tyson and Jenkin, 1974). When haemocyte monolayers were treated with β-1,3-glucan, a trigger of proPO system, a five to seven times higher degree of phagocytosis was observed than untreated control monolayers (Smith and Soderhall, 1983). But the factors, which act as an opsonin in crustacean haemolymph is yet to be isolated.

1.6.2.2. Nodule formation
When the body cavity is invaded by a large number of microorganisms, nodule formation or cell clumping occurs in several invertebrates, including crustaceans. These microorganisms entrapped in several layers of haemocytes, get melanised heavily. Such aggregates have been observed in the gill vasculature of penaeid shrimp Sicyonia ingentis (Martin et al., 1993). However, in other crustaceans haemocyte agglutinations (nodule) have been reported to be dispersed throughout the body as well as in the antennal gland, the heart and the gill (Bauchau, 1981; Johnson et al., 1981). Nodule formation is not an isolated event but occurs in conjunction with phagocytosis and other immune responses to affect a highly efficient clearance mechanism capable of dealing with pathogens. Mode of killing within the nodules is unknown but may involve melanin production and its toxic precursors, lysozyme or release of other enzymes.

1.6.2.3. Encapsulation
In addition to nodule formation and phagocytosis, invertebrate blood cells are capable of immobilizing parasites, that are too large to be ingested by a single blood cell by surrounding them with multicellular sheaths. Considerable confusion exists regarding the types of blood cells involved in encapsulation. Also very little is known about the initiation process of an encapsulation reaction. In crustaceans the only cells to react to foreign
molecules like β-1,3-glucan from fungi or lipopolysaccharides (LPS) from bacteria are the semigranular cells. This cell is also the first one to react to foreign particles and to encapsulate any invading pathogens. Some opsonin factors present in the haemolymph can also mediate the encapsulation process.

1.6.3. Humoral immunity
In many invertebrate species, several kinds of immune-related humoral activities have been reported. Several of these described factors originate and/or reside in the haemocytes and are released during the immune response. These factors are primarily non-self recognition factors that include a variety of defensive enzymes, lectins, lipoproteins, antimicrobial peptides and reactive oxygen intermediates.

1.6.3.1. Lectins
Lectins have been regarded as potential molecules involved in immune recognition and phagocytosis of microorganisms through opsonisation. They are non-enzyme proteins or glycoproteins without catalytic activity that binds to specific carbohydrates expressed on different cell surfaces. These type of carbohydrate binding proteins, which recognize surface structures common for different pathogens, represent a primitive immune response and called pattern recognition proteins (PRPs). Some lectins act as opsonins and bind to foreign particles that facilitate their removal by phagocytosis (Marques and Barracco, 2000). The PRPs recognize targets such as lipopolysaccharides (LPS) or peptidoglycan from bacteria, and β-1,3-glucans or mannans from fungi. Several PRPs recognizing β-1,3-glucans have been found in arthropods. Soderhall et al. (1988) isolated a β-glucan binding protein (BGBP) from plasma of cockroach Balberus canni fer. Lectin activity has been identified in the haemolymph of several penaeid shrimp species (Vargas-Albores et al., 1993). In penaeid shrimp P. monodon, Ratanapo and Chulivatnatol (1992) reported the agglutination of pathogenic Vibrio vulnificus by a purified lectin called monodin. Vargas-Albores et al. (1993)
reported the ability of purified lectin to react with different marine species of *Vibrio*.

1.6.3.2. The proPhenoloxidase system (proPO System)

The best-studied enzymatic system of crustaceans is phenoloxidase cascade (Sritunyalucksana and Soderhall, 2000). This enzyme is a part of complex system of proteinases, pattern recognition proteins and proteinase inhibitors constituting the so called prophenoloxidase (proPO) activating system. It is proposed to be non-self recognition system because conversion of prophenoloxidase to active enzyme can be brought about by miniscule amounts of molecules such as LPS, peptidoglycan and β-1,3-glucan of microbial cell wall. Several components of this system have been isolated and their structure determined. Phenoloxidase (monophenyl L-dopa: oxygen oxidoreductase; EC1.14.18.1) catalyses the oxidation of phenols to quinones followed by several intermediate steps that lead to the production of melanin, a brown pigment. During the formation of melanin, toxic metabolites are formed which have microbicidal activities (Soderhall et al., 1990).

