Review of Literature
Aquaculture is a rapidly expanding industry world wide with a large proportion of the industry comprising of intensive finfish culture. Disease outbreaks resulting from various infections caused to the fish, are recognized as an important factor limiting the productivity, especially in intensive culture systems. Infections caused by the bacteria of the genus *Aeromonas* are the most common disease causing agents in the aquatic environments. Members of this group generally referred to as "aeromonads" have been reported as primary pathogens of numerous cultured fish (Trust *et al*. 1986; Austin and Adams 1996).

Aeromonads are widely distributed in aquatic environments and are reported to be important pathogens of humans and lower vertebrates including amphibians, reptiles and fish (Janda, 1991). The genus is represented by bacteria that are gram negative, short rods with rounded ends, aerobe-anaerobe facultative, motile by a single polar flagellum, with the exception of *A. salmonicida* which is non-motile. The genus comprises of various species that are pathogenic for fish and several cold-blooded animals (Altwegg and Giess, 1989), among them, *Aeromonas hydrophila* is responsible for mass mortalities in several fish species, including Indian major carp, snakehead, gourami and catfish. It is also associated with a variety of fish pathological conditions, altogether named "aeromonosis" (Ghittino, 1985), which occur in nature or in artificial environment. Another important member of this group is *Aeromonas salmonicida*, a fish pathogen which causes a common disease among salmonids, named "forunculosis" or "ulcerative forunculosis".

Aeromonads have been associated with a wide variety of human infective syndromes both as primary pathogens, and as opportunistic agents causing primary and secondary septicemia in immunocompromised persons, serious wound infections in healthy individuals and in patients undergoing medicinal leech therapy, producing infection in skin lesions following exposure to polluted water, bacteremia meningitis, osteomyelitis and conjunctivitis. (Janda *et al*., 1996; Janda and Abbott, 1998). Gastroenteritis, the most common clinical manifestation, still remains controversial (Janda and Abbott, 1998). Since there have been increasing reports of acute diarrhoea caused by these bacteria, *Aeromonas* spp. have reported being responsible for enteric or, less often, extra-enteric infections (Janda and Duffey, 1988) and can now be considered a relatively common enteropathogen.
TAXONOMY

The genus *Aeromonas* has undergone a number of taxonomic and nomenclature revisions over the past 15 years. Although originally placed in the family *Vibrionaceae* (Véron, 1965), which also included the genera *Vibrio*, *Photobacterium*, and *Plesiomonas*, subsequent phylogenetic investigations indicated that the genus *Aeromonas* is not closely related to vibrios but rather forms a monophyletic unit in the γ-3 subgroup of the class *Proteobacteria* (Martinez-Murcia et al, 1992 and Ruimy et al, 1994). These conclusions necessitated the removal of *Aeromonas* from the family *Vibrionaceae* and transfer it to a new family, the *Aeromonadaceae* (Colwell et al., 1986). Similarly, only five species of *Aeromonas* were recognized 15 years ago (Janda and Duffey, 1988), three of which (*A. hydrophila*, *A. sobria*, and *A. caviae*) existed as phenospecies, that is, a named species containing multiple DNA groups, the members of which could not be distinguished from one another by simple biochemical characteristics. Popoff (1984) gave the classical taxonomic scheme according to which there were two main groups. The first group included psychrophilic strains that grow at 22-28°C (*A. salmonicida*) while the second group comprised of mesophilic strains growing at 36-37°C. A new classification scheme based on the classic biochemical tests, and also on results of DNA hybridization tests was proposed by (Abbot 1992). In the most recent classification fifteen species are considered (Janda and Abbott, 1998); among them, six are considered pathogen for man, while nine are non pathogen or "environmental" (Table 1).

Table 1. Current taxonomy of the species belonging to *Aeromonas* genus, grouped according to the pathogenicity for man (modified from Janda and Abbott, 1998)

<table>
<thead>
<tr>
<th>Species associated to disease in man</th>
<th>Environmental species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major Pathogens*</td>
<td>Minor Pathogens*</td>
</tr>
<tr>
<td><em>A. hydrophila</em></td>
<td><em>A. veronii</em></td>
</tr>
<tr>
<td><em>A. caviae</em></td>
<td>(biotype sobria)</td>
</tr>
<tr>
<td><em>A. veronii</em></td>
<td></td>
</tr>
<tr>
<td>(biotype veronii)</td>
<td><em>A. jandaei</em></td>
</tr>
<tr>
<td></td>
<td><em>A. schuberti</em></td>
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* Based on frequency rather than severity of the disease.
INFECTIONS OF AEROMONADS IN FISH

Motile Aeromonas Septicemia (MAS)

This is probably the most common bacterial disease of freshwater fish. This disease has been associated with several members of the genus Aeromonas, including A. hydrophila, A. sobria, A. caviae, A. schuberti, and A. veronii. Such disease conditions have been reported in several fish including striped mullet (Soliman et al., 1989) striped bass, their hybrids, redfish and zebrafish. Over-crowding and stress increased their susceptibility to MAS (Pullium et al., 1999). A. hydrophila has also been diagnosed to infect the fish in China by Nielsen and coworkers (2001). In studies conducted on different bacterial isolates this species accounted for more than 50% of the isolated aeromonands isolated from crucian carp (Carassius carassius) and Wuchang bream (Megalobrama amblycephala). A. hydrophila is the causative agent of acute septicemia in striped mullet (Mugil cephalus) which is characterized by early inflammatory and proliferative changes and later necrotic changes (Soliman et al., 1989).

Clinical signs of motile aeromonas septicemia range from sudden death with high morbidity in peracute cases to superficial to deep skin lesions. In the acute form of disease, a fatal septicemia may occur so rapidly that fish die before they have time to develop anything but a few gross signs of disease. Fish may show exophthalmia, reddening of the skin, and an accumulation of fluid in the scale pockets (Faktorovich, 1969). The abdomen becomes distended as a result of an edema and the scales may bristle out from the skin to give a "washboard" appearance. The gills may hemorrhage and ulcers may develop on the dermis. Severe eye pathology and heavy mortality among yearling and older rainbow trout accompanying a severe outbreak of motile aeromonad septecemia was reported by (Ogara et al., 1998). The condition at first affected one eye, progressed into the other eye, after which the orbits ruptured causing blindness and death. Similarly, Yambot and Inglis (1994) described an acute mortality among Nile tilapia in which the most apparent clinical signs included an opaqueness in one or both eyes, accompanied by exophthalmia and eventual bursting of the orbit. Motile aeromonads were isolated from the eyes, liver and kidneys of affected fish. Histopathological studies by Fuentes and Perez (1998) revealed that fish exhibit epithelial hyperplasia in the foregut; leptomeningeal congestion in the brain, as well as a thrombosis and inflammation in the perisclerotic region and corneal epithelium of the eye. It has also noted that catfish with septecemic or latent infections had enlarged nuclei in the branchial epithelium and
that there was a significant correlation between the presence of these gill lesions and the severity of hepatic and pancreatic lesions (Grizzle and Kiryu, 1993).

*Aeromonas hydrophila* was generally considered to be a secondary invader in red sore disease, in which the primary etiological agent was believed to be the protozoan ciliate *Epistylis* (Rogers, 1971). Recently Hazen *et al.*, (1978b) reexamined the etiology of red sore disease and found that *A. hydrophila* was present in 96% of the initial lesions on fish, whereas *Epistylis* was present in only 35% of such lesions. Furthermore, electron microscopy showed that *Epistylis* lacked structures that produced lytic enzymes and, therefore, could not initiate the development of lesions. This study strongly suggested that *A. hydrophila* is indeed the primary etiological agent of red sore disease and that *Epistylis* is a secondary pathogen that rapidly colonizes the dermal lesions initiated by bacterial proteolytic enzymes.

**INFECTIONS IN HUMANS**

Members of the genus *Aeromonas* have been associated with a wide range of illnesses in humans, including gastrointestinal disorders and systemic infections in both immunocompromised and healthy hosts. Bacteraemia which means the presence of bacteria in the blood is the most common pathogenic manifestation of *Aeromonas* in humans displaying symptoms such as fever and chills. Patients who become severely infected with this bacteria often exhibit abdominal pain, nausea, vomiting, and diarrhea.

The bacteria are distributed worldwide in the primary habitats that are surface water and soil and humans may acquire infections through open wounds resulting in fatal or serious debilitating outcomes, such as amputations. *Aeromonas* wounds fall into three categories, listed in order of increasing severity of damage caused: cellulitis, myonecrosis, and ecthyma gangrenosum.

Cellulitis is an acute inflammation of subcutaneous tissue characterized by redness and induration that may arise from injury or secondary to sepsis (Musher, 1980). The less frequent myonecrosis, or bullous lesions, is characterized by the liquefaction of muscles with blackening of the tissue which may be gangrenous with gas formation. These patients require aggressive antimicrobial therapy and debridement; those individuals that fail to respond to these measures may require amputation (Haburchak, 1996). The third type, ecthyma gangrenosum, is a cutaneous necrotic or gangrenous pustule that occurs secondary to sepsis. Lesions have an erythematous
border surrounding a vesicle which can progress to necrosis of the soft tissue within 24 h. This type of infection is usually fatal (Musher, 1980).

The potential of motile Aeromonas species (A. caviae, A. hydrophila and A. veronii biovar sobria) to cause human gastrointestinal infections has been recognized. Considerable worldwide epidemiological, microbiological and clinical investigations have shown that some strains of the different motile aeromonads are of increasing enteropathogenic significance, especially in children, the elderly and in immunocompromised individuals (Trower et al., 2000).

Meningitis caused by this organism is a rare clinical entity. Aeromonas meningitis may involve all age groups and can be either community or nosocomially acquired (Jacob, 1988; Parras, 1993. Aeromonas meningitis is an uncommon clinical occurrence and accounts for less than 0.15% of Aeromonas septicemia.

Mukhopadhyaya et al., (2003) have reported a case of aspiration pneumonia in an immunocompetent patient with multiple sclerosis, caused by a community acquired, multidrug resistant strain of Aeromonas hydrophila sensitive only to meropenem. This case highlights the clinical significance of Aeromonas hydrophila as a respiratory pathogen, as well as the community origin of multidrug resistance.

There have been reports of infection of burn patients with Aeromonas hydrophila, but it is an uncommon event. The organism was isolated from either skin swabs, tissue samples, blood cultures or cultured lines. In all patients there was a history of immersion in water immediately post burn. Burn injury may induce a state of immunosuppression, making the thermally injured patient a suitable host for aeromonas infection (Barillo et al., 1996; Skoll et al., 1998; Kienzle et al., 2000).

VIRULENCE FACTORS

Aeromonas spp. produce an impressive array of virulence factors, including hemolysins, cytotoxic and cytotoxic enterotoxins, proteases, lipases, leucocidins, endotoxin, adhesions, and an S-layer (Merino, 1995; Chopra et al., 1999; Merino, 1999). Enteropathogenic bacteria including the Aeromonas spp. cause pathogenicity by two main mechanisms which are tissue adherence and toxin production. Tissue adherence is mediated by the S-layers, which can be found in A. salmonicida, in A. hydrophila, and in A. veronii biotype sobria (Janda et al., 1987; Dooley and Trust, 1988).

For the past years a lot of work has been carried out in order to identify the virulence factors or the pathogenetic mechanisms in man and animals. Kay et al., (1991) have
reported that the principal virulence factor of *Aeromonas salmonicida* is its S-layer (A-layer) which is comprised of tetragonally arrayed approximately 50,000 Mr protein subunits tethered to the cell surface via LPS. The detailed composition of its LPS is known, as is the primary sequence, and three-dimensional disposition of the A protein subunits. The A-layer physically protects the cell against bacteriophage, proteases, as well as immune and non-immune complement. The A-layer appears to be uniquely adapted towards binding biologically important molecules such as heme, and to various basement membrane proteins. In addition, the A-layer is required for macrophage infiltration and resistance. Specific mutants containing a disorganized A-layer are avirulent and provide significant protection to salmonids when applied by immersion.

It has been speculated that *A. hydrophila* virulence could involve several extracellular enzymes including proteases, hemolysins, enterotoxins, and acetylcholinesterase. Some of the toxins have been biochemically characterized, but their precise roles in the pathogenicity of *A. hydrophila* have not yet been determined (Chakrabarty *et al.*, 1987; Leung *et al.*, 1988; Rivero *et al.*, 1990; Nieto *et al.*, 1991, Rivero *et al.*, 1991). The toxins of *Aeromonas* can be classified as eso- and endo-toxins. Cytotoxins and enterotoxins (including those with haemolytic activity) are the most important for their pathogenicity; the latter act through the classic cytotoxic mechanism, typical of *V. cholerae* (Merino *et al.*, 1992, 1995). Fang and his associates (2004) reported that *A. hydrophila* causes diseases by adhesive and enterotoxic mechanisms. The adhesion is a prerequisite for successful invasion of the pathogen. Some of the diarrhoeal symptoms of Aeromonas-associated gastroenteritis have also been attributed to enterotoxins (Trower *et al.*, 2000). *A. caviae, A. hydrophila* and *A. veronii biovar sobria*, were tested for their ability to cause fluid accumulation in infant mice. Eight isolates were found to produce enterotoxin. This enterotoxin was reported to be “cholera like” as it cross-reacted with cholera toxin antibodies and it caused marked fluid accumulation in infant mice by the suckling mouse technique.