The proPO is an inactive zymogen stored in the granular haemocytes, which degranulate and release the inactive enzyme into haemolymph. According to the amino acid sequence, proPO belongs to a family of copper containing proteins including haemocyanin and tyrosinases. The activation of proPO is by a proteolytic cleavage mediated by serine protease (proPO activating enzyme, ppA) which itself is seen in an inactive form in the haemolymph. Microbial polysaccharides, like LPS or β-1,3-glucan can mediate the activation of these inactive serine protease to active form, which in turn activate the inactive proPO into active phenoloxidase. Phenoloxidase then oxidises the phenolic group containing amino acids (tyrosine) into semiquinones, which have microbicidal action, and these semiquinones are polymerised into melanin (Cerenius and Soderhall, 2004). Melanisation is involved in the process of tanning of cuticle during the post-molt period, in...
wound healing and in defense reactions (encapsulation of invading microorganisms). This pigment can be recognized as dark brown spots in the cuticle of shrimps that have been injured.

Together with the activation of proPO, another important component of proPO system gets activated. That is a 76 KDa protein that mediate and enhance cell adhesion and degranulation (Johansson and Soderhall, 1989). This is a multifunctional immune factor, which also promotes encapsulation and function as a phagocytosis-stimulating opsonin (when released together with the molecules of the proPO system). Molecular characterisations of this 76 KDa protein were done and it revealed that they belong to the family of peroxidases (Johansson et al., 1995).

The prophenoloxidase system also needs factors that regulate the inappropriate activation and amplification of the response, as unregulated melanisation and protease activities would be disastrous to the animal. This control is partially achieved by synthesising the enzyme as an inactive zymogen that requires proteolytic cleavage in order to become active. To avoid excessive or premature activation of proPO system protease inhibitors like serine proteinase inhibitors have been identified in crustaceans. Many protease inhibitors like serpins and α-macroglobulins have been reported from arthropods, which regulate the unnecessary activation of proPO system (Kanost, 1999). A schematic overview of the important factors in the crustacean defence system is given in Fig 1.1.

1.6.3.3. Antimicrobial peptides

Antimicrobial peptides are widespread in the living kingdom, and a large number of these molecules have been isolated from vertebrates and invertebrates. The production of antimicrobial peptides represents a first line of defence mechanism of innate immunity that is widespread in nature. In crustacean haemolymph, antimicrobial activities have been demonstrated but only a few molecules have been characterised. Three antimicrobial
Fig 1.1 Flow diagram showing the crustacean defence system (Redrawn from Smith et al., 2003)
peptides have been isolated and characterized from *P. vannamei* (Destoumieux *et al.*, 1997 and 2000) and recent studies show that these peptides, named penaeidins, are ubiquitous in crustaceans. These peptides are often broad spectrum in nature and probably act against many infectious agents. They showed activity against the shrimp fungal pathogen, *Fusarium oxysporum* and also to some gram-positive bacteria (Destoumieux *et al.*, 1997). They are classified into three distinct groups based on amino acid sequences, secondary structure and functional similarities (Bachere, 2003). The first and large group is composed of peptides stabilised by intramolecular disulphide bonds, and the other two groups are linear peptides and polypeptides characterized by (1) α-helical structure or (2) a high content of proline residues and/or a high percentage of glycine residues. The haemocytes are found to be the site of production and storage of these peptides. Degranulation of the haemocytes by stress or pathogenic invasion can lead to the release of these peptides into the haemolymph. In most cases, anti-microbial peptides were shown to disrupt microbial membrane by a pore forming action or by a detergent effect.