*Aeromonas* can also produce other extracellular substances, with significance of diffusion factors: proteases, amylase, chitinase, lipase, nuclease (Janda, 1991) and others with unknown pathogenic role (Merino *et al.*, 1995).

The role of aeromonads in Ulcerative Disease Syndrome (UDS) is believed to be opportunistic or secondary and these bacteria are thought to play an important role in this degenerative disease (McGarey, 1991). UDS is an epizootic fish disease
characterized by the presence of severe, open dermal ulcers on the head, midbody, and dorsal regions of the fish. In UDS studies conducted, *A. hydrophila* and *A. sobria* were recovered more often from UDS fish than other bacteria from the genera Vibrio, Alteromonas and Plesiomonas. These aeromonads were reported to produce a wide variety of hydrolytic enzymes and expressed cell surface characteristics linked to virulence whereas the other bacterial species rarely produced the same enzymes or cell surface characteristics.

It has been reported that a secreted protease (AhpB) from *A. hydrophila*, with a high elastolytic activity, is essential for pathogenicity of *A. hydrophila* and should be considered as a virulence factor (Cascón et al., 2000). Studies in this context demonstrated that the LD$_{50}$ of the *A. hydrophila* ahpB mutant is about 100 times higher than that of the wild type.

An aerolysin-related cytotoxic enterotoxin (Act) from *A. hydrophila* that possesses several biological activities has been reported (Ferguson et al., 1997) that causes lysis of red blood cells, is cytotoxic to intestinal and non-intestinal cells, evokes intestinal fluid secretion, and is lethal in nanogram quantities when injected intravenously into mice (Ferguson et al., 1997; Chopra et al., 1999; Sha et al., 2002). Act has also been demonstrated to up-regulate the expression of genes encoding proinflammatory cytokines (tumor necrosis factor-$\alpha$ (TNF-$\alpha$), interleukin-1$\beta$ (IL-1$\beta$), and interleukin-6 (IL-6)) and the inducible nitric-oxide synthase gene in murine macrophages. In other reports, Act activated arachidonic acid metabolism via the induction of group V phospholipase A$_2$ (PLA$_2$) and cyclooxygenase-2 (COX-2), resulting in the production of prostaglandin E$_2$ (PGE$_2$). Act also up-regulated cellular cAMP concentration, which, along with PGE$_2$, contributed to the enterotoxic response associated with Act (Chopra et al., 1992).

More recently, it was demonstrated in macrophages that Act induced the rapid mobilization of calcium from intracellular stores, as well as an influx of calcium from the extracellular medium. The rise in intracellular calcium modulated the production of TNF-$\alpha$ and PGE$_2$ via activation of NF-$\kappa$B (Chopra et al., 2000; Ribardo et al., 2002). In addition to activation of NF-$\kappa$B, Act treatment of macrophages resulted in activation of the transcription factor cyclic AMP-response element-binding protein (CREB). Hence, the data demonstrated a direct role for Act in the induction of the host inflammatory response, which has been confirmed *in vivo* (Sha et al., 2002).

Many bacterial pathogens of animals and plants have been shown to inject anti-host virulence determinants into the hosts via a type III secretion system (TTSS). Yu et al.,
Review of Literature

2004, reported that a TTSS is required for \textit{A. hydrophila} pathogenesis. Insertional inactivation of two of the TTSS genes (aopB and aopD) led to decreased cytotoxicity in carp epithelial cells, increased phagocytosis, and reduced virulence in blue gourami. It is also believed that \textit{Aeromonas hydrophila} causes diseases by adhesive and enterotoxic mechanisms. The adhesion is a prerequisite for successful invasion which is achieved by a 43 kDa major adhesin (Fang et al., 2004).

\textbf{PREVENTION AND TREATMENT}

The best prevention against \textit{Aeromonas hydrophila} infection is to minimize the stress factors to the fish through proper handling, stocking levels, nutrition, transportation, and water quality which requires excellent sanitation and filtration procedures. Four basic measures can be used to reduce or control fish diseases. These include treatment with drugs, culling of diseases fish and only replacing with new stocks after ponds or tanks are disinfected, vaccination of stock or genetic selection to improve disease resistance (Sardar et al., 2001). Therapeutic agents such as antibiotics and chemicals are common treatments for disease control. Antibiotics, namely chloramphenicol, florfenicol, tetracycline, sulphonamide, nitrofuran derivatives, and pyrodoxcarboxylic acids are used for the treatment of \textit{A. hydrophila} infections in fish farms (Aoki and Egusa, 1971; Endo et al., 1973; Katae et al., 1979; Fukui et al., 1987). Although the antibiotic oxytetracycline, fed orally, has been approved for specific bacterial diseases, experimental results show this drug to be immunosuppressive in carp (Rijkers et al., 1980; Grondel and Boesten, 1982) and rainbow trout (Thompson et al., 1995). Other potential problems associated with any antibiotic therapy include inadequate dosage levels, overdosing, drug resistance by the bacteria and the chelating of calcium to hard water in the case of Terramycin used in a dip or bath. In addition there are increasing concerns on the level of antibiotics in flesh of fish destined for human consumption. Chemicals on the other hand have different toxicity controlling ability and therapeutic index (Ward, 1982) and there is the risk of pollution of the aquatic environment. Consequently, farmers are left with few resources other than the use of preventive measures such as early diagnosis, good husbandry techniques and vaccination. The high cost of using chemotherapy, the short-term nature of the protection obtained with antibiotics, the increasing appearance of antibiotic resistant fish pathogens, and, to some extent, concerns about the environmental impacts of antibiotic use has led researchers to investigate the possibility of developing vaccines against major diseases of famed fish.
VACCINATION

Immunization has had a greater impact on the economics of livestock and poultry production than all other therapeutic and prophylactic treatments combined. Thus, even with the significant advancements in modern medicine, vaccination continues to be the most cost-effective method to reduce economic loss and animal suffering from infectious diseases. Vaccination is emerging as an alternate and cost effective measure for protecting fish against diseases. The primary concern in vaccination is efficacy and safety. Thus, vaccination itself must not cause any adverse reactions and should result in greater than 90% efficacy after a single administration regardless of species or type of vaccine.

Vaccines are preparations of antigens from pathogenic organisms which are converted to nonpathogenic form by various means so as to provide resistance from pathogenic infections (Ellis, 1988). A vaccine usually contains multiple disease antigens which work by provoking a protective immune response (production of antibodies) from the fish against the pathogen involved. This imparts a long lasting defence to the fish against infectious agents.

IMMUNIZATION OF FISH

The immune system of the fish is very much comparable to higher vertebrates. Specific and innate responses have been found involving macrophages, natural killer cells, neutrophils and lymphocytes as well as antibodies and cytokines (Iwama and Nakanish, 1996). Molecular data confirm the similarity of the fish specific immune system to that of mammals: polymorphic MHC (major histocompatibility complex) class I and class II genes, as well as T cell receptor (TCR), CD8 and immunoglobulin genes have been described for a number of fish species (Dixon et al., 1995; Stet et al., 1996; Manning and Nakanish, 1996; Rast and Litman, 1998; Hansen and Strassburger, 2000).

The first attempt to vaccinate fish against disease was by Duff (1942) who administered oral vaccine against furunculosis with feed containing chloroform killed Aeromonas salmonicida cells. The first commercial vaccines were for prevention of Enteric Redmouth Disease (EMR) and for Vibrio anguillarum in the mid 1970's in the USA. Vaccines against Aeromonas infections typically consist of dead vaccines, live attenuated vaccines, subunit vaccines and purified antigens.
DEAD VACCINES
Dead vaccines are composed of heat or formalin inactivated pathogens or their extracts termed as bactericins (Lamers, 1986 and Newman, 1993). So far, most commercial vaccines have been inactivated vaccines administered by injection or immersion. Bacterial infections caused by Gram-negative bacteria such as Vibrio sp., Aeromonas sp., and Yersinia sp. have been effectively controlled by such vaccines (Austin and Austin, 1987). Formalin inactivated vaccines have been reported to be superior to heat killed preparations, especially when the bacterins are injected with adjuvants (Anbarasu et al., 1998). However, the efficacy of these preparations has been poor limited to homologous strains only. Moreover the nature of protective antigens in dead vaccines remains to be established and there is a lack of correlation between antibody response and protection (Michel and Faivre, 1982) suggesting that cell mediated immunity may be important.

LIVE ATTENUATED VACCINES
Live vaccines consist of attenuated bacterial strains with low or no virulence have been used successfully against fish bacterial pathogens. Live vaccines stimulate cell mediated immunity better than killed whole cell vaccines. The idea of live vaccines administration is that the organism will grow slowly in the host and induce a strong immune response before it can cause disease. Fortunately it is now possible to identify specific virulent genes from a wide variety of different pathogens and to directly introduce multiple mutations into these genes and to even delete the entire gene in question. If the virulence gene is non-essential gene for replication, the gene deleted pathogen can be used as a vaccine. The protective efficacy of three different avirulent mutants as live vaccines against the fish pathogen, Vibrio anguillarum, was reported by Norqvist (1989). The mutants constructed by transposon insertion mutagenesis (VAN20 and VAN70) or as antibiotic-resistant mutants (VAN1000) were able to induce protective immunity against the homologous as well as a heterologous strain of V. anguillarum. Western immunoblotting showed that strains of V. anguillarum have antigenic determinants in common with Aeromonas strains. Therefore, VAN1000 was also able to induce protective immunity against challenge with Aeromonas salmonicida. The possibility of surface disorganized, attenuated mutants of Aeromonas salmonicida as furunculosis vaccines was reported by Thornton et al., (1991). A slow growing aminoglycoside resistant mutant and a rapidly growing pseudorevertant of A. salmonicida were isolated. Both of them were avirulant and the pseudorevertant strain conferred protection against challenge when
administered by intraperitoneal injection or immersion. To investigate the possibility of an effective live-attenuated vaccine, Vaughan et al. (1993) constructed an aromatic-dependent mutant of aroA gene of *A. salmonicida*. The aroA gene was inactivated by inserting a fragment expressing kanamycin resistance within the coding sequence. The aroA::Kan mutation was introduced into the chromosome of virulent *A. salmonicida* 644Rb and 640V2 by allele replacement by using a suicide plasmid delivery system. The mutation resulted in attenuation when bacteria were injected intramuscularly into Atlantic salmon (*Salmo salar*). Vaccination of brown trout with 10(7) CFU of *A. salmonicida* 644Rb aroA by intraperitoneal injection resulted in a 253-fold increase in the 50% lethal dose (LD50) compared with unvaccinated controls challenged with a virulent clinical isolate 9 weeks later which was enhanced to a further 16-fold by a booster of the same. Hernanz Moral et al., (1998) have shown that vaccination of rainbow trout with the AroA mutant of *A. hydrophila* as a live vaccine confers significant protection against the wild-type strain of *A. hydrophila*. Studies were performed by Marsden et al., (1998) on the use of a live vaccine for immunization of salmonids with *A. salmonicida* (Brivax I) and an unmarked aroA deletion mutant (Brivax II) against the bacterial disease furunculosis and protection elicited.

Massive mortality of koi and common carp – (*Cyprinus carpio*) species have resulted due to an as yet unclassified large DNA virus, designated carp nephritis gill necrosis virus (CNGV) or koi herpes virus (KHV). Ronen et al., (2003) demonstrated that the wild type CNGV lost its pathogenecity following serial transfer in cell culture, and that clones isolated from the attenuated population can be used as a prophylactic vaccine. Further the virus was irradiated in order to increase the number of random mutations in the genome of the attenuated virus, and thus, reduce the possibility of the attenuated virus reverting to pathogenic( Perelberg et al., 2005).

The main disadvantage with the use of attenuated live vaccines is that they sometimes are not sufficiently attenuated, either because the host’s immune system is weak or compromised or because of reversion of the attenuated pathogen to a more virulent live organism. In such cases these vaccines cause the disease that they’re intended to protect against.

Thune and Plumb (1982) reported that cell sonicates evoked the best antibody response. Sonication may disrupt the cell and allow better processing of certain somatic antigens (e.g. – bacterial lipopolysaccharide). Consequently, Baba et al., (1988) were able to evoke better protection against *A. hydrophila* among carp that
Review of Literature

were vaccinated with crude lipopolysaccharide (LPS) rather than whole-cell, formalin killed vaccine. Immersion of fish in the LPS vaccine for 2 h at 25°C was more efficacious and less stressful than injection, but vaccination with crude LPS did not invoke an observed humoral immune response as measured by bacterial agglutination, passive haemagglutination and agar gel diffusion tests.