1.6.3.4. Reactive Oxygen Intermediates (ROI)

Another important defence reaction of haemocytes is the production of a series reactive oxygen intermediates with powerful microbicidal activity. This response termed as respiratory burst, is an aerobic process, which generates highly reactive oxygen species such as superoxide anions (O₂⁻), hydrogen peroxide (H₂O₂), hydroxyl ions (OH⁻) and singlet oxygen (O₂¹) (Reactive Oxygen Intermediates or ROI). Detailed studies were conducted regarding the oxidative metabolism of crustacean haemocytes (Bell and Smith., 1993; Song and Hsieh, 1994). In *P. monodon*, production of ROI has been induced by immunostimulants like β-glucan and zymosan, which confers, enhanced protection against bacterial or viral infections (Song and Hsieh, 1994).
1.6.4. The concept of vaccination in shrimps

Vaccination, a strategy developed for generating immunity to the lethal smallpox virus, is based on the memory capacity of adaptive immune system. One of the most important attributes of an adaptive immune response is the establishment of a state of immunological memory. Adaptive secondary memory immune response of vertebrates depends on immunoglobulins (Igs), T Cell receptors (TCRs), Major Histocompatibility Complex (MHC) and memory T cells (Klein, 1989). It is the ability of the immune system to respond more rapidly and effectively to pathogens that have been encountered previously. It is very evident that there exists an anticipatory (memory) and non-anticipatory immune response in vertebrates, whereas only non-anticipatory immune responses were observed in invertebrates (Klein, 1997).

In vaccination, a harmless inactivated form of a pathogen is used to stimulate the primary antibody response, so that when the real pathogen is met, there is pre-existing immunity and the secondary response can be evoked to boost the level of immunity very quickly. As in other arthropods, crustaceans have a non-adaptive (innate) immune system, which means that there is little logic in trying to immunize these animals whereas it is possible to enhance the immune capacity for a limited period of time by vaccination.

Shrimps possess non-specific immune system, which is substantially different from that of vertebrates. Attempts have been made to vaccinate shrimps and lobsters. Adams (1991) reported vaccination of shrimp by exposure to heat killed preparations of pathogens. It has been reported that treatment of *P. monodon* with β-1,3-glucan (Kenkyu, 1994), killed vibrios (Teunissen *et al.*, 1998) significantly enhanced resistance to infection by vibrios. It shows that treatment with dead *Vibrios* and β-1,3-glucan is more effective in the protection against vibriosis than treatment with dead vibrios alone. Keith *et al.* (1992) reported that "vaccination" against gaffkemia
infection in lobsters was effective with inactivated bacteria. But all these responses are short-lived and usually last for a few hours or a day or so. It was observed that treatment with β-1,3-glucan induced a higher percentage of haemocytes with superoxide anions than with other immunostimulants in *P. monodon* (Song and Hsieh, 1994). Chaves and Sequeira (2000) observed a secondary immune response in *P. japonicus* which can be fit into the designation of immune memory stated by Hildemann (1984). It remains unclear whether such results are due to the existence of an adaptive immune response in invertebrates homologous to that observed in vertebrates or to a distinct type of immunoprotective pathway.

1.7. Immunostimulants in aquaculture

Immunostimulants are chemical compounds that activate the immune system of animals and render them more resistant to infections by viruses, bacteria, fungi, and parasites. It has been known for many years that cell wall fragments of microorganisms render animals more resistant to microbial infections (Kiser *et al.*, 1956). The ability of the immune system to respond to microbial surface components is the result of an evolutionary process whereby animals have developed mechanisms to detect common and highly conservative chemical structures of potential pathogenic microorganisms and to use those structures as “alarm signals” to switch on the defence against infection. The immune system will therefore respond to an immunostimulant as if challenged by a pathogenic microbe. Administration of an immuno-stimulant prior to an infection may protect the animal against an infection which otherwise would have become severe or lethal.

Immunostimulants have been obtained from diverse natural sources and a large number have been synthesised chemically with the natural products as structural models, wherein the main natural source of immunostimulants is microbial cell wall. The active principles of immunostimulatory cell wall preparations are various muramylpeptide fragments, lipopolysaccharides (LPS), lipopeptides, acyloligopeptides (Azuma, 1987). The immuno-
stimulants present in the cell walls of mushrooms and yeast are mainly β-glucans.