RECENT ADVANCES IN FISH VACCINATION – SUBUNIT AND RECOMBINANT VACCINES

Because of safety considerations regarding killed or inactivated vaccines and live attenuated vaccines, there is an increase in demand for more elaborate and defined approaches for developing vaccines to combat diseases of fish. These new approaches include subunit vaccines comprising of purified cell components and recombinant protein technologies. Subunit vaccines are defined as those containing one or more pure or semi pure antigens. The advantage of using subunit vaccines is that by selecting the correct antigen which induces protective immunity the possibility of the presence immunosuppressive proteins in the whole pathogen can be eliminated. The most recent armamentarium of vaccinologists is the polynucleotide or recombinant DNA vaccination approach.

Subunit vaccines based on E. coli-expressed IPNV (infectious pancreatic necrosis virus) proteins have been tested in rainbow trout and Atlantic salmon. Vaccination by immersion in rainbow trout fry with bacterial lysate from E. coli expressing the IPNV Sp strain gene segment A, induced protection against challenge with the IPNV Buhl strain. By injection of Atlantic salmon parr with partly purified E. coli-expressed rVP2 (N1 strain), increased resistance against IPN infection was demonstrated by challenge. In field trials it is shown that vaccination of pre-smolt with rVP2 included in a commercial oil/glucan adjuvanted multivalent bacterial vaccine gives protection against IPN in natural outbreaks, compared to fish vaccinated with the same vaccine without the IPNV component (Christie, 1997). Infectious haematopoietic necrosis (IHN) is one of the most important viral diseases of salmonids, especially among juvenile fish where losses can be high. Subunit vaccines using part of the IHNV glycoprotein gene cloned into E. coli or into an attenuated strain of A. salmonicida have been tested and these vaccines appear to be safe, inexpensive and could provide some protection when delivered by immersion (Winton, 1997).

The first successful vaccination rainbow trout with a conserved purified outer membrane porin of A. salmonicida was reported by Lutwyche et al., (1995). The protein with an apparent M(r) of 28,000, isolated from outer membrane preparations
of *A. salmonicida*, was tested for the ability to form pores, using a planar lipid bilayer model membrane system. The porin purified by preparative SDS-PAGE was used to immunize Rainbow trout (*Oncorhynchus mykiss*), which showed significant protection from experimental *A. salmonicida* challenge. Further the immune response of Indian major carp, *Labeo rohita*, to antigens of motile aeromonads was studied by Karunasagar et al., (1997). The fish showed immunological memory and secondary response on booster administration and the extent of protection showed good correlation with titres of agglutinating antibody. When polyvalent vaccine was used, the fish showed antibody titres against all the components of the strains. However, the level of antibody was less compared to immunization by monovalent vaccines. Cross-reacting antibodies induced by monovalent vaccines showed varying degrees of protection.

Rahman et al., (2002) assessed the efficacy of the vaccine based on the antigenic outer membrane fraction (OMF) of *Flavobacterium psychrophilum* (the causative agent of coldwater disease). The OMF induced significantly higher protection against coldwater disease in both rainbow trout (*Oncorhynchus mykiss*) and ayu (*Plecoglossus altivelis*) compared to inactivated whole cell *F. psychrophilum* bacterin. Coincident with higher protection, sera of fish immunised with the OMF vaccine had higher agglutination titres than those of fish immunised with inactivated whole cell *F. psychrophilum*.

In the recombinant vaccine approach proteins or glycoproteins that are important in inducing protective immunity from many families of pathogens are investigated. Once the putative protein (to be used as a vaccine) is identified, it is expressed in efficient prokaryotic expression systems in case of a bacterial protein. Either gram-negative or gram positive bacteria can be engineered to secrete the proteins into the growth media, target the product into the periplasm, or have the bacteria retain the product as an insoluble mass intracellularly called inclusion bodies (Pillai et al., 1996; Kleid et al., 1981; Amann et al., 1985; Babuiik, 1999). *E. coli*, which is the most commonly used bacteria for the expression of proteins, offers a number of advantages. The methods are relatively inexpensive, proteins can be over-expressed with high yields, and expression can be easily regulated. Immobilized metal ion chromatography has opened new prospects for efficient purification of proteins with engineered poly-histidine tags (Gaberc-Porekar and Menart, 2001). By identifying the proteins involved in inducing protective immunity and isolating the gene coding for these proteins it is possible to use recombinant DNA technology or synthetic peptide
technology to produce sufficient quantities of these protective epitopes for incorporation into vaccines. Recombinant proteins and synthetic peptides are considered to be safer potential vaccine sources than inactivated or live attenuated microorganisms.

Only one vaccine containing recombinant products is commercially available for aquaculture use (Grudding et al., 1999) while effective synthetic peptide and protein vaccines have been prepared against animal viruses and human microbial diseases (Borras-Cuesta et al., 1987; Partidos et al., 1991). The gene encoding the envelope glycoprotein of a salmonid rhabdovirus, viral haemorrhagic septicaemia virus (VHSV) was cloned and expressed in *Escherichia coli* by Lorenzen et al., (1993). The glycoprotein, without the N-terminal leader sequence and C-terminal hydrophobic anchor segment, was expressed in *E coli* as a factor Xa protease-cleavable fusion protein. When injected in rainbow trout the purified and renatured viral part of the recombinant protein was able to elicit VHSV-specific antibodies and neutralizing antibody activity in serum. Lecocq-Xhonneux et al., (1994) reported for the first time that a recombinant viral haemorrhagic septicaemia virus glycoprotein expressed in insect cells induces protective immunity in rainbow trout. The VHS virus (VHSV) glycoprotein, which induces neutralizing antibodies in rainbow trout, was chosen for expression in insect cells using a baculovirus vector. The recombinant protein displayed different degrees of glycosylation and was recognized in ELISA by neutralizing antibodies. It was transported to the plasma membrane of insect cells where its ability to induce membrane fusion was preserved. The efficacy of the recombinant protein as a vaccine was compared with those of an inactivated and an attenuated vaccine. When injected intraperitoneally into rainbow trout, the baculovirus-encoded protein was shown (i) to induce the synthesis of VHSV-neutralizing antibodies and (ii) to confer protection against virus challenge. This is the first report of a recombinant vaccine that protects fish against VHSV.

Husgag et al., (2001), studied the immune response to a recombinant capsid protein, rT2, of striped jack nervous necrosis virus (SJNNV) in turbot (*Scophthalmus maximus*) and Atlantic halibut (*Hippoglossus hippoglossus*). A specific humoral immune response was recorded in both species, and the levels of rT2-specific antibodies and evaluation of a vaccine against SJNNV. A vaccine efficacy test performed on juvenile turbot, employing oil emulsified rT2 as a test vaccine and intramuscular inoculation of SJNNV showed significant protection was observed when the challenge was performed 10 wk post-vaccination.
A. hydrophila, 43 kDa major adhesin gene (prerequisite for successful invasion of the bacterium) was cloned and expressed using the T-5 expression system. The purified recombinant adhesin when used to immunise blue gourami, could confer significant protection to fish against experimental A. hydrophila challenge (Fang et al., 2004).

Recently, Sommerset et al., (2005) showed that recombinant capsid protein vaccination elicited protection in turbot against Atlantic halibut nodavirus. A recombinant protein vaccine and a DNA vaccine were produced, based on the same capsid-encoding region of the Atlantic halibut nodavirus (AHNV) genome, and tested for protection in juvenile turbot (Scophthalmus maximus). Vaccine efficacy was observed in the fish vaccinated with recombinant capsid protein but not in the DNA-vaccinated fish.

DNA VACCINES

Genetic immunisation is currently a new approach to vaccination of the aquacultured species. In 1990, Wolff and his colleagues were the first to report the successful expression of naked plasmid DNA in mouse muscle tissue. A few years later it was reported that the injection of DNA encoding an antigenic protein of influenza virus conferred protective immunity in mice (Ulmer et al., 1993). It mimics the manner by which live attenuated viruses deliver antigens and offers advantages over conventional, live attenuated, whole killed, or subunit protein vaccines. Predominantly, it places the immunised host at no risk from infection as the vaccine consists of plasmids containing pathogen specific genes for antigenic proteins, representing a novel means of expressing antigens in vivo, for both humoral and cellular immune responses (Donnelly et al., 1997). Introduction of naked plasmid DNA induces a strong, long-lived immune response to the antigen encoded by the gene vaccine (Corr et al., 1996). After introduction the host cells take up the plasmid DNA and the resultant foreign protein is produced within the host cell and then processed and presented appropriately to the immune system, inducing a specific immune response (Donnelly et al., 1997 and Cohen et al, 1998). Genetic or DNA vaccines have been used to deliver viral (Ulmer et al., 1993 and Davis et al., 1996), bacterial (Anderson et al., 1996), and parasite (Doolan et al., 1996 and Rothel et al., 1997) antigens to a variety of animal models from mice through to cattle (Cox et al., 1993) and sheep (Rothel et al., 1997). In fish, antigen processing and presentation is predominantly conducted by macrophages (Secombes, 1996) and re-expressed on antigen presenting cell surfaces for presentation to lymphocytes (Vallejo et al., 1996).
DNA vaccines offer several advantages over classical antigen vaccines (i.e., live attenuated, whole killed and subunit vaccines). They are inexpensive, easier to clone and modify, and do not require a cold chain like proteins. Mixing together different plasmids, or including more than one antigen-encoding gene in single vector for collinear expression can also prepare multivalent vaccines. Thus DNA vaccinations is an attractive approach to fish vaccine manufacturers.

The administration of a simple plasmid can induce a broad spectrum of immune responses (Kowalczyk et al., 1999). They include the activation of CD8+ T lymphocytes, implicated in host defense against intracellular pathogens via cytotoxic T lymphocytes (CTL), and CD4+ T lymphocytes, which secrete cytokines and play a role in B cell production of specific antibodies. The mechanism has been depicted in the figure shown.

Principle of DNA vaccination. (Adopted from DNA vaccines: new applications for veterinary medicine by Vinciane Dufour; 15 May 2001)
Plasmid constructs used for expression of foreign genes in fish usually contain a mammalian expression vector backbone. Sequences for transcriptional control in vectors i.e promoter, enhancer etc have been assessed for the expression of foreign genes in fish tissue. The immediate early promoter of the cytomegalovirus (CMV) gives the best results and is the most widely used in fish vaccination trials (Anderson et al., 1996; Lorenzen et al., 1998; Lorenzen et al., 1999).

Fish specific expression vector are now being considered in place of the CMV promoter. DNA plasmid vectors were constructed by Alonso et al., (2003) which had inducible rainbow trout promoters. DNA fragments containing the promoter regions upstream of the rainbow trout Mx1 and interferon regulatory factor 1A (IRF1A) genes were obtained and their nucleotide sequences were determined. Vectors containing the G gene of IHNV linked to the trout promoters were found to be very effective as vaccines in rainbow trout.

Expression of the G protein from infectious haematopoietic necrosis virus (IHNV) (Anderson et al., 1996) and viral haemorrhagic septicaemia virus (VHSV) (Heppell et al., 1998), production of trout antibodies against G (Bourdinot et al., 1998) and trout protection against viral challenges have been demonstrated following intramuscular injection of plasmids coding for the corresponding rhabdoviral genes (Lorenzen et al., 1998; Traxler et al., 1999; Corbeil et al., 2000).

The relative efficacy of various routes of immunisation with pIHNVw-G (glycoprotein (G) of IHNV) was evaluated by Corbeil et al., (2000) using rainbow trout fry vaccinated via intramuscular injection, scarification of the skin, intraperitoneal injection, intrabuccal administration, cutaneous particle bombardment using a gene gun, or immersion in water containing DNA vaccine-coated beads. Results of the virus challenge showed that the intramuscular injection and the gene gun immunisation induced protective immunity in fry, while intraperitoneal injection provided partial protection. Neutralising antibodies were not detected in sera of vaccinated fish regardless of the route of immunisation used, suggesting that cell mediated immunity may be at least partially responsible for the observed protection.

Recently the Immunogenic and protective effects of a DNA vaccine for Mycobacterium marinum infection in Juvenile hybrid striped bass (Morone saxatilis x M. chrysops) was reported by Pasnik and Smith (2005). A DNA vaccine was constructed using the Mycobacterium marinum Ag85A gene (encoding one of the major secreted fibronectin-binding proteins of Mycobacterium spp. Juvenile hybrid striped bass (Morone saxatilis x M. chrysops) were immunized by i.m. and i.p.
injection with the resulting construct and as a result produced specific immune responses towards the Ag85A. Increasing concentrations of humoral antibodies to the Ag85A antigen were generated in all DNA vaccine groups, while macrophage phagocytosis and respiratory burst functions failed to exhibit upregulation after vaccination. Fish receiving the DNA vaccine developed a protective response to a live M. marinum challenge 90 days post-inoculation, as demonstrated by increased survival of vaccinated fish over control fish and by reduced splenic bacterial counts in vaccinated fish. Furthermore, humoral immune responses and protective effects were significantly increased at higher vaccine doses using the i.m. injection route.