1.7.1. Yeast cell wall glucan as immunostimulant

β-1,3-glucans appear to be the most promising of all immunostimulants so far experimented in fish and shrimp. Glucan belongs to the class of drugs known as Biological Response Modifiers (BRMs). β-Glucans are polyglucose molecules linked through β-1,3 bonds in a long chain and with β-1,6 branches consisting of single glucose molecule or chains of glucose molecules (Fig. 1.2). Such glucans can exist in various structural forms and may be in the form of water-soluble oligomers, water soluble or insoluble macromolecules or particulates.

![Fig1.2 Structure of β-1,3-glucan with 1-6 branching](image)

There are well-defined receptors for β-1,3-glucans on the macrophages of warm-blooded animals, fish and on shrimp haemocytes. The β-1,3-glucan receptor on macrophages is highly specific in their action and can “recognise” a β-1,3-glucan chain with more than 3 to 5 glucose units.

Glucan is, from an evolutionary point of view, the most widely and most commonly observed macrophage activator in nature. β-1,3-glucan has been
proven to both stimulate and activate macrophage cells, which can overcome the negative effects of immunosuppression. Activation of macrophages results in increased non-specific phagocytic activity, killing pathogens more efficiently and thereby preventing disease outbreaks.

The phenoloxidase system is an important element in the disease resistance of crustaceans. It is, however, of crucial biological significance that this latent defence apparatus is able to identify a real infection and not be switched on by signals other than those unique to pathogens. Crustaceans use LPS and the β-1,3-glucan structure as specific signals to activate the prophenoloxidase system. The haemolymph of crustaceans contain proteins that specifically bind to β-1,3-glucans. When this protein has reacted with β-1,3-glucan, it can bind to a specific receptor on the haemocytes and induce degranulation and release of the prophenoloxidase, leading to the proPO cascade and formation of melanin (Cerenius and Soderhall, 2004). Various authors reported that glucan derived from yeast cell wall act as an immunostimulant in penaeid shrimps (Sung et al., 1994; Song et al., 1997; Chang et al., 1999, 2000, 2003).

1.7.2. Bacterial cell wall products as immunostimulants

1.7.2.1. Lipopolysaccharides (LPS)

The bacterial cell wall carbohydrates including Lipopolysaccharides (LPS) and peptidoglycan are the two other most common immunostimulants in aquaculture practice. Gram-negative bacteria consist of a complex LPS structure that is anchored to the underlying peptidoglycan (Fig. 1.3). Animals have evolved mechanisms to detect the presence of LPS, and this molecular structure has diverse nonspecific action on the immune system, activating both macrophages and lymphocytes (Burrell, 1990).

A low dose of LPS enhances the disease resistance and act as a prophylactic agent (Noworthy, 1983), but their high toxicity to warm blooded animals may limit their use in practice (Bone, 1991). But LPS is less toxic to
fish and shrimps and has been shown to be active as an immunostimulatory complex that increases the disease resistance of fish and shrimps (Jorgensen, 1994; Song and Sung, 1990).

Fig. 1.3. Structure of lipopolysaccharides (LPS) of Gram-negative bacteria

1.7.2.2. Peptidoglycan

Peptidoglycan is a cell wall component of many bacteria, though it is found in greater amount in Gram-positive bacteria (Fig 1.4). Matsuo and Miyazono (1993) have reported that oral administration of peptidoglycans from *Bifidobacterium thermophilum*, protected juvenile rainbow trout challenged with *Vibrio anguillarum*. Boonyaratpalin et al. (1995) reported the effects of PG from *Brevibacterium lactofermentum* on the growth, survival, immune response and tolerance to stress in *P. monodon*. Itami *et al.* (1998) noted that peptidoglycan from *Bifidobacterium thermophilum* protected shrimps against White Spot Syndrome Virus (WSSV).

Fig. 1.4. Structure of peptidoglycan (PG) of bacteria

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Apart from these, various other cell wall preparations of bacteria namely, BCG, Freunds (Complete) Adjuvant, curdlan, and other cellular glycans from mycelial fungi and plants have been in use as immunostimulants with limited success (Sakai, 1999).