Potential of DNA vaccination in spotted sand bass (Paralabrax maculatofasciatus) was evaluated by Vazquez-Juarez et al., (2005). Immunization of the fish with the outer membrane proteins (OMP 38 and OMP 48) of Aeromonas veronii resulted in weak protein expression, as determined by immunoblotting, was observed after transfection of eukaryotic cells with the DNA vaccines. Challenge with A. veronii recorded a relative percent survival (RPS) between 50 and 60% indicating the protective (partial) potential DNA plasmids encoding the major OMPs from A. veronii.

Gene expression libraries have also been assessed for the effect of immune response in fish. Miquel and coworkers (2003) used the expression library immunization technology to study the protection of Coho salmon (Oncorhynchus kisutch) to the infection with Piscirickettsia salmonis. Purified DNA from this bacterium was sonicated and the fragments were cloned in the expression vector pCMV-Bios and two expression libraries prepared. Intramuscular administration of the plasmid DNA isolated from the expression library to the fish followed by a second booster dose was resulted in 20% survival of the fish as compared to the controls.

**PORINS**

Gram-negative bacteria are characterized by the construction of their cell walls. The cytoplasm is surrounded by the cytoplasmic or inner membrane, and mechanical stability and shape is supported by a a murein layer. The cell is surrounded by a second outer layer which makes few contacts with the inner membrane. In between is the periplasm; in this compartment there are some metabolic activities which would disturb the metabolism within the cell proper - e.g. reactions dealing with toxic substances. The exchange of substances between cytoplasm and periplasm is regulated by highly specific transport systems. The exchange between periplasm and environment occurs via outer membrane proteins. Nakae characterised such a
protein for the first time in 1976 and named it porin, which may be unspecific or specific for groups of substances.

About 50% of the outer membrane mass consists of protein, either in the form of integral membrane proteins or as lipoproteins that are anchored to the membrane by means of N-terminally attached lipids. More than a dozen different outer membrane lipoproteins have been identified in *E. coli* (Blattner *et al.*, 1997). A few integral membrane proteins, such as OmpA and the general porins, are expressed at high levels. Besides these, there are minor proteins whose synthesis in some cases is strongly induced when they are needed, such as porins (e.g. PhoE and LamB), TonB-dependent receptors (e.g. FhuA and FepA), components of several protein export systems, referred to as autotransporters and systems I to III (comprising the ABC transporters, the general secretion pathway, the type III secretion apparatus; Pugsley, 1993), proteins involved in the biogenesis of flagella and pili (Macnab, 1999; Soto and Hultgren, 1999), and enzymes (e.g. OmpT protease, Mangel *et al.*, 1994; and phospholipase A, Dekker, 2000), whose function is still very much in the dark.

Depending on their physicochemical properties and their conformational and environmental characteristics, bacterial porins can display either a trimeric or monomeric organisation (Koebnik *et al.*, 2000). The folding pattern of each monomer is constituted by 16 or 18 antiparallel β-strands crossing the outer membrane. Loops connect the β-strands on either side of the membrane. The trimer is an antiparallel β-barrel with large loops of variable length on the external surface of the bacterial membrane and short periplasmic turns. The barrel spans the entire outer membrane, with the three barrel axes approximately parallel to one another and perpendicular to the membrane plane. The lumen of the pore is restricted by one large loop that folds back into the channel and narrows it, determining the effective size of the pore and thus the molecular exclusion limit as well as its physiological and conductivity properties. These restricted entry checkpoints allow the selective uptake of required nutrients (Schulz, 2002). The three monomer units making up the functional trimer associate through numerous protein–protein interactions inside the bilayer and interactions between surface-exposed loops.

Porin loops are potential sites for the binding of bactericidal compounds to the surface of gram-negative bacteria. Altogether these interactions give porins a considerable stability, and only drastic conditions, e.g. harsh detergent such as sodium dodecyl sulfate at high temperatures (up to 70°C), can dissociate them. This tight conformation, inserted into the outer membrane, organizes a compact molecule
with specific and highly variable cell-surface-exposed domains involved in various activities (Koebnik et al., 2000).

The high level of expression ($10^4$–$10^6$ copies per cell) of the major outer membrane protein is the most striking feature of porin which depends on class, bacterial species and the environmental conditions (Koebnik et al., 2000). The synthesis of porins may be up- or down-regulated by the presence or absence of special molecules in the medium. Maltose and equivalent dextrins induce the expression of maltoporin LamB, and sucrose or fructose similarly regulates ScrY. Phosphate starvation allows the full expression of PhoE, the phosphoparin.

**FUNCTIONS OF PORINS**

Porins due to their structure and high level of expression may affect several biological functions apart from their regular tasks, which allows them to adapt rapidly to surrounding conditions and survival of bacterial cells. Being privileged in their location and high copy number, porins have been selected during evolution to take up various functions, phage receptors, bacteriocins, sites for antibody production by the immune system, binding sites for components of complement cascades, sites involved in the uptake of various nutrient transport complexes.

**PORIN AS AN IMMUNOGEN**

Isolation of carbohydrate-reactive outer membrane proteins (CROMPs) from *A. hydrophila* A6 strain by affinity chromatography on the basis of their reactivity with trisaccharide structures analogous to the terminal trisaccharide of the H antigen of the human ABO(H) blood group system, characterized by using antisera raised against the isolate has been reported by Quinn et al., 1993. The isolated products contained at least three components (M(r)s, 43,000, 40,000, and < 14,000), as detected by immunoblot analysis with a polyvalent, polyspecific rabbit antiserum to *A. hydrophila* A6 (serum 3/83). A specific antiserum (serum 3/91) prepared in a rabbit by repeated immunizations with nitrocellulose containing the 43,000-Da band reacted with three bands (M(r)s, 43,000, 40,000, and < 14,000) in immunoblot analysis of solubilized outer membranes of *A. hydrophila* A6, suggesting that the 40,000- and < 14,000 Da elements are immunologically related to components of the 43,000-Da protein. CROMPs act as adhesions and possibly contribute to the virulence of this organism. A conserved *Aeromonas salmonicida* porin with an apparent M(r) of 28,000 was isolated by Lutwyche et al., (1995). A polyclonal antibody was raised using the purified protein. Immunoblot analysis showed that an immunologically cross-reactive protein was present in other *Aeromonas* strains but not in strains of
Vibrio or Yersinia Rainbow trout (Oncorhynchus mykiss) immunized intraperitoneally with the purified porin protein were significantly protected from experimental A. salmonicida challenge.

Porins have been observed to be involved in the interaction and adherence of certain pathogenic bacteria with the leukocytes. Negm and Pistol (1999) showed that the OmpC porin of S. typhimurium mediates adherence to macrophages by using transposon mutagenesis to develop an OmpC-deficient mutant. Indications from this study support a contribution of the OmpC in initial recognition by macrophages, allowing the identification of regions of this protein that potentially participate in host cell recognition of bacteria by phagocytic cells (Galdiero et al., 1999). In the case of Pasteurella multocida, porin and LPS are able to modulate inflammatory and immunological responses by affecting the release of several cytokines and the expression of their genes. They up-regulate the mRNA expression of IL-1α, IL-6, TNF-α, INF-γ and IL-12 p40 (Lovane et al., 1998). Porin proteins from different bacterial species, such as S. typhimurium, Salmonella minnesota, K. pneumoniae and Aeromonas hydrophila have been shown to activate complement via the classical or alternative pathway, after C1q binding (Alberti et al., 1996; Merino et al., 1998). Nogueras et al., (2000) cloned and sequenced the structural gene for Aeromonas hydrophila porin II from strain AH-3 and defined the role of porin II as an important surface molecule involved in serum susceptibility and C1q binding in these strains.

ROLE OF PORINS IN ANTIBiotic RESISTANCE

Porins play a significant role in antibiotic resistance by modulating the pore size. This is achieved by expression of variable levels of porin and have been reported to exhibit antibiotic resistance, thus favouring onset of disease. Nosocomial outbreaks are mainly due to extended-spectrum β-lactamase (ESBL)-producing enterobacteria. Production of β-lactamase by these microorganisms inactivates most of the β-lactam antibiotics, and their resistance to other antibiotic groups, such as aminoglycosides and quinolones, prevent their eradication owing to the very restricted choice of drug (Achouak et al., 2001). In the presence of some antibiotics, bacterial pathogens switch off or decrease the expression of the porins concerned. There have been reports which suggest the relationship between antibiotic resistance and loss of porins OmpC, OmpF and OmpK35 especially for E. coli, Salmonella typhimurium and K. pneumoniae strains respectively (Chavaller et al., 1992). The alteration of the functional structure of the pore has been demonstrated for β-lactam-resistant
isolates (Gill et al., 1998; Hernandez-Allés et al., 1999). Porins have also been reported to be involved in bacterial resistance to carbapenems in P. aeruginosa and E. cloacae (Livermore, 1995).

ROLE OF PORINS IN PATHOGENESIS

The role of porins in bacterial virulence has been reported in many bacteria. Outer membrane porins, such as OmpD from S. typhimurium (Dorman et al, 1989), OmpK36 from K. pneumoniae (Alberti et al., 1996), and OmpC from Shigella flexneri (Bernardini et al., 1993) may act as virulence factors during the invasive processes of these bacteria. Provenzano et al, 2000 reported that the transcriptional activator ToxR-dependent modulation of the outer membrane porins OmpU and OmpT seems to be critical for V. cholerae bile resistance, virulence factor expression, and intestinal colonisation, suggesting a relationship between porin and pathogenesis functions.

An interesting member of porin family is the PorB, of Neisseria gonorrhoeae, which causes sexually transmitted disease in humans. This porin is unique for the fact that it can translocate from the outer membrane of bacteria into the host cell membrane where it modulates the process of infection (Weel et al., 1991). The insertion process leads to the formation of a functional channel which is under regulation of the eukaryotic host cell. The translocation of the neisserial porin induces apoptosis by causing a rapid calcium influx, followed by the activation of the calcium-dependent cysteine protease calpain, and the central apoptosis-executing molecules, the caspases (Müller et al., 1999).

MALTOSE INDUCIBLE PORIN (LamB)

In case of stressed conditions like lack of nutrition, the pure diffusion process is too slow and the bacteria need to improve the efficiency of the translocation. For those cases, specific and highly sophisticated membrane channels are present in the outer membrane of the bacteria. The most extensively studied examples of specific porins are the maltooligosaccharide-specific channel Maltoporin of E. coli (Szmelcman et al., 1975; Schmid et al., 1988; Kullman et al., 2002). Maltoporin forms ion-conducting channels when reconstituted into lipid bilayers (Szmelcman et al., 1976; Benz et al., 1986). The 3D structure of Maltoporin shows that the monomer of Maltoporin of E. coli consists of an 18 stranded β-barrel with short turns at the periplasmic side and large irregular loops at the outside of the cell (Dutzler et al., 1996). Molecular cloning and nucleotide sequence analysis of the maltose-inducible porin gene of Aeromonas salmonicida (maltoporin) was reported by Dodsworth et al., (1993). The gene had a high degree of similarity with Escherichia coli LamB, and codon usage was very
similar to that of another A. salmonicida outer membrane protein but markedly different from those of extracellular proteins. A LamB-like maltoporin from Aeromonas hydrophila A6 strain was first identified in both carbohydrate-reactive outer membrane proteins CROMP and carbohydrate-reactive porin preparations on the basis of pore-forming properties in planar lipid bilayers and channel inhibition with maltotriose solutions by Quinn et al., (1994).

The bacteriophage Lambda is a virus that recognizes the maltoporin channel at the outer cell surface of the bacteria (Roa and Scandella, 1976), hence the name LamB. In absence of this membrane channel, phage Lambda cannot recognize the bacteria. The bacteria can defend themselves against viral attacks in the presence of any minor mutations. The virus itself can, in turn mutate to restore binding ability.

Most of the immunological studies have been conducted with native outer membrane proteins isolated from bacterial membranes. However, only few reports are available on the immunogenicity of recombinant porins. Most immunogenic studies have been conducted with Nesseral porins (Ward et al., 1996; Wright et al., 2002). The outer membranes proteins are quite difficult to express, still several reports of have been put forth on expression of outer membrane porins from a number of bacteria (Aguero et al., 1987; Coulton et al., 1992; Koehler et al., 1992; Ward et al., 1996; Fenno et al., 1996; Fang et al., 2000; Rahman et al., 2000; Wright et al., 2002).

In aquaculture vaccination with outer membrane proteins has been shown to be effective against A. salmonicida and A. hydrophila (Hirst and Ellis, 1994; Lutwyche et al., 1995; Bricknell et al., 1999; Fang et al., 2000; Rahman et al., 2000). Yet there is hardly any report on the use of recombinant vaccines for fish. As infections of Aeromonas sp. are common among the Indian freshwater fishes, the present study is directed to apply the recombinant and DNA vaccine approach against this infection. The gene candidate selected is maltose-inducible porin which has been shown to elicit protective response in rainbow trout.