1.7.3. Rationale of immunostimulants in shrimp aquaculture

It is an inevitable part of intensive aquaculture to stress the animals by containment, transport, handling, sorting, periodic low oxygen or high ammonia etc and as a result create a physiological condition characterized by a suppressed immune system (Raa, 1996). The development of disease particularly in shrimp aquaculture, resulted not only from an intensification of production, based on zootechnological progress, but also from ecological and environmental disturbances, pollution and nutritional imbalance (Kautsky et al., 2000).

Avoidance of pathogen is not practically sound where a large-scale conventional open culture system exists in a country like India. Nowadays closed culture system or recirculating culture systems are being practiced in many countries, where no or limited water exchange takes place. In shrimp farming system, horizontal transmission of the virus occurs via oral ingestion and the waterborne route (Corsin et al., 2001) whereas, in hatcheries vertical transmission has been identified to be the major route (Mushiake et al., 1999). Therefore disease control in aquaculture should focus first on preventive measures related to water quality, technology and husbandry thus significantly eliminating disease promoting factors. Disease risk may be reduced further by the use of improved feeds, higher disease resistance through selective breeding and by the use of immunostimulants, probiotics and vaccines. But in shrimp aquaculture, vaccination is not a relevant option, either because the pathogenic agent is unknown and therefore beyond the scope of a classical approach to vaccine development or because of impaired performance and mortality as a result of environmental stress, which elicit infection by opportunistic pathogens in the environment.
Immunostimulants may reduce the risk of disease under such circumstances. Since there is no obvious biological basis for making vaccines for crustaceans; the use of immunostimulants to increase their disease resistance may be a rewarding option (Raa, 1996). Currently many commercial products are available in shrimp aquaculture sector under the label of immunostimulants and are extensively used by the shrimp farmers. But the nature of these compounds, the dose required for eliciting immunostimulation at optimum level, duration of protection conferred, route of application etc. are still uncertain. These lacunae in the scientific knowledge pertaining to such practices necessitate a comprehensive study on products before they are launched into the market. Present work is aimed at the identification and utilisation of marine yeast as a source of immunostimulant to penaeid prawn culture system. Indian white prawn *Fenneropenaeus indicus* is selected as model organisms to study the immunostimulatory effect of the yeast/yeast products. In this context present study was undertaken with the following objectives

1. Screening of marine yeasts to identify potent strains showing immunostimulant and growth enhancing property in *Fenneropenaeus indicus*.

2. Optimisation of yeast biomass concentration in feed for effective protection against experimental infection with White Spot Syndrome Virus.

3. Optimisation of culture conditions of the selected marine yeast for biomass production.

4. Extraction and partial H-NMR structural characterization of (1→3)-β-D-glucan from selected yeasts

5. Efficacy of (1→3)-β-D-glucan from marine yeast as an immunostimulant to Indian white prawn *Fenneropenaeus indicus*. Optimisation of the dose, frequency and mode of glucan administration to *Fenneropenaeus indicus*.

6. Assessing the immunological profile of *Fenneropenaeus indicus* on administration of whole cell marine yeast/cell wall glucan
First chapter present a general introduction of the topic. The results of the present study are presented in eight chapters. Screening of selected marine yeast for growth enhancing and immunostimulants property in *Fenneropenaeus indicus* is presented in chapter 2. Third chapter deals with the optimisation of culture conditions of selected marine yeast for biomass production. Immunological profile of *Fenneropenaeus indicus* on oral administration of marine yeast is presented in chapter 4. The fifth chapter deals with extraction and partial H-NMR characterisation of cell wall (1→3)-\(\beta\)-D-glucan from selected yeasts. The H-NMR spectra obtained for marine yeast glucan was compared with that of a cell wall glucan extracted from baker's yeast *Saccharomyces cerevisiae*. In chapter 6, the efficacy of cell wall glucan from marine yeast as an immunostimulant to Indian white prawn *Fenneropenaeus indicus* is presented. The dose, frequency and mode application are also presented this chapter. In chapter 7 the dose and frequency of glucan to be administered was confirmed by studying the immunological profile of *Fenneropenaeus indicus*. Chapter 8 describes a comparative study of whole cell marine yeast, it's glucan and a glucan obtained from baker's yeast as an immunostimulant to *Fenneropenaeus indicus*. This is followed by summary, list of references and appendices.